

RESEARCH PAPER

Comparative neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylone

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BACKGROUND AND PURPOSE

Here, we have compared the neurochemical profile of three new cathinones, butylone, mephedrone and methylone, in terms of their potential to inhibit plasmalemmal and vesicular monoamine transporters. Their interaction with 5-HT and dopamine receptors and their psychostimulant effect was also studied.

EXPERIMENTAL APPROACH

Locomotor activity was recorded in mice following different doses of cathinones. Monoamine uptake assays were performed in purified rat synaptosomes. Radioligand-binding assays were carried out to assess the affinity of these compounds for monoamine transporters or receptors.

KEY RESULTS

Butylone, mephedrone and methylone (5–25 mg·kg⁻¹) caused hyperlocomotion, which was prevented with ketanserin or haloperidol. Methylone was the most potent compound inhibiting both [³H]5-HT and [³H]dopamine uptake with IC₅₀ values that correlate with its affinity for dopamine and 5-HT transporter. Mephedrone was found to be the cathinone derivative with highest affinity for vesicular monoamine transporter-2 causing the inhibition of dopamine uptake. The affinity of cathinones for 5-HT_{2A} receptors was similar to that of MDMA.

CONCLUSIONS AND IMPLICATIONS

Butylone and methylone induced hyperlocomotion through activating 5-HT_{2A} receptors and increasing extra-cellular dopamine. They inhibited 5-HT and dopamine uptake by competing with substrate. Methylone was the most potent 5-HT and dopamine uptake inhibitor and its effect partly persisted after withdrawal. Mephedrone-induced hyperlocomotion was dependent on endogenous 5-HT. Vesicular content played a key role in the effect of mephedrone, especially for 5-HT uptake inhibition. The potency of mephedrone in inhibiting noradrenaline uptake suggests a sympathetic effect of this cathinone.

Abbreviations

AUC, area under the curve; pCPA, p-chlorophenylalanine; VMAT2, vesicular monoamine transporter

Introduction

A decrease in the illegal availability of chemical compounds used for the synthesis of methamphetamine and 3,4-

methylenedioxymethamphetamine (MDMA) or Ecstasy, coupled with a more than 50% decrease in the purity of ecstasy or cocaine (Measham *et al.*, 2010; Winstock *et al.*, 2011), has resulted in the appearance on the black market of

a new generation of designer drugs known as 'cathinones' or 'beta-keto amphetamines' (the latter name deriving from the characteristic presence of a ketone in the side chain). These derivatives include a wide range of substances such as butylone, ethylone, methylone and mephedrone (4-methylmethcathinone). The most commonly available cathinones sold on the illegal market up until 2010 were mephedrone and methylone (Brunt *et al.*, 2011).

The popularity of mephedrone (with street names such as 'meow meow', 'plant food', 'bubbles' and 'MCAT') rose sharply in 2009 after it came to be seen as a legal, cheap and easily available alternative to MDMA. Mephedrone is predominantly used by teenagers and young adults (Vardakou *et al.*, 2011). It is sometimes sold mixed with methylone in a product called 'bubbles' and may also be mixed with butylone.

Methylone emerged under the trade name 'Explosion' around 2004 and was one of the first products to be marketed online and via head shops (Bossong *et al.*, 2005). In recreational users, a subjective comparison of the effects of this new drug suggested that it exhibited subtle differences when compared with MDMA. Little is known about any potential detrimental effects of methylone, however, given the similarities between this drug and MDMA, risks commonly associated with MDMA cannot be excluded. Butylone is also closely related to methylone. Butylone acts as an entactogen, psychedelic and stimulant and shares the same relationship to methylbenzodioxylbutanamine (MBDB) as methylone does to MDMA.

The first new beta-keto amphetamine to be banned (in 2009) was mephedrone; following this ruling, different European countries banned some of these derivatives, such as methylone but butylone was banned only in the UK and Denmark. Very recently, the Drug Enforcement Administration (DEA) temporarily classified mephedrone and methylone, but not butylone, as Schedule I under the Controlled Substances Act (Drug Enforcement Administration, 2011).

Previous reports have noted that users of cathinone derivatives loosely compared the effects with those of amphetamines and cocaine (Winstock *et al.*, 2011), but there was a greater similarity of effects with MDMA. These observations may explain the drug's rapid rise in popularity before its ban. In fact, many users consider the effects of cathinones to be superior to cocaine and MDMA (Winstock *et al.*, 2010; Vardakou *et al.*, 2011). Moreover, the abuse potential of cathinone derivatives is comparable with that of cocaine or Ecstasy (McElrath and O'Neill, 2011).

Based on their chemical structure, it could be postulated that the stimulant and empathogenic effects of cathinones are similar to those of amphetamine derivatives (Schifano *et al.*, 2011). However, very little is known about the pharmacology of the new cathinones. Cozzi *et al.* (1999) performed some *in vitro* studies with methcathinone and methylone, resulting in a hypothesis that the mechanism of action was similar to that of amphetamine. In addition, some cathinones, such as methylone, are able to bind to some monoamine transporters (Nagai *et al.*, 2007). Recently, some preliminary studies on the pharmacological targets of mephedrone have been published (Martínez-Clemente *et al.*, 2012; Hadlock *et al.*, 2011; Kehr *et al.*, 2011; Motbey *et al.*, 2012). Cathinone overdose results in cardiovascular disturbances

(Wood and Dargan, 2010; Regan *et al.*, 2011) and the studies of Meng *et al.* (2012), characterizing the effects of mephedrone on cardiac ion channels, concluded that this drug acts as a sympathomimetic agent. Given what is known about the neuropharmacology of amphetamine derivatives, primarily methamphetamine and MDMA, and because of the chemical structural similarity of the cathinone derivatives, it has been suggested that these new compounds possess a monoaminergic mechanism of action.

In order to understand the effects of these psychostimulant beta-keto amphetamines, it is essential to determine which transporter or neurotransmitter systems are most affected. A thorough understanding of the pharmacological profile for each psychostimulant drug is essential in the development of treatment protocols for stimulant overdose and dependence.

The aim of the present study was to compare the neurochemical profile of butylone, mephedrone and methylone in terms of their abilities to inhibit plasma membrane and vesicular monoamine uptake transporters. We focused our attention on the dopamine and 5-HT transporters because these proteins are the most frequently implicated in the reinforcing properties and abuse potential of amphetamines. In addition, a comparative study of the interaction of these drugs with 5-HT and dopamine receptors and their psychostimulant effect was also carried out. In summary, this study represents the first comparison of the neuropharmacology of the three cathinone derivatives and suggests the likely effects of these compounds on individuals who abuse them.

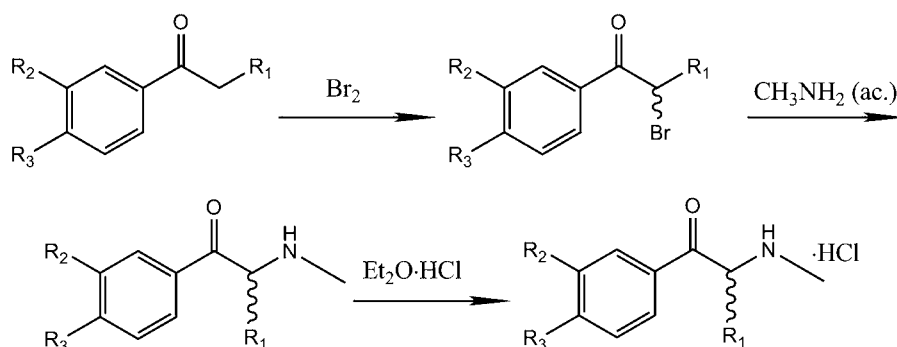
Methods

Animals

All animal care and experimental protocols in this study complied with the guidelines of the European Community Council (86/609/ECC) and were approved by the Animal Ethics Committee of the University of Barcelona under the supervision of the Autonomous Government of Catalonia. Efforts were made to minimize suffering and reduce the number of animals used. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010). Adult Swiss CD-1 mice (Charles River, Lyon, France) weighing 25–30 g (total = 215) and male Sprague Dawley rats (Janvier, Le Genest, France) weighing 225–250 g (total = 58) were used. The animals were housed at $22 \pm 1^\circ\text{C}$ under a 12 h light/dark cycle with free access to food (standard laboratory diet, Panlab SL, Barcelona, Spain) and drinking water.

