

RESEARCH PAPER

Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats

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BACKGROUND AND PURPOSE

The designer drug 1-(4-methylphenyl)-2-methylaminopropan-1-one (4-methylmethcathinone, mephedrone) is reported to possess psychostimulant, entactogenic and hallucinogenic effects. The purpose of this study was to examine the effects of acute administration of mephedrone on extracellular levels of dopamine (DA) and 5-HT in the nucleus accumbens of awake rats and compare these effects with those induced by 3,4-methylenedioxyamphetamin (MDMA, ecstasy) and amphetamine.

EXPERIMENTAL APPROACH

Microdialysis sampling was performed while simultaneously recording locomotor activity in rats and the monoamines were determined by HPLC with electrochemical detection.

KEY RESULTS

Mephedrone (3 mg·kg⁻¹ s.c.) and (+)-amphetamine (1 mg·kg⁻¹ s.c.) caused rapid increases in extracellular DA levels of 496% and 412%, respectively, whereas MDMA (3 mg·kg⁻¹ s.c.) showed only a moderate effect (235%). The corresponding 5-HT levels increased to 941% (mephedrone) and 911% (MDMA), but only to 165% following amphetamine. The calculated $t_{1/2}$ values for elimination rate of mephedrone, MDMA and amphetamine-induced increases in extracellular DA levels were 25, 303 and 51 min, the corresponding $t_{1/2}$ values for 5-HT were 26, 48 and 84 min, respectively. Locomotor activity was increased most by amphetamine, whereas both mephedrone and MDMA showed about three times lower and shorter-lasting effects.

CONCLUSIONS AND IMPLICATIONS

The neurochemical and functional properties of mephedrone resemble those of MDMA, but it also shows an amphetamine-like effect in that it evokes a rapid release and elimination of DA in the brain reward system, a feature that may contribute to its potent re-inforcing properties.

Abbreviations

5-HIAA, 5-hydroxyindolacetic acid; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; MDMA, (±)-3,4-methylenedioxyamphetamin hydrochloride; NA, noradrenaline; NAcc, nucleus accumbens

Introduction

Mephedrone (1-(4-methylphenyl)-2-methylaminopropan-1-one, 4-methylmethcathinone, 4-MMC) is a substituted phenethylamine, structurally a cathinone derivative that possesses powerful psychostimulant, entactogenic and hallucinogenic effects (see Europol-EMCDDA, 2010; Schifano *et al.*, 2011). Recently, mephedrone has gained growing popularity as a 'designer drug' or a 'research compound' offered for sale under various names via Internet sites, head shops and recently, by street dealers (Schifano *et al.*, 2011; Winstock *et al.*, 2010a,b; 2011). During the last 2 years, mephedrone has been banned in most of the EU countries. However, the unified international legislation and control of mephedrone as a substance of abuse is still missing. The users of mephedrone have described its psychomimetic effects being comparable to amphetamine, cocaine and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) causing euphoria, elevated mood, stimulation, enhanced appreciation for music, decreased hostility, improved mental function and mild sexual stimulation (Europol-EMCDDA, 2010). A recent web-based survey from 1006 responders revealed that mephedrone users consider its effects best compared with those of MDMA (Carhart-Harris *et al.*, 2011). However, mephedrone is associated with a high risk of triggering repetitive and uncontrolled drug intake subsequent to its initial administration. Excessive intake of mephedrone leads to acute intoxication, displaying the clinical features of acute sympathomimetic toxidrome (Wood *et al.*, 2010a,b); this has been linked to an increasing number of fatalities in European countries as reported recently (Gustavsson and Escher, 2009; Dickson *et al.*, 2010; Winstock *et al.*, 2010a; Lusthof *et al.*, 2011). Based on the spectrum of psychostimulant effects described by the drug users and the chemical similarity of mephedrone to substituted methcathinone and methamphetamine it has been speculated that mephedrone may act via increased release and re-uptake inhibition of 5-HT (serotonin) and dopamine (DA) (Schifano *et al.*, 2011). However, there are no data available to provide neurochemical evidence for the *in vivo* effects of mephedrone on DA and 5-HT transmission in the brain areas implicated in drug reinforcement.

The chemical structure of mephedrone, being a β -ketoamphetamine, indicates that it is closely related to cathinone, the active constituent of khat known for its psychostimulant properties (see Kalix, 1994). Indeed, cathinone was shown to exert similar effects to amphetamine, increasing locomotor activity (Kalix, 1992) and extracellular DA levels in the nucleus accumbens (NAcc) and caudate-putamen of rats (Pehek *et al.*, 1990). These findings offer an initial hypothesis that mephedrone, besides its similarity to MDMA that affects mostly 5-HT release and 5-HT₂ receptors (see Gudelsky and Yamamoto, 2008), may also act by increasing the release of DA and/or inhibiting its re-uptake in the mesolimbic structures including the NAcc. Both animal and human studies revealed that the acute re-inforcing effects of drugs, as well as their incentive and reward seeking behaviour, are anatomically linked to the NAcc and mesolimbic DA system (see Koob and Volkow, 2010). Further, the abuse potential of a drug is believed to be governed primarily by its $t_{1/2}$, and the potency and the kinetics of the pharmacodynamic responses induced (see Nutt *et al.*, 2007).

The rationale behind this study was to examine, by use of *in vivo* microdialysis and simultaneous recordings of locomotor activity, the effects of acute administration of mephedrone on extracellular levels of DA and 5-HT and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA) in the NAcc of awake rats and compare these effects with those induced by a single dose of (\pm)-3,4-methylenedioxymethamphetamine (MDMA) and (+)-amphetamine.

Methods

Chemicals and test substances

Dopamine.HCl (DA), 5-HT, the metabolites DOPAC, 5-HIAA and MDMA, as well as chemicals for mobile phase and perfusion medium preparations including sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate, sodium chloride, potassium chloride, calcium chloride, citric acid, sodium acetate and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). EDTA-2Na was obtained from Dojindo (Kumamoto, Japan). (+)-Amphetamine sulphate salt was purchased from Apoteket AB (Stockholm, Sweden), mephedrone.HCl (illicit formulation, purity >95%) was kindly provided by Swedish National Laboratory of Forensic Science (Linköping, Sweden). De-ionized water was obtained from a MilliQ water purification system (Millipore, MA, USA). Standard solutions of monoamines, metabolites and drugs were prepared in water and kept frozen (-20°C) in amber-coloured test tubes. All drugs were dissolved in saline and administered s.c. in a volume of $1\text{ mL}\cdot\text{kg}^{-1}$ in the scruff of the neck at the following doses (all in the respective salt form): mephedrone (1 or $3\text{ mg}\cdot\text{kg}^{-1}$), MDMA ($3\text{ mg}\cdot\text{kg}^{-1}$) and (+)-amphetamine ($1\text{ mg}\cdot\text{kg}^{-1}$). The nomenclature of drugs and molecular targets conforms to 'Guide to Receptors and Channels' (Alexander *et al.*, 2008).

Surgery and microdialysis experiments

Male Sprague-Dawley rats (weighing 300–350 g) were used in the study. The microdialysis experiments were carried out on awake rats following the protocol described elsewhere (Kehr, 1999; Kehr and Yoshitake, 2006). All animal care and experimental procedures were approved by the local ethical committee and complied with the guidelines of the 'Principles of Laboratory Animal Care' (NIH publication no. 8023) and the Council of the European Communities (86/809/EEC). The rats (three animals per cage) were maintained on a 12 h light-dark cycle (light at 7:00 h), room temperature $22 \pm 2^{\circ}\text{C}$ and humidity 50–55%. All efforts were made to minimize animal suffering and the number of animals used for the study.

Rats were anaesthetized with isoflurane (Forene®, Abbott Laboratories, Abbott Park, IL, USA) using a Univentor 400 anaesthesia unit (AgnThos, Lidingö, Sweden). The rat was placed in a stereotaxic frame (David Kopf Instruments, CA, USA) in a flat skull position with the incisor bar set to -3.2 mm . The isoflurane anaesthesia was maintained at 2% in the mixture of air, the air flow was $500\text{ mL}\cdot\text{min}^{-1}$, the body temperature of the rat was controlled by a rectal thermometer and maintained at $+37^{\circ}\text{C}$ using a CMA/150 temperature controller (CMA/Microdialysis, Stockholm, Sweden). The middle

scalp incision of 2–3 cm was made and the flaps were kept aside using the homeostatic forceps. After exposure of the skull, a hole for a probe and two holes for the fixing screws were drilled using a fine trephine drill. The guide cannula for a microdialysis probe (Eicom Corp., Kyoto, Japan) was implanted into the NAcc (AP +2.2 mm, L +1.1 mm, V –5.3 mm; from bregma and the dural surface, according to the stereotaxic atlas of Paxinos and Watson (2007). The guide cannula was fixed firmly to the skull surface using dental cement. Following 5–7 days of recovery, a microdialysis probe (Eicom CX-I; 0.22 mm outside diameter, 2 mm membrane length with 50 000 Da cut-off) was inserted into the guide cannula of the awake rat. The probe was perfused with Ringer's solution (NaCl, 147 mM; KCl, 4 mM; CaCl₂, 2.3 mM) at a flow rate of 1 $\mu\text{L}\cdot\text{min}^{-1}$. After the initial stabilization period of 2–3 h, the microdialysis samples were collected in 20 min intervals. The first three samples were used for estimation of basal levels of DA, 5-HT, and DOPAC, homovanillic acid (HVA) and 5-HIAA. Thereafter, mephedrone (1 or 3 $\text{mg}\cdot\text{kg}^{-1}$), MDMA (3 $\text{mg}\cdot\text{kg}^{-1}$) (+)-amphetamine (1 $\text{mg}\cdot\text{kg}^{-1}$) or vehicle were injected s.c. to separate groups of rats and the fractions were collected for 180 min. At the end of the experiment, the animals were killed by an overdose of isoflurane and dislocation of the neck. The brains were removed and examined for correct placement of the probe (the probe track) in the rat brain.

Locomotor activity test

Locomotor activity was monitored by use of a single-beam activity frame (44 \times 30 cm ACTIMO 10, Shintech, Japan) placed around the lower part of the Macrolon III cage. This arrangement allowed for simultaneous recordings of locomotor activity and microdialysis sampling. The data were collected by counting and summarizing the overall activity (number of beam crossings) in 5 min intervals and further pooled into 20 min bins, thereby matching the frequency of microdialysis sampling.

HPLC determination of monoamines and acidic metabolites

Concentrations of DA and 5-HT in the brain microdialysis samples were determined by HPLC with electrochemical detection as described elsewhere (Kehr and Yoshitake, 2006). Briefly, the HPLC system consisted of a HTEC500 unit (Eicom, Kyoto, Japan), and a CMA/200 Refrigerated Microsampler (CMA Microdialysis, Stockholm, Sweden) equipped with a 20 μL loop and operating at +4°C. The potential of the glassy carbon working electrode was +450 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 200 \times 2.0 (inside diameter) mm Eicompak CAX column (Eicom) protected with a guard column CAX-GC2/20 (Eicom). The mobile phase was a mixture of methanol and 0.1 M phosphate buffer (pH 6.0) (30:70, v v⁻¹) containing 40 mM potassium chloride and 0.13 mM EDTA-2Na. The chromatograms were recorded and integrated by use of a computerized data acquisition system Clarity (DataApex, Prague, Czech Republic).