Synthesis of butylone, mephedrone and methylone

We synthesized cathinones in our organic chemistry laboratory, with permission from our University, following the procedures described by Chad and Copeland (2010) with minor modifications. Briefly, butylone, mephedrone and methylone were obtained by adding 3,4-methylenedioxybutyrophenone, 4-methylpropiofenone and 3,4-methylenedioxypropiofenone dissolved in CH_2Cl_2 to bromine, to give 3',4'-methylenedioxy-2-

**Figure 1**

Chemical synthesis of butylone from 3,4-methylenedioxybutyrophenone, mephedrone from 4-methylpropiofenone and methylone from 3,4-methylenedioxypropiofenone. Butylone: $R_1 = -CH_2CH_3$; $R_2/R_3 = -OCH_2O-$. Mephedrone: $R_1 = -CH_3$; $R_2 = -CH_3$; $R_3 = -CH_3$. Methylone: $R_1 = -CH_3$; $R_2/R_3 = -OCH_2O-$.

bromobutyrophenone, 4'-methyl-2-bromopropiofenone and 3',4'-methylenedioxy-2-bromopropiofenone respectively. These compounds were dissolved in CH_2Cl_2 and added to an aqueous solution of methylamine (40%). HCl was then added. The aqueous layer was removed and made alkaline using sodium bicarbonate. Ether was used to extract the amine. Finally, a drop of ether-HCl solution was added to produce β -keto-*N*-methylbenzodiololylpropylamine hydrochloride (butylone), 4-methylmethcathinone hydrochloride (mephedrone) and 3,4-methylenedioxy-*N*-methylcathinone hydrochloride (methylone) (Figure 1). The identification of the three compounds obtained was assessed by proton nuclear magnetic resonance (1H NMR) (CD_3OD) yielding the following results: butylone: δ 7.87 (dd, 1H) $J = 8.1$ Hz $J = 1.8$ Hz; 7.67 (d, 1H) $J = 1.8$ Hz; 7.18 (d, 1H) $J = 8.1$ Hz; 6.29 (s, 2H); 5.19 (t, 1H) $J = 5.1$ Hz; 2.88 (s, 3H); 2.22 (m, 2H); 1.07 (t, 3H) $J = 7.5$ Hz; mephedrone: δ 7.62 (d, 2H) $J = 8.5$ Hz; 7.42 (d, 2H) $J = 8.5$ Hz; 5.09 (q, 1H) $J = 7.2$ Hz; 2.77 (s, 3H); 2.45 (s, 1H); 1.57 (d, 3H) $J = 7.2$ Hz; methylone: δ 7.86 (dd, 1H) $J = 8.1$ Hz $J = 1.8$ Hz; 7.66 (d, 1H) $J = 1.8$ Hz; 7.18 (d, 1H) $J = 8.1$ Hz; 6.29 (s, 2H); 5.15 (q, 1H) $J = 7.2$ Hz; 2.91 (s, 3H); 1.73 (d, 3H) $J = 7.2$ Hz.

Chemical purity of the obtained compounds was also assessed by melting point determination, thin layer chromatography, 1H NMR and mass spectrometry. All analytical data were consistent with the assigned structures with over 98% purity for the three cathinone derivatives.

Drug-induced spontaneous locomotor activity in mice

Before experimentation, all the mice ($n = 9$ per group) received two habituation sessions (48 and 24 h before testing) that were intended to reduce the novelty and stress associated with handling and injection. During these sessions, each mouse was given a subcutaneous injection of saline and placed in a Plexiglas cage. This cage constituted the activity box that was later placed inside a frame system of two sets of 16 infrared photocells (LE8811, Panlab SL) mounted according to the x , y axis coordinates and 1.5 cm above the wire mesh floor. Occlusions of the photo beams were recorded and sent to a computerized system (SedaCom32, Panlab SL). The interruption counts, over a 10 min period, were used as a measure of

horizontal locomotor activity. The locomotor activity was monitored for 360 min. All experiments were conducted between 8:30 a.m. and 2:30 p.m. On the testing day, the animals received the cathinone derivative subcutaneously, at different doses, and were immediately placed in the activity box. Registration of horizontal locomotor activity then began. Antagonists were administered i.p., 20 min before cathinone derivative treatment. Treatment with *p*-chlorophenylalanine (pCPA) was carried out at a dose of 300 mg·kg⁻¹ given daily i.p., over the course of 3 days. Eighteen hours after the last administration of pCPA, the animals received the cathinone. Each animal was used only once. Results are expressed as area under the curve (AUC), which was measured as the total changes from baseline at each recorded interval over 360 min or cumulative breaks for 120 min.

Rat synaptosome preparation

Pure synaptosome suspensions were prepared as described elsewhere (Pubill *et al.*, 2005) with minor modifications. Briefly, on the morning of each day of the experiment, two rats were decapitated and their striata or cortex were homogenized and centrifuged at 1000× g at 4°C for 10 min. The supernatant was recovered and sucrose buffer was added to a final sucrose concentration of 0.8 M. Samples were then centrifuged at 13 000× g for 30 min at 4°C. The supernatant was discarded and the synaptosome layer was separated from mitochondria by carefully adding 1 mL of ice-cold 320 mM sucrose buffer and gently shaking. Finally, the synaptosome fraction was diluted in HEPES-buffered solution (composition in mM: 140 NaCl, 5.37 KCl, 1.26 CaCl₂, 0.44 KH₂PO₄, 0.49 MgCl₂·6H₂O, 0.41 MgSO₄·7H₂O, 4.17 NaHCO₃, 0.34 Na₂HPO₄·7H₂O, 5.5 glucose and 20 HEPES-Na) containing pargyline (20 μ M) and ascorbic acid (1 mM) [HEPES pargyline ascorbic buffer (HPAB)].

Plasmalemmal 5-HT, dopamine and noradrenaline uptake

In order to obtain evidence of the direct blockade (competitive inhibition) of [3H]5-HT uptake in the presence of cathinone derivatives, synaptosomes from rat cortex were prepared as described earlier so that the final protein content

was approximately equivalent to 10 mg of tissue (wet weight) per mL. Reaction tubes were composed of 0.85 mL of butylone, mephedrone or methylone at different concentrations in buffer and 0.1 mL of synaptosome suspension. Tubes were warmed 10 min at 37°C before the addition of 0.05 mL of [3 H]5-HT (final concentration 15 nM), after which incubation was carried out for a further 5 min. The uptake reaction was stopped by rapid vacuum filtration through Whatman GF/B glass fibre filters (Whatman Intl Ltd., Maidstone, UK) pre-soaked in 0.5% polyethyleneimine. Tubes and filters were washed rapidly three times with 4 mL ice-cold 50 mM Tris-HCl. The radioactivity trapped on the filters was measured by liquid scintillation spectrometry. Non-specific uptake was determined at 4°C in parallel samples containing fluoxetine (10 μ M).

Synaptosomes from the rat striatum were obtained in order to measure the direct blockade of [3 H]dopamine uptake by cathinone derivatives. Competitive blockade of [3 H]dopamine uptake was assessed in the presence of butylone, mephedrone and methylone at different concentrations. The experiments were carried out as described earlier, using a final concentration of [3 H]dopamine of 5 nM. Non-specific uptake was determined at 4°C in parallel samples containing cocaine 100 μ M.

Similarly, the direct blockade of [3 H]noradrenaline uptake by cathinone derivatives was measured in synaptosomes from the rat cortex. The experiments were carried out as described earlier, using a final concentration of [3 H]noradrenaline of 20 nM. Non-specific uptake was determined at 4°C in parallel samples containing 10 μ M desipramine.

To measure persistent inhibition of [3 H]5-HT or [3 H]dopamine uptake, fresh synaptosomes were pre-incubated in a shaking water bath at 37°C for 1 h with butylone, mephedrone or methylone at different concentrations. Following pre-incubation, synaptosomes were centrifuged at 13 000 $\times g$ for 20 min, resuspended in 5 mM Tris-HCl/320 mM sucrose buffer and re-centrifuged. Final pellets were resuspended in HPAB buffer. The suspension was warmed for 10 min at 37°C. Then, incubation was carried out for 5 min as described. The remaining synaptosomal preparation (i.e. that was not used for the uptake assay) was kept and the protein concentration was determined using a Bio-Rad protein reagent (Bio-Rad Labs, Inc., Hercules, CA, USA). Specific [3 H]5-HT or [3 H]dopamine uptake for each condition was normalized by dividing by the protein concentration.

Previous studies (Hrometz *et al.*, 2004; Jones *et al.*, 2004) demonstrated that dopamine can enter 5-HT nerve terminals via the 5-HT transporter. To measure this uptake, experiments were carried out as described but using synaptosomes from the rat cortex. In these experiments [3 H]dopamine (5 nM), d-amphetamine (1 μ M) and cathinone derivatives were present in the medium. Inhibition by fluoxetine (10 μ M) was used to confirm that, in this preparation, [3 H]dopamine uptake was carried out only by the 5-HT transporter.