The detection limit (signal-to-noise ratio = 3) for DA and 5-HT was 0.05 nM, that is, 0.75 fmol in 15 μL injected onto the column respectively. Concentrations of DOPAC, HVA and 5-HIAA in the brain microdialysis samples were determined

by a separate HPLC system with electrochemical detection (HTEC500). The potential of the glassy carbon working electrode was +750 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 150 \times 3.0 (inside diameter) mm Eicompak SC-50DS column (Eicom) protected with the guard column OPTI-GUARD C18 (Optimize Technologies, Oregon, OR, USA). The mobile phase was a mixture of methanol and 0.1 M citrate/0.1 M sodium acetate buffer solution (pH 3.5) (16:84, v v⁻¹) and contained 210 $\text{mg}\cdot\text{L}^{-1}$ octanesulphonic acid sodium salt and 5 $\text{mg}\cdot\text{L}^{-1}$ EDTA-2Na. The detection limit (signal-to-noise ratio = 3) for DOPAC and 5-HIAA was 2 nM, that is, 10 fmol in 5 μL injected onto the column. The chromatograms were recorded and integrated by use of the computerized data acquisition system Clarity (DataApex).

Data presentation and statistical analysis

The basal concentrations of monoamines and metabolites were calculated from the mean values of three fractions collected from each individual animal during the pre-drug period (–60 to 0 min) and then expressed as a mean \pm SEM, $n = 4$ rats for each treated group. The mean concentrations were taken as 100% and all values were recalculated as a percentage of these basal levels. The overall effects of drug treatments on DA and 5-HT levels, as well as on the levels of metabolites DOPAC and 5-HIAA were expressed for each analyte as the area under the curve (AUC_(0–180 min)) calculated as the sum of relative changes in extracellular levels of DA, 5-HT, DOPAC and 5-HIAA over the 180 min post-treatment period (nine samples) and subtracted from the mean relative AUC_(0–180 min) value of the vehicle-treated group. Statistical analysis was performed using Prism 5 (GraphPad Software, San Diego, CA, USA) statistical software. Mean basal levels were compared by use of one-way ANOVA followed by Newman–Keuls multiple comparison test. Differences between the groups of treatment and interaction of treatment and time were analysed by repeated measures two-way repeated measures ANOVA followed by Bonferroni's post-test. Differences between the AUC_(0–180 min) values of DA, 5-HT, DOPAC, 5-HIAA and the ratio 5-HT : DA of the vehicle- and drug-treated groups were analysed by one-way ANOVA followed by Newman–Keuls multiple comparison test. Elimination rates expressed as the $t_{1/2}$ of the decays in drug-induced increases in extracellular DA and 5-HT levels and locomotor activity were calculated by use of a non-linear fit to the one phase exponential decay curve.

Results

Basal extracellular levels of DA, 5-HT, DOPAC and 5-HIAA in the rat NAcc

A typical placement of the guide cannula and the microdialysis probe in the NAcc is illustrated in Figure 1. As seen, the membrane of the microdialysis probe was positioned preferentially in the NAcc shell but protruded also to the core part of the nucleus. The basal concentrations (expressed in nM) of DA, 5-HT and metabolites DOPAC and 5-HIAA in the rat NAcc are summarized in Table 1. The basal levels of monoamines and metabolites did not significantly differ within the respective treated groups.

Table 1

Basal levels of DA, 5-HT, DOPAC and 5-HIAA

	Vehicle	Mephedrone (1 mg·kg ⁻¹)	Mephedrone (3 mg·kg ⁻¹)	MDMA (3 mg·kg ⁻¹)	Amphetamine (1 mg·kg ⁻¹)
DA	1.73 ± 0.34	1.22 ± 0.16	1.48 ± 0.36	0.92 ± 0.39	1.91 ± 0.49
5-HT	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.10 ± 0.02	0.09 ± 0.01
DOPAC	418.8 ± 144.4	366.3 ± 52.6	632.1 ± 58.4	399.3 ± 123.0	448.4 ± 34.9
5-HIAA	81.1 ± 27.5	114.3 ± 22.7	74.8 ± 17.9	77.2 ± 13.5	64.5 ± 9.3

The results were calculated as nM and expressed as mean ± SEM, *n* = 4, in the microdialysates from rat nucleus accumbens. The basal levels of monoamines and metabolites did not differ significantly within the respective treatment groups.

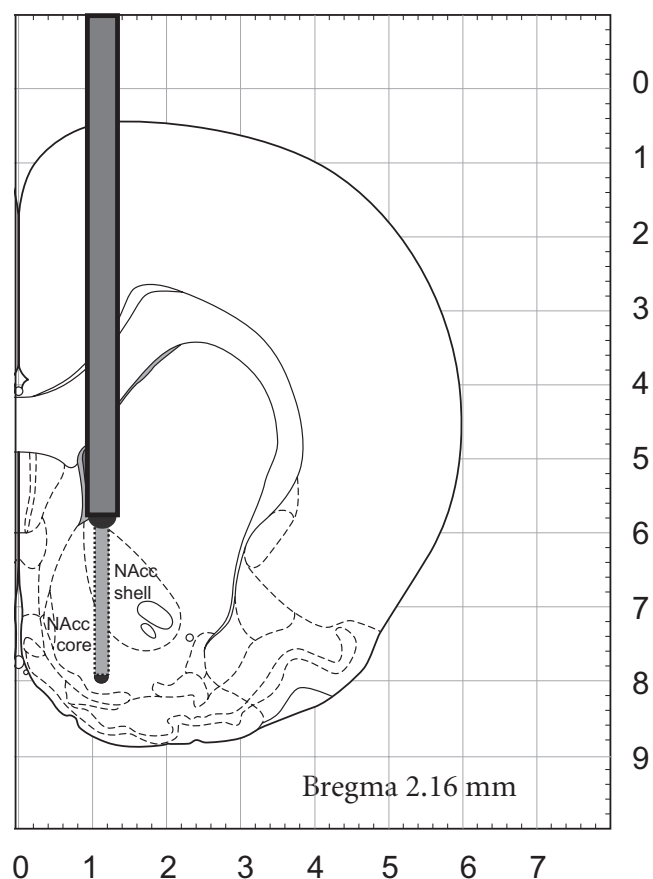


Figure 1

A representative placement of the microdialysis probe in the nucleus accumbens. The membrane of the microdialysis probe is targeting preferentially the shell but protruded also to the core part of the nucleus. Adapted from Paxinos and Watson (2007).