Vesicular dopamine uptake

In order to measure [3 H]dopamine uptake via the vesicular monoamine transporter 2 (VMAT2; transporter and receptor nomenclature follows Alexander *et al.*, 2011), the method described by Hansen *et al.* (2002) was used with minor modifications. Briefly, rat striatal synaptosomes were obtained as

previously described and resuspended and lysed in ice-cold deionized water. Osmolarity was restored by the addition of HEPES and potassium tartrate to final concentrations of 245 and 100 mM, respectively, and samples were centrifuged for 20 min at 20 000 $\times g$ (4°C) to remove synaptosomal membranes. MgSO₄ (1 mM) was added to the supernatant, which was then centrifuged for 45 min at 100 000 $\times g$ (4°C). The resulting vesicular pellet was resuspended in wash buffer (see later section for composition), at a concentration of 50 mg·mL⁻¹ (wet tissue weight). Vesicular [3 H]dopamine uptake measurement was performed by incubating 100 μ L of vesicles at 30°C for 3 min in assay buffer (25 mM HEPES, 100 mM potassium tartrate, 1.7 mM ascorbic acid, 0.05 mM EGTA, 0.1 mM EDTA, and 2 mM ATP-Mg, pH 7.5) in the presence of 30 nM [3 H]dopamine. The reaction was terminated by addition of 1 mL of ice-cold wash buffer (assay buffer containing 2 mM MgSO₄ instead of ATP-Mg, pH 7.5) and rapid filtration followed by three 1 mL washes. Reserpine (10 μ M) was tested in each experiment as a positive control of vesicular uptake inhibition. Non-specific incorporation was determined by measuring uptake at 4°C in wash buffer containing reserpine (20 μ M).

Rat tissue membrane preparation

The rats were killed by decapitation under isoflurane anaesthesia and the brains were removed rapidly from the skull and the striatum and cortex were quickly excised, dissected out, frozen on dry ice and stored at -80°C until later use (Chipana *et al.*, 2008a). When required, samples were thawed and homogenized in a 10-volume buffer: 5 mM Tris-HCl, 320 mM sucrose and protease inhibitors (aprotinin 4.5 μ g· μ L⁻¹, 0.1 mM phenylmethylsulfonyl fluoride and 1 mM sodium orthovanadate), pH 7.4, with a Polytron homogenizer (Heidolph, Schwabach, Germany). The homogenates were centrifuged at 15 000 $\times g$ for 30 min at 4°C. The resulting pellets were washed twice and the final pellets were resuspended in the appropriate buffer and stored at -80°C for use in radioligand binding experiments.

Interaction with 5-HT and dopamine transporters

[3 H]Paroxetine binding was used to label the cortical 5-HT transporter. Competition binding experiments were carried out using the membrane preparations from rat cortex. These experiments were performed in glass tubes containing 0.05 nM [3 H]paroxetine, butylone, mephedrone or methylone at increasing concentrations, and 150 μ g of brain membranes. Incubation was carried out at 25°C for 2 h in a Tris-HCl buffer (50 mM, pH 7.4) containing 120 mM NaCl and 5 mM KCl to a final volume of 1.6 mL. Clomipramine (100 μ M) was used to determine non-specific binding. Binding was terminated by filtration and data were treated as explained earlier.

[3 H]WIN35428 binding was used to label striatal dopamine transporters. Membrane preparations from rat striatum (Chipana *et al.*, 2008b) were resuspended in phosphate-buffered 0.32 M sucrose, pH 7.9 at 4°C to a concentration of 1 mg·mL⁻¹. Binding assays were performed in glass tubes containing 200 μ L of [3 H]WIN35428 dilution in phosphate-buffered 0.32 M sucrose (final radioligand concen-

tration: 5 nM), butylone, mephedrone or methylone at increasing concentrations and 50 µL of membrane suspension. Samples were incubated for 2 h at 4°C. Non-specific binding was determined in the presence of 30 µM bupropion. Binding was terminated by filtration and data were analysed as previously described.

Interaction with 5-HT and dopamine receptors

[³H]Ketanserin binding was used to label cortical 5-HT_{2A} receptors. Competition binding experiments were carried out using the membrane preparations from rat cortex. These experiments were performed in tubes containing 1 nM [³H]ketanserin, cathinone derivatives at increasing concentrations and 100 µg of brain membranes. Incubation was carried out at 37°C for 30 min in a Tris-HCl buffer to a final volume of 0.5 mL. Methysergide (10 µM) was used to determine non-specific binding. Binding was terminated by filtration and data were analysed as previously described.

[³H]Raclopride binding was used to label striatal dopamine D₂ receptors. Competition binding experiments were carried out using the membrane preparations from rat striatum. These experiments were performed in tubes containing 2 nM [³H]raclopride, cathinone derivatives at increasing concentrations and 50 µg of brain membranes. Incubation was carried out at 25°C for 1 h in a Tris-HCl buffer to a final volume of 0.5 mL. Sulpiride (300 µM) was used to determine non-specific binding. Binding was terminated by filtration and data were analysed as previously described.

Data analysis

All data are expressed as mean ± SEM. One-way ANOVA was used to determine overall treatment effects on locomotor activity. The Tukey-Kramer multiple comparisons test was used for *post hoc* analysis in all instances following a significant ANOVA ($P < 0.05$) to assess the difference between treatment groups and the control group. Competition-binding curves were plotted and calculated by nonlinear regression using GraphPAD Prism (GraphPAD software, San Diego, CA, USA). Data were best fitted to a one-site competition model and an IC₅₀ value (the concentration that induces 50% displacement) and the Hill coefficient (n_H) was obtained. The K_i values (the concentration that occupies 50% of the receptor population) for competing drugs were calculated using the equation by Cheng and Prusoff: $K_i = IC_{50} / [1 + (L/K_d)]$, where L is the total radioligand concentration and K_d is the dissociation constant of the radioligand. The Hill coefficient (n_H) was calculated by linear regression with data transformed according to Hill function.

Materials

All drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA) with the exception of cocaine and MDMA (National Health Laboratory, Barcelona, Spain). SB-216 641 was from Tocris Bioscience (Bristol, UK). 3,4-Methylenedioxybutyrophenone and 3,4-methylenedioxypropiofenone were from Alfa Aesar GmbH (Karlsruhe, Germany). [³H]dopamine, [³H]5-HT, [³H]ketanserin, [³H]noradrenaline, [³H]paroxetine, [³H]raclopride and

[³H]WIN35428 were from Perkin Elmer Life Sci. (Boston, MA, USA). All buffer reagents were of analytical grade.

Results

Effect of cathinone derivatives on spontaneous locomotor activity

Overall, analysis of the data (by ANOVA) demonstrated an extremely significant effect of cathinone treatment in the increase of spontaneous locomotor activity (expressed as AUC value) in mice [$F(9,35) = 48.892$, $P < 0.001$]. The *post hoc* Tukey-Kramer test to analyse each drug treatment revealed that subcutaneous administration of butylone [$F(3,17) = 98.267$, $P < 0.001$], mephedrone [$F(3,17) = 37.606$, $P < 0.001$] and methylone [$F(3,17) = 96.515$, $P < 0.001$] increased the locomotor activity in a dose-dependent manner (Table 1).

At the highest dose tested, both butylone and methylone induced a very significant hyperlocomotion compared with mephedrone. Furthermore, at 10 mg·kg⁻¹, a similar hyperlocomotion effect for methylone and mephedrone was found, whilst butylone induced a greater stimulant effect. The duration of the hyperlocomotive effect induced by cathinone

Table 1

Effect of butylone, mephedrone and methylone on the behavioural parameters in mice

Drug (dose in mg kg ⁻¹)	Locomotor activity	Rearings
Saline	72 829 ± 9524	8657 ± 1764
MDMA (5)	164 408 ± 29 558 ^a	3405 ± 1695
Methamphetamine (5)	723 295 ± 35 137 ^{c,h}	40 025 ± 7860 ^{b,h}
Butylone (5)	229 910 ± 22 684 ^c	12 945 ± 2465
Butylone (10)	400 775 ± 4026 ^{c,f,g}	24 800 ± 1571 ^a
Butylone (25)	756 330 ± 84 909 ^{c,e}	26 735 ± 7686 ^a
Mephedrone (5)	201 806 ± 21 894 ^c	11 470 ± 1457
Mephedrone (10)	203 650 ± 35 616 ^b	7185 ± 2703
Mephedrone (25)	319 265 ± 19 834 ^c	7010 ± 1450
Methylone (5)	163 340 ± 20 019 ^a	13 295 ± 3523
Methylone (10)	227 780 ± 10 635 ^b	13 685 ± 6825
Methylone (25)	689 705 ± 73 385 ^{c,d}	13 785 ± 4604

Results are expressed as mean ± SEM and represent the measurement of the area under the curve (total AUC) over a period of 360 min.

The number of animals in each group was nine with the exception of saline, which numbered 15.

^a $P < 0.05$ versus saline.

^b $P < 0.01$ versus saline.

^c $P < 0.001$ versus saline.

^d $P < 0.05$ versus mephedrone (25).

^e $P < 0.01$ versus mephedrone (25).

^f $P < 0.01$ versus mephedrone (10).

^g $P < 0.01$ versus methylone (10).

^hFrom Camarasa *et al.* (2009).

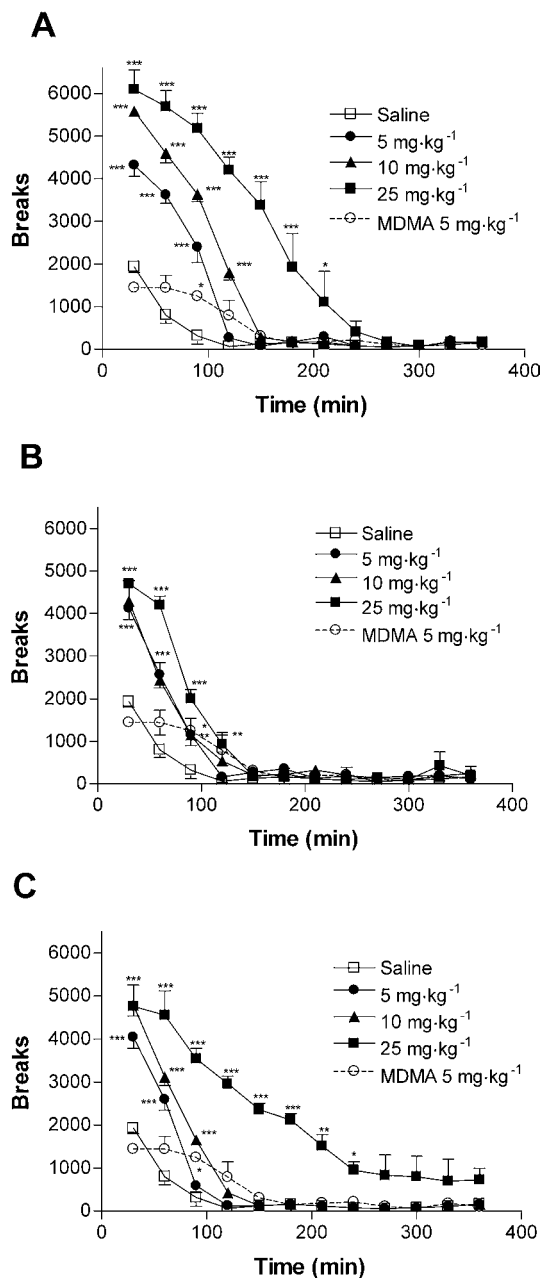


Figure 2

Effect of a single s.c. administration of (A) butylone, (B) mephedrone and (C) methylone (5, 10 and 25 mg·kg⁻¹) compared with MDMA (5 mg·kg⁻¹) and saline. For locomotor activity, the interruption counts in the lower frame of the apparatus were registered, displayed in a 30 min block and monitored for 360 min. Data are expressed as the mean \pm SEM of values from 9 mice. * P < 0.05, ** P < 0.01, *** P < 0.001; significantly different from saline.

treatment was also dose-dependent. At the highest dose tested, the stimulant effect of mephedrone lasted for 150 min after administration, whilst the effect of butylone and methylone persisted for 240 min and 270 min respectively (Figure 2).

In addition to our analysis of locomotor activity, we simultaneously recorded the number of rearings as a measure

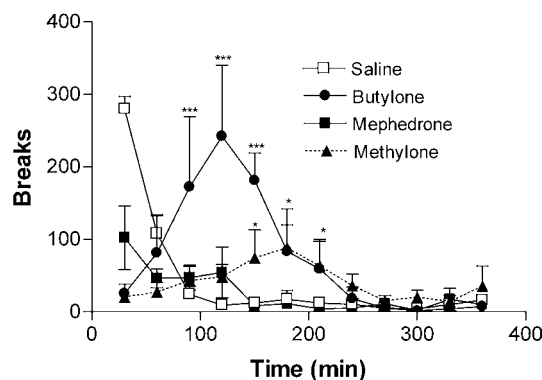


Figure 3

Time course of rearings induced by a single s.c. administration of 25 mg·kg⁻¹ of butylone, mephedrone and methylone compared with saline. For this behaviour, the interruption counts in the upper frame of the apparatus were registered, displayed in a 30 min block and monitored for 360 min. Vertical axis shows these counts per animal in 30 min intervals. Data are expressed as the mean \pm SEM of values from nine mice. * P < 0.05, *** P < 0.001; significantly different from saline. Significance at 180 min refers only to butylone.

to determine the habituation of animals to the new environment (Figure 3). As Table 1 indicates, the animals treated with different doses of mephedrone or methylone showed no change in this behavioural activity (the total AUC rearing values were not significantly different from those of saline-treated mice). This observation contrasts with the results found for butylone at doses of 10 and 25 mg·kg⁻¹.

Pretreatment with ketanserin (0.5 and 4 mg·kg⁻¹), a 5-HT₂ receptor antagonist, and haloperidol (0.1 and 0.25 mg·kg⁻¹), a non-selective dopamine receptor antagonist, at doses that did not affect basal locomotor activity, fully and dose-dependently inhibited the hyperlocomotion induced by butylone and methylone. However, these antagonists partly inhibited the effect of mephedrone on locomotor activity (by about 53% and 65% respectively). Furthermore, pretreatment of animals with SB-216 641, a selective antagonist of 5-HT_{1B} receptors, at a dose of 8 mg·kg⁻¹, significantly reduced (by about 37%) the effect of butylone and fully inhibited the increase in number of rearings induced by this cathinone. In contrast, SB-216 641 failed to antagonize the increase in locomotor activity induced by mephedrone and methylone. Additionally, pCPA, an inhibitor of 5-HT synthesis, administered in a regime that fully inhibited the effect of MDMA (5 mg·kg⁻¹) (data not shown), significantly reduced the effect of mephedrone (by about 53%) and failed to antagonize the effect of butylone and methylone (Figures 4–7). Surprisingly, this antagonist potentiates the methylone effect at later test times (Figure 6).

Effect of cathinone derivatives on plasmalemmal 5-HT, dopamine and noradrenaline uptake

Butylone and methylone reduced [³H]5-HT uptake in synaptosomes in a concentration-dependent manner, similar to mephedrone (Martínez-Clemente *et al.*, 2012). At the same