Effects of mephedrone, MDMA and amphetamine on DA and 5-HT levels

Administration of mephedrone at a dose of 3 mg·kg⁻¹ s.c. caused a rapid increase in extracellular levels of DA reaching the peak levels of 496 ± 140% (*P* < 0.001) for DA at 40 min (Figure 2A) and 941 ± 102% (*P* < 0.001) for 5-HT levels already at 20 min (Figure 2B).

Mephedrone given at the lower (1 mg·kg⁻¹) dose caused reduced but still significant increases in 5-HT levels to 709 ± 107% (*P* < 0.001) and DA levels to 295 ± 54% (*P* < 0.05). The DA levels between the groups were significantly different both for the treatment [*F*_(4,15) = 5.26; *P* < 0.01] and the interaction of time and treatment [*F*_(44,165) = 3.28; *P* < 0.001]. The corresponding values for the differences in 5-HT levels for the treatment were [*F*_(4,15) = 9.45; *P* < 0.001] and the interaction of time and treatment [*F*_(44,165) = 11.89; *P* < 0.001].

The increased concentrations of DA and 5-HT returned rapidly within the next 100–120 min to the basal levels. A single injection of MDMA (3 mg·kg⁻¹ s.c.) caused only a moderate increase in DA levels to 235 ± 16%, which was not significant (two-way ANOVA) from the vehicle group (Figure 2A). However, MDMA caused a massive increase in extracellular 5-HT levels with a peak value of 911 ± 180% (*P* < 0.001) at 40 min as shown in Figure 2B. Lastly, a single dose of amphetamine (1 mg·kg⁻¹ s.c.) increased the DA levels to 412 ± 101% (*P* < 0.001) at 40 min, and the 5-HT levels to 165 ± 12% (not significant from the vehicle group) shown in Figure 2A and B, respectively.

Effects of mephedrone, MDMA and amphetamine on DOPAC and 5-HIAA levels

The same samples analysed for DA and 5-HT concentrations were also analysed for the content of metabolites DOPAC and 5-HIAA. The graph representing the overall effect on the metabolites DOPAC and 5-HIAA following acute treatments with mephedrone at 1 and 3 mg·kg⁻¹, MDMA (3 mg·kg⁻¹) and amphetamine (1 mg·kg⁻¹), expressed as relative AUC_(0–180 min) values, is shown in Figure 3. Following administration of mephedrone at the higher dose, the DOPAC levels were significantly reduced by 23.3 ± 4.2% (*P* < 0.01) compared with the AUC_(0–180 min) value of the control group. A similar decrease by 27.6 ± 5.5% (*P* < 0.001) was observed for the MDMA effect, whereas amphetamine caused the most significant decrease of the DOPAC levels by 42.5 ± 3.6% (*P* < 0.001) of the corresponding value of the vehicle-treated group. Mephedrone (3 mg·kg⁻¹) and MDMA decreased non-significantly the AUC_(0–180 min) values of 5-HIAA by 14.9 ± 12.8% and 30.9 ± 3.0% respectively, whereas amphetamine caused a significant increase in 5-HIAA levels by 32.9 ± 14.3% (*P* < 0.05) of the vehicle-treated group.

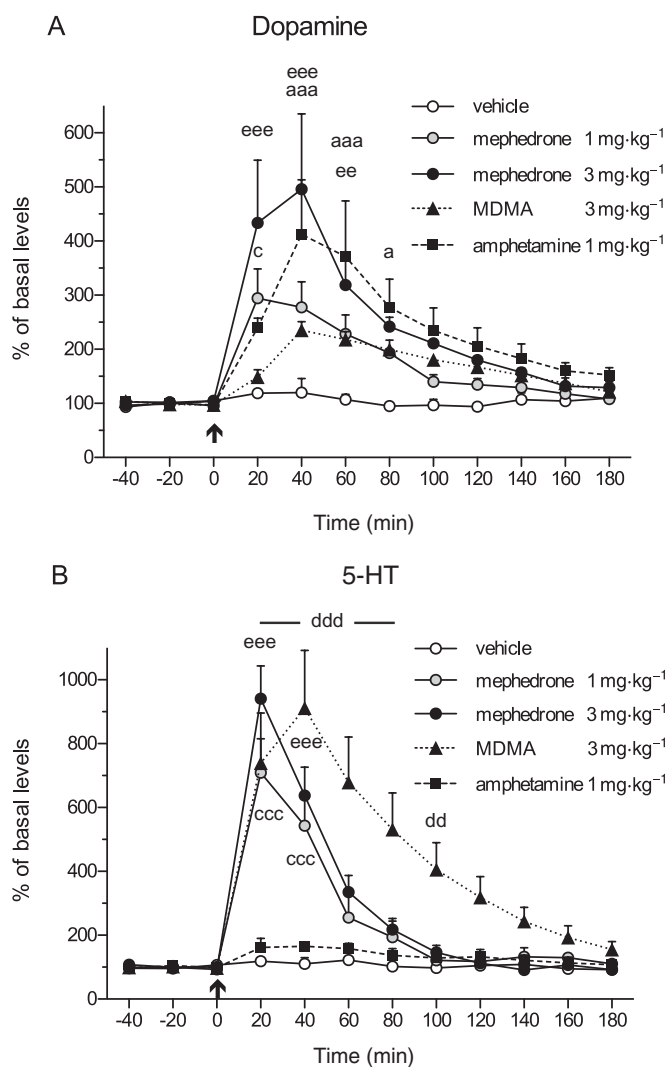


Figure 2

Effects of a single s.c. administration of mephedrone (1 and 3 mg·kg⁻¹), MDMA (3 mg·kg⁻¹) and amphetamine (1 mg·kg⁻¹) on (A) extracellular levels of DA and (B) extracellular levels of 5-HT in the NAcc of awake rats. The arrow indicates the time of drug or vehicle administration. Repeated measures two-way ANOVA followed by Bonferroni post-test; the drug-treated groups were compared with the vehicle group: mephedrone (1 mg·kg⁻¹) ^{ccc}*P* < 0.001, ^c*P* < 0.05; mephedrone (3 mg·kg⁻¹) ^{eee}*P* < 0.001, ^{ee}*P* < 0.01; MDMA ^{ddd}*P* < 0.001, ^{dd}*P* < 0.01; amphetamine ^{aaa}*P* < 0.001, ^a*P* < 0.05; mean ± SEM, *n* = 4.