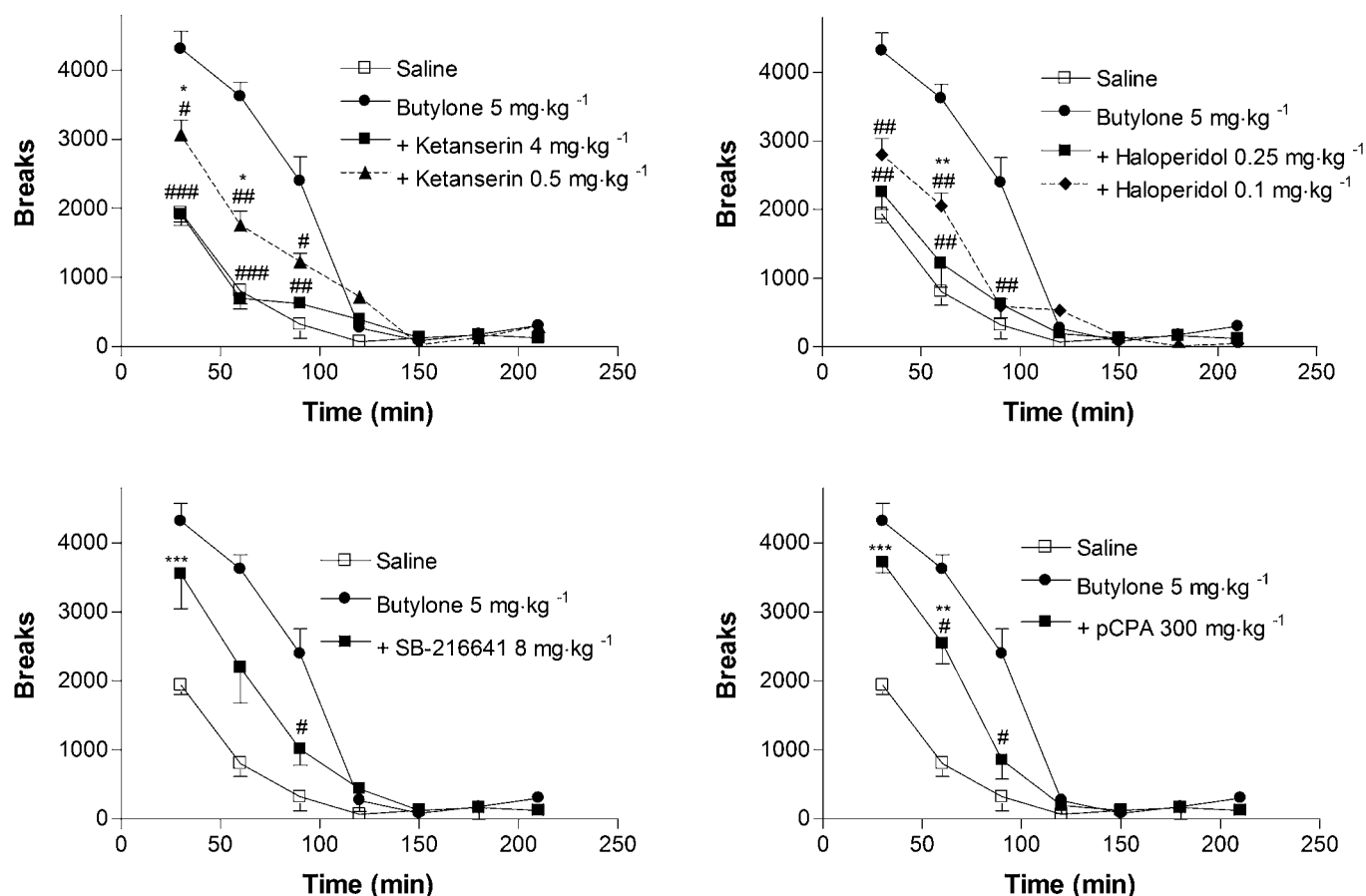


Figure 4

Effect of ketanserin, haloperidol, SB-216 641 and p-chlorophenylalanine (pCPA) on the time course of butylone-induced hyperlocomotion. Data are expressed as the mean \pm SEM of values from nine mice. * $P < 0.05$, *** $P < 0.001$; significantly different from saline; # $P < 0.05$, ### $P < 0.01$, ### $P < 0.001$; significantly different from butylone. Differences between butylone data and saline are displayed in Figure 2A.

Table 2

Calculated IC_{50} values (μM) of the three cathinone derivatives inhibiting the plasmalemmal and vesicular monoamine uptake

Drug	Monoamine transporter			
	5-HT	Dopamine	Noradrenaline	VMAT2
Butylone (10^{-8} M– 10^{-4} M)	0.68 ± 0.13	1.71 ± 0.32	0.92 ± 0.13	81.84 ± 3.33
Mephedrone (10^{-8} M– 10^{-4} M)	0.31 ± 0.08^a	0.97 ± 0.05^a	0.18 ± 0.01	3.40 ± 0.20
Methylone (5×10^{-8} M– 10^{-4} M)	0.23 ± 0.03	0.56 ± 0.05	0.53 ± 0.05	21.83 ± 1.77

Data are mean \pm SEM from three different experiments.

^aFrom Martínez-Clemente *et al.* (2012).

concentration range, the three cathinone derivatives also reduced both [3H]dopamine and [3H]noradrenaline uptake (Table 2)

When measuring persistent inhibition of monoamine uptake, only at the highest concentration tested (1 mM) was [3H]5-HT uptake inhibited by mephedrone ($22 \pm 1\%$ $P < 0.01$) but not by butylone. Methylone inhibited [3H]5-HT uptake at a concentration of 10 μM and reached a maximum of 71 \pm

0.8% inhibition ($P < 0.001$) at a concentration of 1 mM. [3H]dopamine uptake was also persistently inhibited by 1 mM butylone ($35 \pm 1.3\%$, $P < 0.05$) and methylone ($75 \pm 1.8\%$, $P < 0.01$) but not by mephedrone.

[3H]Dopamine at a concentration of 5 nM enters the serotonergic terminal through the 5-HT transporter (90% inhibition was found with fluoxetine 1 μM). Butylone inhibited (by $27 \pm 2.2\%$ $P < 0.01$) this uptake only at the highest

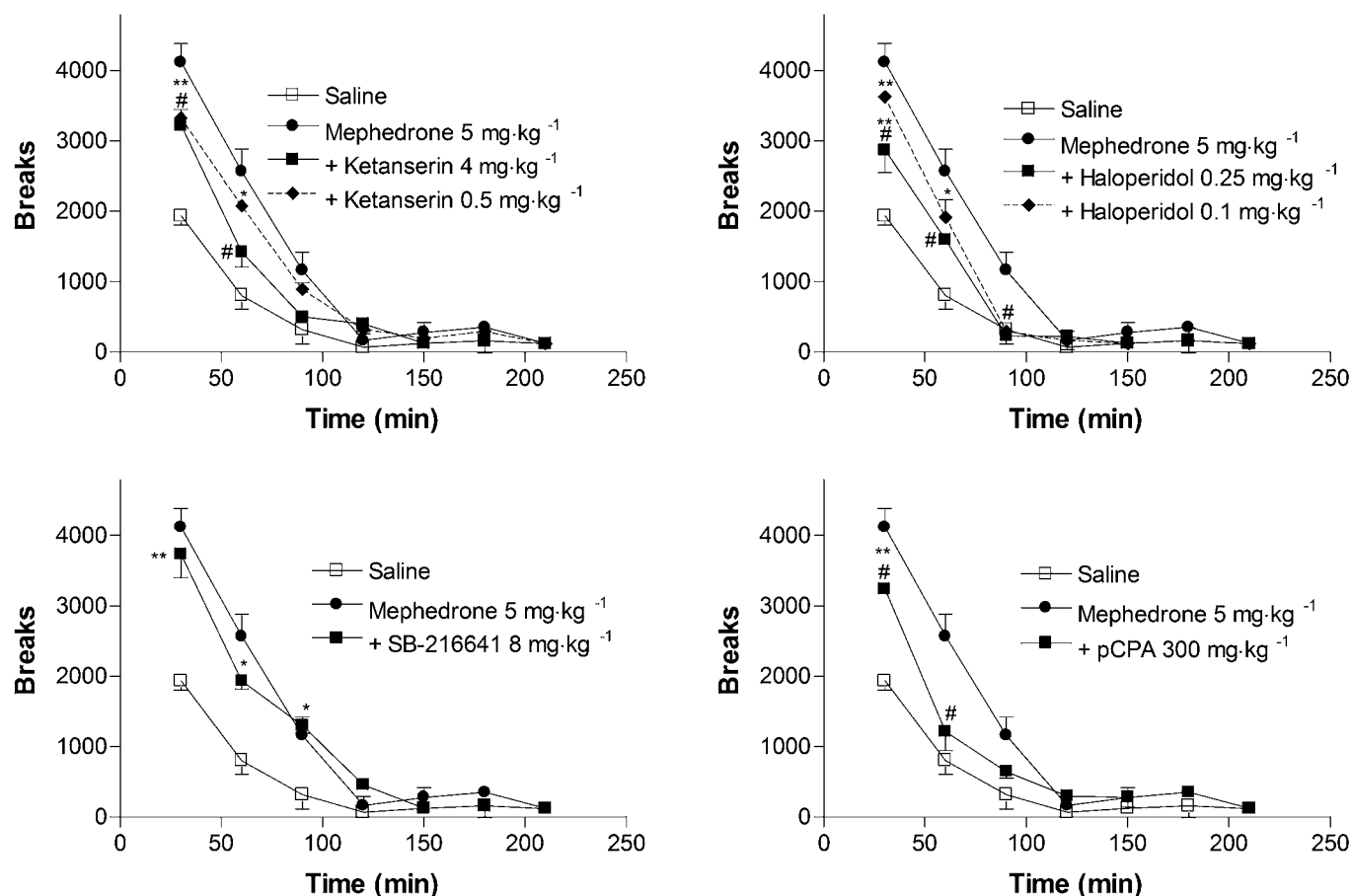


Figure 5

Effect of ketanserin, haloperidol, SB-216 641 and p-chlorophenylalanine (pCPA) on the time course of mephedrone-induced hyperlocomotion. Data are expressed as the mean \pm SEM of values from nine mice. * $P < 0.05$, *** $P < 0.001$, significantly different from saline; # $P < 0.05$, significantly different from mephedrone. Differences between mephedrone data and saline are displayed in Figure 2B.

Table 3

Inhibition of monoamine uptake (in %) by the three cathinone derivatives, at a concentration of 10^{-4} M, in rat synaptosome preparations in the presence or the absence of reserpine (20 μ M)

Drug	5-HT Without reserpine	With reserpine	Dopamine Without reserpine	With reserpine
Butylone	94 \pm 1.6	57 \pm 1.6 ^a	97 \pm 0.7	54 \pm 0.9 ^a
Mephedrone	95 \pm 0.1	28 \pm 3.9 ^a	98 \pm 0.1	35 \pm 1 ^a
Methylone	96 \pm 0.5	64 \pm 0.7 ^a	100 \pm 0.2	67 \pm 0.7 ^a

Data are mean \pm SEM from three different experiments.

^a $P < 0.001$ significantly different from the corresponding value without reserpine. Student's *t*-test (independent samples).

concentration tested (500 μ M). Mephedrone and methylone inhibited this process at a lower concentration (100 μ M: by $28 \pm 3.2\%$ $P < 0.05$ and $17 \pm 0.5\%$ $P < 0.05$ respectively; 500 μ M: by $57 \pm 0.8\%$ $P < 0.001$ and $28 \pm 3.6\%$ $P < 0.05$ respectively).

We also wished to determine the role of endogenous neurotransmitters in our studies and so we measured [3 H]5-HT and [3 H]dopamine uptake in synaptosomes from

cortex and striatum in the presence of reserpine (20 μ M), thereby ensuring the blockade of VMAT2. Under these experimental conditions, we measured the effect of butylone, mephedrone and methylone at a concentration range from 10^{-7} to 10^{-4} M. The three cathinone derivatives inhibited both [3 H]5-HT and [3 H]dopamine uptake; nonetheless, this inhibition was lower than in the absence of reserpine (Table 3). The

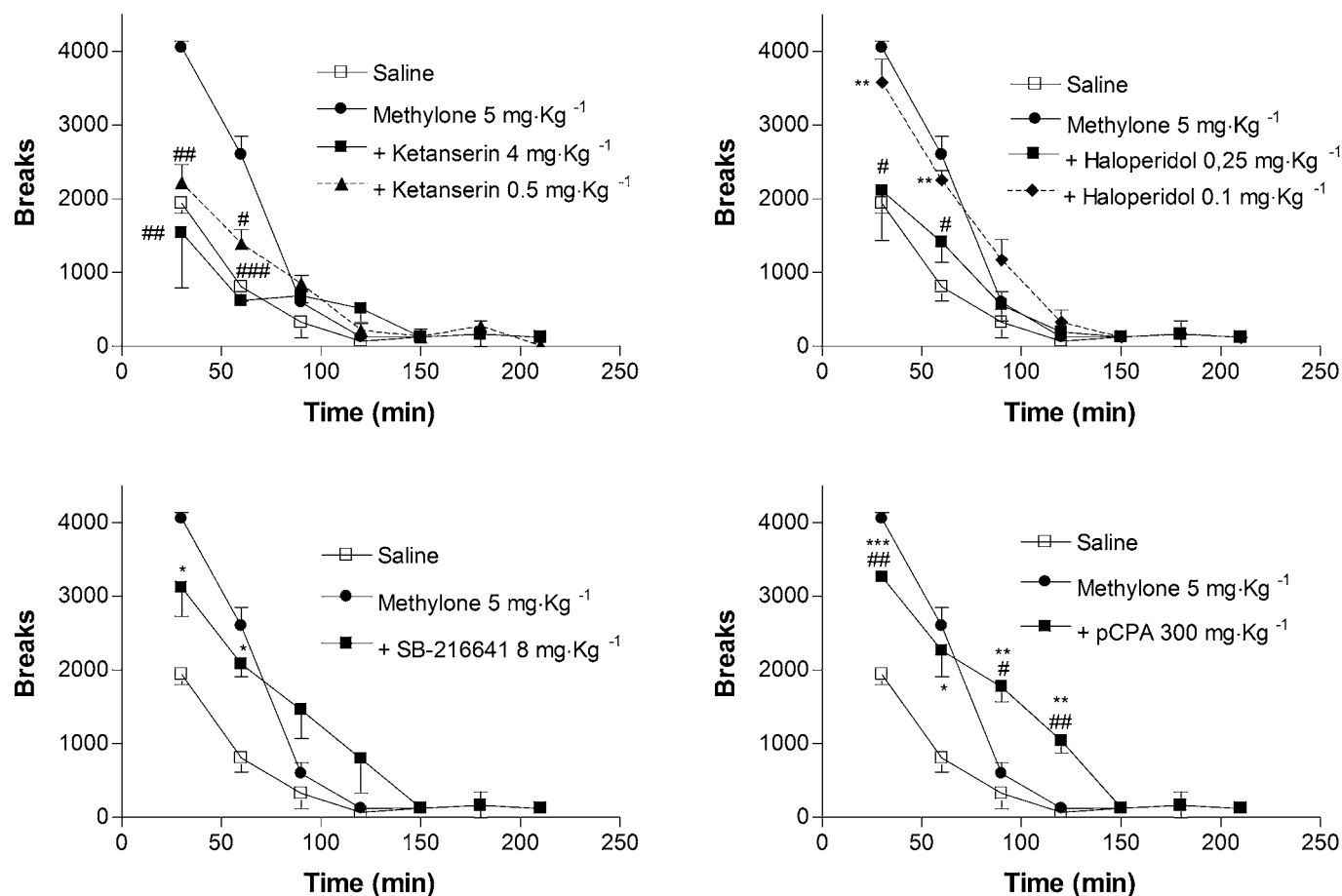


Figure 6

Effect of ketanserin, haloperidol, SB-216 641 and p-chlorophenylalanine (pCPA) on the time course of methylone-induced hyperlocomotion. Data are expressed as the mean \pm SEM of values from nine mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; significantly different from saline; # $P < 0.05$, ### $P < 0.01$, #### $P < 0.001$; significantly different from methylone. Differences between methylone data and saline are displayed in Figure 2C.

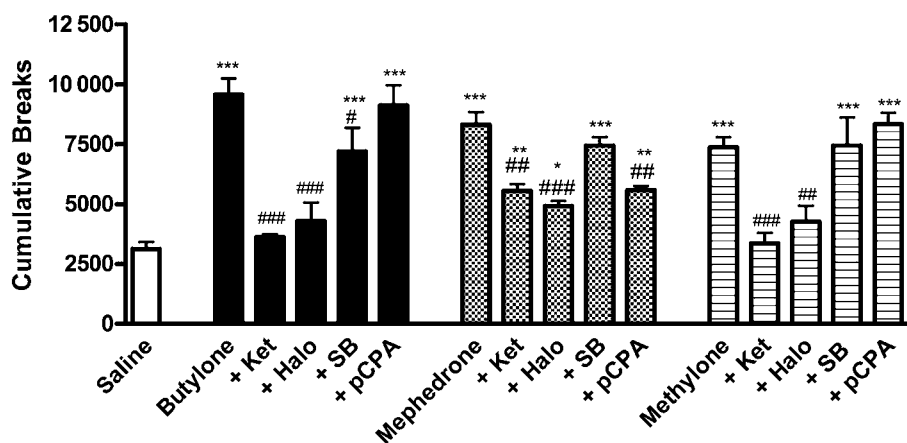


Figure 7

Cumulative breaks after 120 min for the effect of ketanserin (Ket) ($4 \text{ mg} \cdot \text{kg}^{-1}$), haloperidol (Halo) ($0.25 \text{ mg} \cdot \text{kg}^{-1}$), SB-216 641 (SB) ($8 \text{ mg} \cdot \text{kg}^{-1}$) and p-chlorophenylalanine (pCPA) ($300 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 3 days) on butylone, mephedrone and methylone ($5 \text{ mg} \cdot \text{kg}^{-1}$)-induced hyperlocomotion. Data are expressed as and represent mean \pm SEM from three separate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from saline and ## $P < 0.01$, ### $P < 0.001$, significantly different from the corresponding cathinone derivative. One-way ANOVA and *post hoc* Tukey–Kramer multiple comparisons test.

greatest difference in results obtained with the two protocols was found with mephedrone.

Effect of cathinone derivatives on vesicular dopamine uptake

Vesicular uptake of dopamine through the VMAT2 was assayed in the presence of different concentrations of butylone, mephedrone and methylone, using reserpine 10 μ M as positive control. Under these experimental conditions, butylone, mephedrone and methylone significantly inhibited [3 H]dopamine uptake in a concentration-dependent manner. The corresponding IC_{50} calculated values for each compound are shown in Table 2.

Interaction of butylone, mephedrone and methylone with the 5-HT and dopamine transporters

All three cathinones displaced bound [3 H]paroxetine in a concentration-dependent manner. This displacement occurred with K_i values in the low micromolar range for butylone ($K_i = 2.86 \pm 1.48 \mu$ M; $n_H = 0.86 \pm 0.04$), mephedrone ($K_i = 17.55 \pm 0.78 \mu$ M; $n_H = 0.72 \pm 0.04$, $P < 0.05$ vs. unity) and methylone ($K_i = 6.49 \pm 1.66 \mu$ M; $n_H = 0.47 \pm 0.08$, $P < 0.05$ vs. unity) (Figure 8A).