The overall effects of mephedrone, MDMA and amphetamine on DA and 5-HT levels

The overall effects of mephedrone, MDMA and amphetamine on the relative AUC_(0-180 min) values of DA and 5-HT are depicted in Figure 4. Mephedrone (3 mg·kg⁻¹) caused a marked and significant increase in the AUC_(0-180 min) value of DA by 155.6 ± 35.5% (*P* < 0.01), which was similar to that induced by amphetamine: 148.5 ± 43.8% (*P* < 0.01). Further, mephedrone at 3 mg·kg⁻¹ caused a marked increase in the AUC_(0-180 min) of 5-HT by 197.8 ± 34.3% (*P* < 0.05), whereas the

same dose of MDMA increased the 5-HT value up to 364 ± 89.4% (*P* < 0.001) of the vehicle group. The AUC_(0-180 min) ratios of the 5-HT and DA values, calculated as mean ± SEM for each treatment group were: 1.01 ± 0.04 (vehicle), 1.42 ± 0.13 (mephedrone 1 mg·kg⁻¹), 1.22 ± 0.2 (mephedrone 3 mg·kg⁻¹), 2.68 ± 0.5 (MDMA 3 mg·kg⁻¹) and 0.62 ± 0.14 for amphetamine (1 mg·kg⁻¹). As seen, the 5-HT : DA values for mephedrone, and MDMA, indicate that these drugs are preferential releasers of 5-HT, whereas the 5-HT : DA ratio for amphetamine confirms its preferential effect on DA release.

Elimination rate of DA and 5-HT release induced by mephedrone, MDMA or amphetamine

The elimination rates of drug-induced increases in extracellular DA and 5-HT levels in the rat NAcc were calculated by use of a one-phase exponential decay curve fit to the experimental data and calculating the respective *t*_{1/2} values for DA and 5-HT. The calculated *t*_{1/2} values for the elimination rates of mephedrone-induced release of DA and 5-HT were 24.5 min and 25.5 min, respectively, for the dose of 3 mg·kg⁻¹. The corresponding values for 1 mg·kg⁻¹ mephedrone were 37.4 min and 24.8 min; for MDMA 302.5 min and 47.9 min, respectively, and for elimination of amphetamine-induced DA and 5-HT release, their respective *t*_{1/2} values were 51 min and 84.1 min. The calculated correlation factors (*r*²) for the curve fit of the DA and 5-HT decays for the mephedrone, MDMA and amphetamine-treated groups were for the DA curves: 0.595, 0.705 and 0.438, and for the 5-HT curves: 0.898, 0.664, 0.254, respectively.

Effects of mephedrone, MDMA and amphetamine on locomotor activity

Locomotor activity of vehicle- and drug-treated rats was monitored simultaneously during the microdialysis sampling period (Figure 5). The mephedrone-induced motor activation showed a peak level of 220.8 ± 34.7 counts in 20 min and returned to basal levels during the following 40 min. MDMA caused a similar, not significant motor activation (208.8 ± 29.3 beam crossings) in 40 min. Amphetamine induced a robust and long-lasting locomotor activation with a maximum of 554.5 ± 169.3 counts (*P* < 0.001) in the 40–60 min bin and the activation remained significantly elevated during the following 60 min, and, thereafter, slowly decreased until the end of the sampling period. Two-way repeated measures ANOVA followed by Bonferroni multiple comparison test revealed significant differences for the treatment (*F*_(4,15) = 6.04; *P* < 0.01) and the interaction of time and treatment (*F*_(44,165) = 4.69; *P* < 0.001). The overall value of locomotor activation (AUC_(0-180 min)) by amphetamine was 4.47 and 5.37 times larger than the corresponding AUC_(0-180 min) values of MDMA and mephedrone, respectively. The rate of decline of drug-induced motor activation expressed as the *t*_{1/2} of each exponential curve calculated by the nonlinear regression analysis of the experimental data revealed a *t*_{1/2} of 7.3 min (*r*² 0.663) for mephedrone, 23.6 min (*r*² 0.702) for MDMA and 43.9 min (*r*² 0.252) for amphetamine.

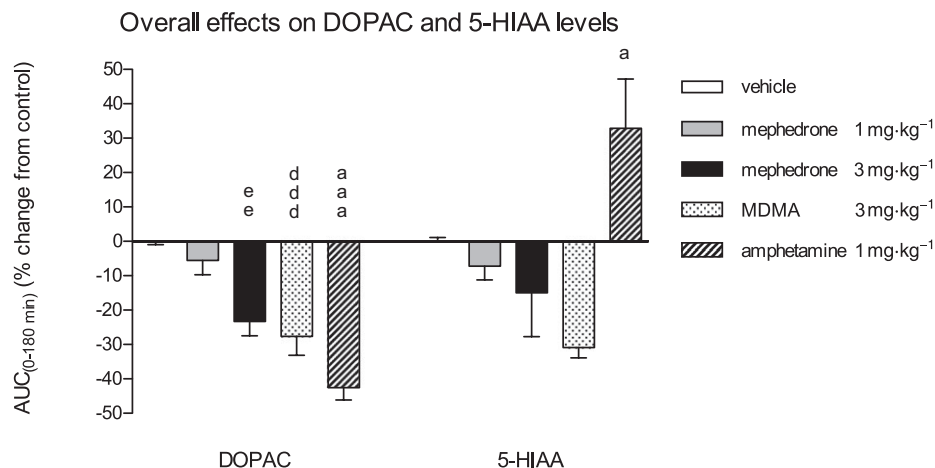


Figure 3

Overall effects of mephedrone (1 and 3 mg·kg⁻¹), MDMA (3 mg·kg⁻¹) and amphetamine (1 mg·kg⁻¹) on DOPAC, and 5-HIAA levels in the NAcc of awake rats. The columns represent the AUC_(0-180 min) values calculated as the differences in relative changes in DOPAC and 5-HIAA levels over a 3 h period between the drug- and vehicle-treated groups. One-way ANOVA followed by Newman-Keuls multiple comparison test; drug-treated groups were compared with the vehicle group: mephedrone (3 mg·kg⁻¹) ^{ee}*P* < 0.01; MDMA ^{ddd}*P* < 0.001; amphetamine ^{aaa}*P* < 0.001; ^a*P* < 0.05; mean ± SEM, *n* = 4.

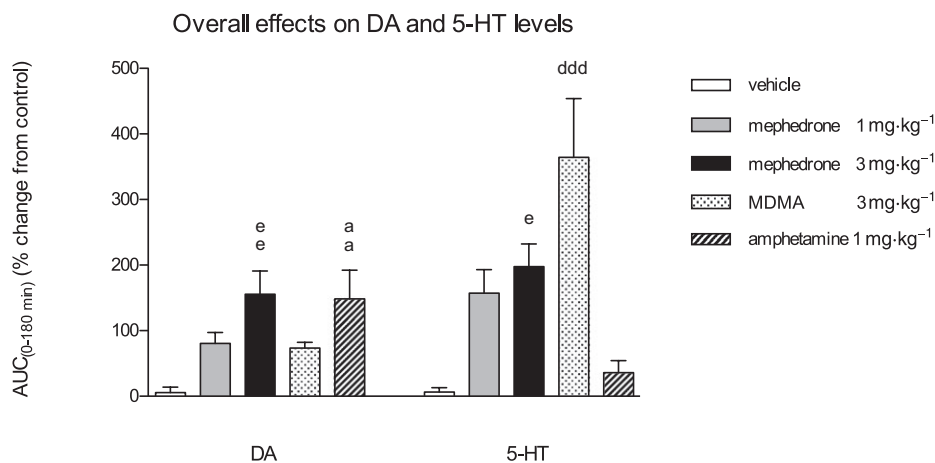


Figure 4

Overall effects of mephedrone (1 and 3 mg·kg⁻¹), MDMA (3 mg·kg⁻¹) and amphetamine (1 mg·kg⁻¹) on DA, and 5-HT levels in the NAcc of awake rats. The columns represent the AUC_(0-180 min) values calculated as the differences in relative changes in DA and 5-HT over a 3 h period between the drug- and vehicle-treated groups. One-way ANOVA followed by Newman-Keuls multiple comparison test; drug-treated groups were compared with the vehicle group: mephedrone (3 mg·kg⁻¹) ^{ee}*P* < 0.01, ^e*P* < 0.05; MDMA ^{ddd}*P* < 0.001; amphetamine ^{aa}*P* < 0.01; mean ± SEM, *n* = 4.

Discussion

Recently, numerous reports, comments and correspondence in medical literature have discussed and pointed out the health problems associated with growing use of novel synthetic stimulant drugs, so-called ‘legal highs’, which are relatively cheap and distributed under various names mostly via the Internet. Cathinone derivatives and, in particular, mephedrone has gained wide popularity as a research chemical and a party drug in several European countries (see Europol-EMCDDA, 2010) including Sweden (Gustavsson and Escher, 2009), England (Brandt *et al.*, 2010a,b,c; Dargan *et al.*, 2010;

Schifano *et al.*, 2011; Winstock *et al.*, 2010a,b; 2011), France (Debruyne *et al.*, 2010), Ireland (McNamara *et al.*, 2010; Nicholson *et al.*, 2010), Scotland (Torrance and Cooper, 2010) and the Netherlands (Brunt *et al.*, 2010). The users of mephedrone have compared its powerful psychostimulant, entactogenic and hallucinogenic properties to other abuse substances of this class including amphetamine, methamphetamine, cocaine and ecstasy (Europol-EMCDDA, 2010; Winstock *et al.*, 2010a,b; 2011). In a survey among dance drug users in the UK (Winstock *et al.*, 2011), mephedrone was the sixth most frequently used drug after tobacco, alcohol, cannabis, cocaine and MDMA. However, the use of mephedrone is associated

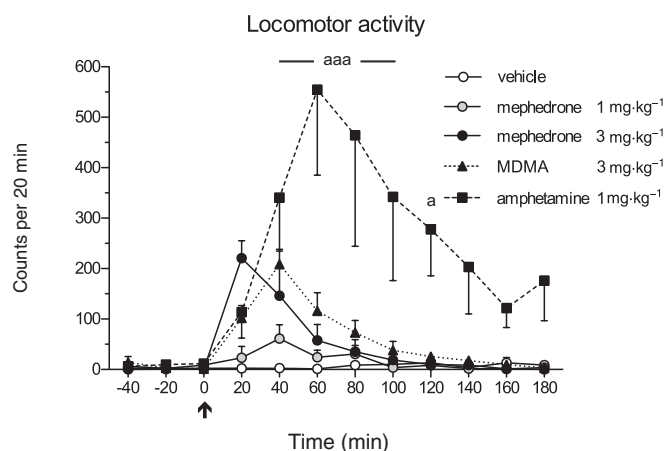


Figure 5

Effects of mephedrone (1 and 3 mg·kg⁻¹), MDMA (3 mg·kg⁻¹) and amphetamine (1 mg·kg⁻¹) on locomotor activity recorded simultaneously with microdialysis sampling. The drug or vehicle was administered at time 0 min (arrow). Repeated measures two-way ANOVA followed by Bonferroni post-test; the drug-treated groups were compared with the vehicle group: amphetamine: ^{aaa} $P < 0.001$, ^a $P < 0.05$; mean \pm SEM, $n = 4$.

with a high risk of overdose, leading to uncontrolled and often fatal drug intoxication (Gustavsson and Escher, 2009; Dickson *et al.*, 2010; James *et al.*, 2010; Regan *et al.*, 2010; Wood *et al.*, 2010a,b; 2011; Lusthof *et al.*, 2011).