Butylone, methylone and mephedrone displaced bound [3 H]WIN35428 in a concentration-dependent manner. This displacement occurred with K_i values in the very low micromolar range for all drugs: butylone ($K_i = 0.39 \pm 0.06 \mu$ M; $n_H = 1.28 \pm 0.15$); mephedrone ($K_i = 1.53 \pm 0.47 \mu$ M; $n_H = 0.93 \pm 0.04$); and methylone ($K_i = 0.86 \pm 0.24 \mu$ M; $n_H = 0.94 \pm 0.09$) (Figure 8B).

Interaction of butylone, mephedrone and methylone with 5-HT and dopamine receptors

Butylone, mephedrone and methylone displaced [3 H]ketanserin binding in a concentration-dependent manner. Mephedrone showed the highest affinity for this receptor type followed by methylone and lastly by butylone. Calculated values of K_i for these drugs were: butylone ($K_i = 37.49 \pm 6.41 \mu$ M; $n_H = 0.84 \pm 0.03$, $P < 0.05$ vs. unity), mephedrone ($K_i = 3.96 \pm 0.22 \mu$ M; $n_H = 0.94 \pm 0.09$) and methylone ($K_i = 11.12 \pm 2.89 \mu$ M; $n_H = 0.77 \pm 0.03$, $P < 0.05$ vs. unity) (Figure 9A).

The three cathinone derivatives displaced bound [3 H]raclopride in a concentration-dependent manner with K_i values in the high micromolar range, mephedrone being the compound with highest affinity. K_i values obtained for these drugs were: butylone ($K_i = 57.09 \pm 11.46 \mu$ M; $n_H = 1.00 \pm 0.03$), mephedrone ($K_i = 50.86 \pm 3.45 \mu$ M; $n_H = 0.83 \pm 0.21$) and methylone ($K_i = 191.28 \pm 9.44 \mu$ M; $n_H = 0.63 \pm 0.06$, $P < 0.05$ vs. unity) (Figure 9B).

Discussion

In this study, we compared the *in vitro* neuropharmacology of three cathinone derivatives as well as demonstrating, in laboratory animals, the psychostimulant effect which has been described by recreational users. The mesolimbic dopamine

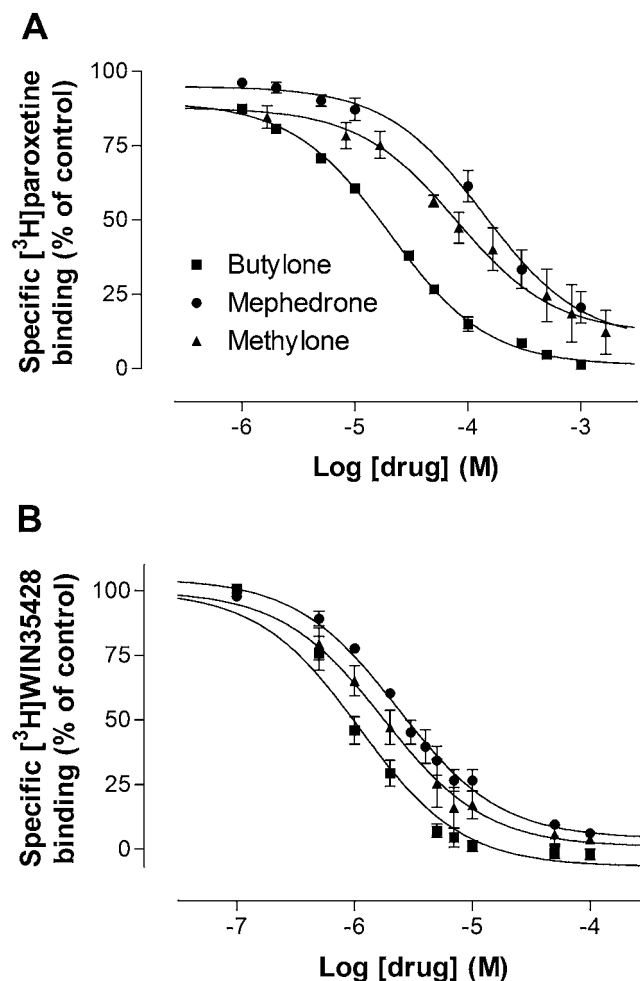


Figure 8

(A) Competition curves of the inhibition of [3 H]paroxetine binding by butylone, mephedrone and methylone in cortical membranes from Sprague Dawley rats. Membranes were incubated at 25°C for 2 h with 0.05 nM [3 H]paroxetine in the presence of increasing concentrations of cathinone derivatives. (B) Inhibition of [3 H]WIN35428 binding by these drugs in striatal rat membranes incubated for 2 h at 4°C with 5 nM [3 H]WIN35428 also in the presence of increasing concentrations of cathinone derivatives. Inhibition curves were calculated using the nonlinear least squares method and adjusted to a one-site model. Data shown are mean \pm SEM of duplicates and the experiments were performed in triplicate.

system is a final common pathway through which amphetamines exert their psychostimulant effect. It is well known that amphetamine derivatives produce dose-dependent increases in locomotor activity in rodents (Izawa *et al.*, 2006), which reflects increased dopaminergic transmission in the nucleus accumbens (Ljungberg and Ungerstedt, 1985). This hyperlocomotor activity of amphetamines is directly correlated with blockade of dopamine uptake and with a non-exocytotic, transporter-mediated, dopamine release (Leviel, 2001; Escubedo *et al.*, 2005). Our results demonstrate that all three cathinone derivatives, given subcutaneously to mice, induced a dose-dependent increase in locomotor activity that reached its peak shortly after administration.

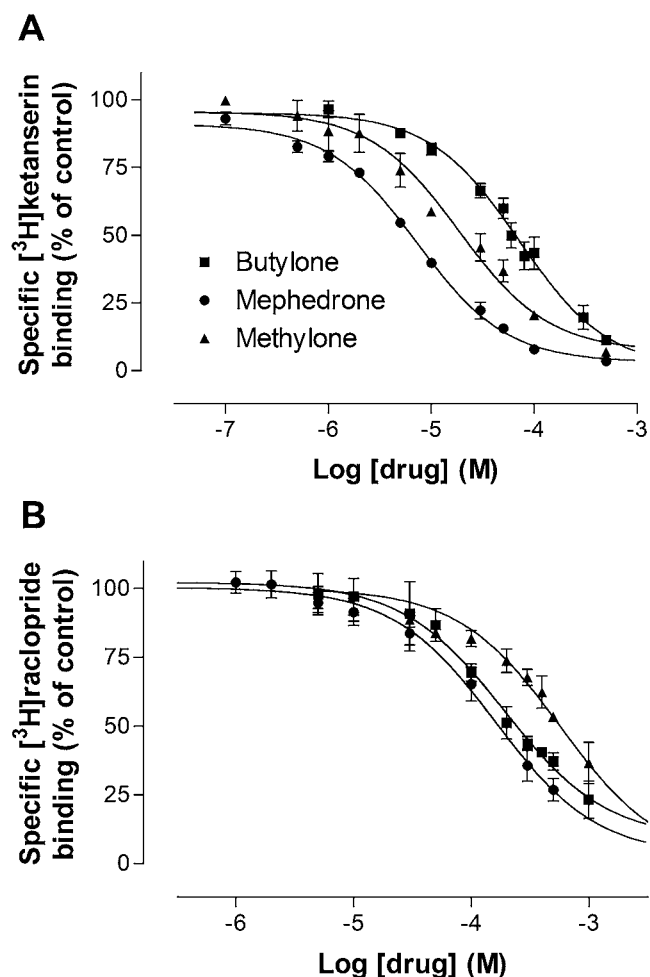


Figure 9

(A) Competition curves of the inhibition of [^3H]ketanserin binding by butylone, mephedrone and methylone in cortical membranes from Sprague-Dawley rats. Membranes were incubated at 25°C for 2 h with 1 nM [^3H]ketanserin in the presence of increasing concentrations of cathinone derivatives. (B) Inhibition of [^3H]raclopride binding by these drugs in striatal rat membranes incubated for 2 h at 4°C with 2 nM [^3H]raclopride also in the presence of increasing concentrations of cathinone derivatives. Inhibition curves were calculated using the nonlinear least squares method and adjusted to a one-site model. Data shown are mean \pm SEM of duplicates and the experiments were performed in triplicate.