Mephedrone as a substituted β -ketoamphetamine is expected to act primarily by increasing the release of monoamines and possibly via inhibition of their re-uptake (Schifano *et al.*, 2011). The major finding of the present study is that mephedrone causes significant, rapid and dose-dependent increases in both 5-HT and DA levels in the NAcc. The overall effects of mephedrone injected at a higher dose on the 5-HT levels were comparable to the effects induced by the same dose of MDMA. Both mephedrone and MDMA preferentially increased 5-HT over the DA levels; however, the proportion of 5-HT increase (ratio 5-HT : DA) was at least two times higher for MDMA than for mephedrone. In addition, mephedrone but not MDMA, still potentially increased the accumbal DA release to a level that was comparable to the effect induced by amphetamine. Amphetamine had only a minor effect on extracellular 5-HT concentrations.

The effects of MDMA and amphetamine on the release of DA and 5-HT in the rat NAcc observed in this study are in good agreement with earlier reports on the effects of these two drugs given at similar doses. Thus, a single i.p. injection of MDMA (2.5 or 3 mg·kg⁻¹) increased accumbal DA to about 200 to 250% (Kankaanpää *et al.*, 1998; O'Shea *et al.*, 2005; Panos and Baker, 2010) and 5-HT levels only to about 350% (Kankaanpää *et al.*, 1998) and 200% (O'Shea *et al.*, 2005) of the baseline. However, in another paper, Kurling *et al.* (2008) reported that MDMA (5 mg·kg⁻¹ free base, i.p.) increased the DA levels to about 700% and 5-HT levels to more than 1400% in NAcc of awake rats. Amphetamine sulphate increased accumbal DA to about 250% following a dose of 0.75 mg·kg⁻¹ i.p. (Auclair *et al.*, 2004) and to about 300% at a s.c. dose of 0.63 mg·kg⁻¹ free base (Millan *et al.*, 1999), to 650% following

an i.p. injection of 1.6 mg·kg⁻¹ sulphate salt (Pehek *et al.*, 1990), to 350–550% in the Lewis and Fisher rats, respectively, following a s.c. dose of 1 mg·kg⁻¹ sulphate salt (Cadoni and Di Chiara, 2007) and up to 700% at the same dose but calculated as a free base (Kankaanpää *et al.*, 1998). The effects of amphetamine on accumbal 5-HT levels are negligible (Kankaanpää *et al.*, 1998) or only moderate, increasing the 5-HT levels to about 130% of control values (Millan *et al.*, 1999). The differences between the reported effects of systemic amphetamine on accumbal DA levels can be explained by the different routes of administration, doses given as salts or free bases, racemic or (+)-form of amphetamine and variations in the microdialysis protocols used by different investigators.

Microdialysis data provide valuable information on the *in vivo* pharmacodynamics of neurotransmitter release; however, the data do not allow a direct evaluation of a potential mechanism of action of drugs such as mephedrone. Mephedrone caused only minor and temporal decreases in the DOPAC levels, whereas the values of 5-HIAA were not significantly affected. These effects are similar to those induced by MDMA, whereas amphetamine significantly decreased the DOPAC levels, while the 5-HIAA levels significantly increased. Thus, the moderately reduced levels of DOPAC and 5-HIAA further indicate that the effect of mephedrone resembles that of MDMA, that is, it acts preferentially as a releaser of 5-HT, NA and DA, as reported for MDMA using *in vitro* methods (Rothman *et al.*, 2001).

Addition of substituents to the ring of phenethylamine and its related derivatives at the ethylamine moiety including amphetamine causes a shift in the psychostimulant properties of these agents from acting as preferential releasers of NA and DA to release also 5-HT and/or exhibit binding affinity to 5-HT₂ receptors, the features responsible for increased hallucinogenic effects of such compounds (Shulgin and Shulgin, 2007). Strategies based on increased 5-HT transmission, for example, by use of amphetamine analogues that release both DA and 5-HT (Rothman *et al.*, 2001; Rothman and Baumann, 2006; Baumann *et al.*, 2011), co-administration of 5-HT releasing drug fenfluramine (Wee and Woolverton, 2006) or co-administration of 5-HT_{2A} antagonists or 5-HT_{2C} receptors agonists (Bubar and Cunningham, 2008; Fletcher *et al.*, 2011) were proposed to counteract self-administration of psychostimulant drugs in animal models. In a recent study, Baumann and colleagues (Baumann *et al.*, 2011) compared four amphetamine analogues displaying similar *in vitro* potency as DA releasers but marked differences as 5-HT releasers. Although the microdialysis data on DA release in NAcc did not correlate with the *in vitro* predictions, the authors found good correlations between extracellular DA levels and locomotor activity. The increases in 5-HT release in NAcc were proportional to the decreased DA release and decreased locomotion, the most significant effect was observed for the p-methylamphetamine (PAL-313) analogue (Baumann *et al.*, 2011). These data support the findings in our study, demonstrating that substituted phenethylamines mephedrone and MDMA markedly increase 5-HT release, but lower DA release and reduce locomotor activity when compared with the effects of amphetamine.

In this respect, it could be predicted that mephedrone and MDMA are weaker re-inforcers than amphetamine or cocaine.

This conclusion is in line with a current report on mephedrone and cocaine users in the UK where about 56% of 947 responders (dance drug users) evaluated mephedrone as less addictive but giving a better high than cocaine, whereas 30% and 14% reported that it was equally or more addictive than cocaine (Winstock *et al.*, 2011). On the other hand, it was reported that some users compulsively redose mephedrone, consuming their whole supply during a session (Europol-EMCDDA, 2010). One possible explanation for this behaviour could be a short duration (2–3 h) of the mephedrone effects (Europol-EMCDDA, 2010). This conclusion is in agreement with our data showing that mephedrone, given at a same dose as MDMA, is a more potent DA releaser than MDMA, whereas the elimination rate of mephedrone-induced DA release in the NAcc was almost 10 times faster than that of induced by MDMA and two times faster than that induced by amphetamine.

The calculated elimination rates of extracellular DA and 5-HT levels correlate well with the pharmacokinetic profiles of MDMA and amphetamine reported elsewhere. Thus, the estimated $t_{1/2}$ of MDMA was 47.4 min following a s.c. injection of 2 mg·kg⁻¹ MDMA in the rat (Baumann *et al.*, 2009). This value is in good agreement with the $t_{1/2}$ of 48 min reflecting the rate of elimination of MDMA-induced 5-HT efflux but does not agree with the $t_{1/2}$ of 303 min for decay of MDMA-induced DA release. This discrepancy can be due to a large standard error in estimated $t_{1/2}$ for elimination rate of MDMA-induced DA, alternatively, the slow elimination rate of DA may imply the effects of MDMA are evoked by its active metabolites such as 3,4-methylenedioxyamphetamine (MDA) (Baumann *et al.*, 2009). The estimated $t_{1/2}$ of (+)-amphetamine following an i.v. bolus injection in the rat was 67 min (Hutchaleelaha *et al.*, 1994). This can be compared with the pharmacodynamic effects of amphetamine expressed as $t_{1/2}$ of elimination rates of extracellular levels of DA (51 min) and 5-HT (84 min) reported in this study. There are no pharmacokinetic data available on mephedrone. Six mephedrone (phase I) metabolites have been identified in rat urine and seven in human urine (Meyer *et al.*, 2010). The initial metabolic step for both species is N-demethylation of mephedrone to normephedrone. It is not known whether normephedrone possesses any psychomimetic properties. This possibility cannot be excluded, particularly when considering an analogous N-demethylation of methamphetamine to its active metabolite amphetamine (see Schep *et al.*, 2010) and N-demethylation of MDMA to its active metabolite MDA (Baumann and Rothman, 2009). Interestingly, the calculated $t_{1/2}$ for (+)-methamphetamine and its metabolite (+)-amphetamine in the male rat following i.v. administration of 1 mg·kg⁻¹ (+)-methamphetamine were 73 min and 93 min, respectively (Milesi-Hallé *et al.*, 2005), and in another study (Rivière *et al.*, 1999), 63 min and 98 min, respectively. The estimated $t_{1/2}$ of the cardiotoxic metabolite MDA formed from MDMA was 175 min, which is almost four times lower than the $t_{1/2}$ value for MDMA (Baumann *et al.*, 2009). In conclusion, by referring to metabolism of methamphetamine (Rivière *et al.*, 1999) and MDMA (Baumann *et al.*, 2009), it can be hypothesized that a potential accumulation of mephedrone metabolites including normephedrone could contribute to the clinically-reported, toxicity of mephedrone binges.

The peak effect of mephedrone on DA release was similar to the effect of a 1 mg·kg⁻¹ s.c. dose of amphetamine, which in turn, could be compared with the i.v. dose of 0.1 mg·kg⁻¹ inducing self-administration in rats (Di Ciano *et al.*, 1995). Here, the rats self-administered at about 0.7 mg amphetamine within the first 30 min of the session, which was accompanied by an increase in accumbal DA to the peak level of 550% in the 15–30 min fraction (Di Ciano *et al.*, 1995), levels similar to those observed for the amphetamine and mephedrone doses in our study. However, further studies are necessary to demonstrate whether mephedrone can induce self-administration in rats.

From a comparison of the time courses of locomotor activation induced by mephedrone, MDMA and amphetamine it was concluded that the overall effect of mephedrone was equipotent to MDMA; however, the mephedrone-induced motor activation diminished about three, and six times faster than that induced by MDMA and amphetamine, respectively. Amphetamine caused a marked increase in locomotor activity that lasted for about 120 min; this finding is in good agreement with data reported elsewhere (Cadoni and Di Chiara, 2007; Kurling *et al.*, 2008). Compared to its ability to release of DA, (+)-amphetamine is an even more potent at inducing the release of NA, while possessing similar efficacies for inhibition of DA and NA uptake (Rothman *et al.*, 2001). This suggests that the differences in locomotor activation observed between (+)-amphetamine and mephedrone or MDMA could also account for the differences in activation of the noradrenergic system. Indeed, it was demonstrated that (+)-amphetamine-induced locomotor hyperactivity is markedly reduced in mice lacking α_{1B} -adrenoceptors (Drouin *et al.*, 2002). On the other hand, the amphetamine-induced locomotion could be almost completely abolished by the blockade of 5-HT_{2A} receptors in the ventral tegmental area of the rat (Auclair *et al.*, 2004). These data indicate a complex interplay between NA and 5-HT and their respective receptors in controlling the release of DA in the NAcc induced by various psychostimulant drugs.

In conclusion, the present data demonstrate for the first time that acute administration of mephedrone induces a rapid release of both 5-HT and DA in the NAcc of awake rats and this effect is accompanied by a short-lasting increase in locomotor activity. These results support the notion that mephedrone resembles the key neurochemical and functional properties of MDMA, confirming the similarities between mephedrone and MDMA effects reported by drug users. In addition, mephedrone-induced release and rapid elimination of DA in the NAcc were similar to the effect of amphetamine given at a dose relevant to its addictive properties. However, further studies are needed to elucidate the detailed mechanisms behind the reported risk of a compulsive binge intake of mephedrone and the risk for tolerance development.

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Conflict of interest

The authors declare no conflict of interest.

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