Mephedrone elicits a moderate increase in locomotor activity lasting for 150 min. The limited information available in humans comes from user self-reports, which suggest that the onset of the psychostimulant effects of mephedrone occurs 15–45 min after oral ingestion, and the duration of the desired effects appears to last 2–3 h. Whilst we used a different route of administration in our studies, our results are in general agreement with the psychostimulant effects of mephedrone seen in humans. To our knowledge, there are no published data about the locomotor stimulatory activity of butylone or methylone. In this regard, our results demonstrate a dose-dependent psychostimulant effect induced by these drugs which is greater than that of mephedrone. This

effect persists for 4 h after administration at the highest dose. It should be noted that the methylone effect dramatically increases between the doses of 10 and 25 mg·kg⁻¹. Although we have no data on methylone metabolism, this result could be explained by a saturation of the metabolic pathways of methylone leading to increased and persistent plasma levels of this drug. In our mouse locomotor studies, cathinones exhibited a different profile of activity from that of MDMA in that they showed a higher maximal activity which returned more rapidly to basal values. These observations are in agreement with results obtained by Kehr *et al.* (2011) in rats.

High doses (25 mg·kg⁻¹) of amphetamine induces a climbing effect in test animals that impairs the proper measurement of locomotor activity. However, the beta-keto amphetamines, at this high dose, show a marked increase in locomotor activity, with an AUC value for butylone and methylone that does not differ from that of methamphetamine at 5 mg·kg⁻¹.

MDMA increased dopamine release by activating 5-HT₂ receptors, which may also contribute to the hyperlocomotion effect (Yamamoto *et al.*, 1995). When the animals were pre-treated with ketanserin or haloperidol (at doses that did not affect basal locomotor activity), the hyperlocomotion following cathinone derivatives was inhibited, suggesting a 5-HT and dopamine involvement. The affinity of these cathinones for 5-HT_{2A} receptors supports this hypothesis. However, their relatively low affinity for dopamine D₂ receptors rules out a direct effect on this receptor type and hence, we suggest that these drugs can induce an increase in the synaptic dopamine concentration that finally interacts with this dopamine receptor. Only mephedrone displayed a pCPA-dependent effect, suggesting the involvement of endogenous 5-HT. The hyperactivity of test animals treated with methylone in the presence of pCPA and seen at later time points could be due to an interaction of pCPA with the proposed cathinone-saturable metabolism. Kot and Daniel (2011) described an alteration of liver CYP activity when animals were treated with pCPA.

An activation of 5-HT_{1B} receptors is suggested to be responsible for the increase in the locomotor and rearing behaviour induced by MDMA in mice (McCreary *et al.*, 1999). These terminal autoreceptors regulate 5-HT release from dorsal raphe nucleus projections throughout the rat forebrain (Clark *et al.*, 2002). The results obtained with a selective 5-HT_{1B} receptor antagonist suggest a role for these receptors in the hyperlocomotive effect of butylone but not in the effects of mephedrone and methylone.

Mice treated with mephedrone or with methylone did not demonstrate novel-environment behaviour (absence of significant difference in rearings from saline-treated animals), but animals treated with butylone, demonstrated an increase in the number of rearings that appeared when hyperlocomotion was decreasing. This behaviour seems to be a consequence of the activation of 5-HT_{1B} receptors by this drug, given that the increase was fully inhibited by pretreating the animals with SB-216 641.

Amphetamines are substrates for the dopamine and 5-HT transporters, but the action of amphetamines is not a mere inhibition of the uptake transporter. Following its transport into the synaptic terminal, amphetamines stimulate a reversal of the transporter thereby eliciting monoamine

efflux (Fischer and Cho, 1979; Fleckenstein *et al.*, 2007). This reverse operation results in a net inhibition of monoamine uptake into the terminal and a prolonged extra-cellular concentration, which is responsible for their psychostimulant effects (Levi and Raiteri, 1993; Levie, 2001). Incubation of synaptosomes with low concentrations of butylone, mephedrone and methylone induced a significant concentration-dependent reduction in all [^3H]5-HT, [^3H]noradrenaline and [^3H]dopamine uptake. It is important to stress the low IC_{50} value of mephedrone inhibiting [^3H] NA uptake. Methylone is the most potent compound inhibiting both [^3H]5-HT and [^3H]DA uptake. This cathinone also induced the most persistent inhibition of 5-HT uptake. Nagai *et al.* (2007) reported higher IC_{50} values for methylone than those reported in our study. However, the higher purity of the synaptosomal preparation used in our study could result in greater sensitivity to the compound.

The direct interaction of cathinones with both plasmalemmal dopamine and 5-HT transporters can be correlated with the displacement of [^3H]WIN35428 or [^3H]paroxetine binding. In our studies, butylone and methylone demonstrated a close relationship between the IC_{50} values of their inhibition of [^3H]5-HT and [^3H]dopamine uptake and the K_i values obtained for their interaction with the respective transporters. Consequently, the inhibition of these transporters by cathinones is the result of a direct interaction with these proteins, competing with the endogenous substrate. However, mephedrone does not seem to interact directly with the 5-HT transporter, suggesting an additional mechanism.

We have limited information about pharmacokinetics of cathinones. Hadlock *et al.* (2011) found plasma mephedrone levels of about 5 μM one hour after a dosing regimen that mimics binge consumption. Present results from *in vitro* uptake experiments demonstrate that monoamine uptake inhibition is a probable mechanism of action of the *in vivo* psychostimulant effect of cathinones.

Cathinones also inhibit the entry of dopamine through the 5-HT transporter, but only at very high concentrations. Consequently, at concentrations reached *in vivo*, cathinones cannot prevent the excessive dopamine released from being transported and oxidized in the depleted 5-HT terminals.

The biggest differences among these compounds were observed in their effects on the transporter VMAT2. When measuring dopamine uptake through this transporter, we found a sevenfold and 27-fold reduction in potency of methylone and butylone, respectively, as compared with mephedrone. The inhibition of VMAT2-mediated uptake by amphetamines has been attributed to the ability of these compounds to act as substrates for VMAT2 (Schuldiner *et al.*, 1993), although another potential inhibitory mechanism could be through dissipation of the pH gradient. Given that the IC_{50} values obtained for cathinone derivatives are substantially lower than the concentration of MDMA required for this dissipation to take place (Rudnick and Wall, 1992), this mechanism can be ruled out. The results on VMAT2 inhibition and those obtained with reserpine-treated synaptosomes suggest that plasmalemmal and vesicular components are involved in the final inhibitory effect of cathinones. The vesicular component is especially important in explaining the effects of mephedrone. This suggestion is in agreement with results obtained by Kehr *et al.* (2011) in rats.

Putative phosphorylation sites for protein kinases exist in dopamine and 5-HT transporters. For example, protein kinases A and C have been implicated in various aspects of dopamine transporter function and regulation, such as trafficking, transport activity and direct phosphorylation (Jayanthi *et al.*, 2005; Foster *et al.*, 2006; Ramamoorthy *et al.*, 2011). Thus, a substance acting on specific intracellular pathways can induce a persistent inhibition of the transporter. We found that methylone caused prolonged inhibition of [^3H]5-HT uptake suggesting that alterations in the transporter functionality are more complex than a simple blocking of the carrier. In our *in vitro* model, mephedrone did not inhibit dopamine uptake. Nonetheless, it persistently inhibited 5-HT uptake, by about 22%, but only at a high concentration. This observation is in accordance with recent reports stating that repeated mephedrone administrations causes persistent 5-hydroxytryptaminergic, but not dopaminergic deficits (Hadlock *et al.*, 2011).

Results obtained in the present work, using radioligand binding assays, confirm our initial hypothesis that cathinone derivatives interact with both dopaminergic and 5-hydroxytryptaminergic targets in the CNS. We demonstrate a great affinity of butylone, mephedrone and methylone for the 5-HT transporter as these drugs displaced [^3H]paroxetine binding with K_i values in the micromolar range, with the following order of affinity: butylone > methylone >> mephedrone. The interaction of butylone with the plasma membrane 5-HT transporter occurs in a single class of binding sites (the Hill coefficient value did not differ from unity), whilst the interaction of mephedrone and methylone occur in a more complex manner. Similarly, we demonstrate a high affinity of butylone, mephedrone and methylone for the dopamine transporter. This interaction occurs in a single class of binding sites.

5-HT $_{2A}$ receptors are a pharmacological target of amphetamine compounds, mediating not only dopamine release but also locomotor responses (Auclair *et al.*, 2004). Hallucinogens potentially stimulate the 5-HT $_{2A}$ receptors, and their agonistic properties are responsible for the behavioural effects of these compounds (Erritzoe *et al.*, 2011). All three compounds displaced bound [^3H]ketanserin, with the lowest K_i value being for mephedrone, which interacts with a single class of binding sites. This low value is similar to that of MDMA acting as a 5-HT $_{2A}$ receptor agonist. Moreover, the affinity values of cathinone derivatives for dopamine D $_2$ receptors are in the high micromolar range. This feature is shared with MDMA, which has an affinity for this dopamine receptor of 95 μM (Battaglia *et al.*, 1988), and rules out the possibility of a direct activation of these receptors *in vivo*, by these cathinones.

In summary, we provide evidence that some cathinone derivatives interact with dopamine and 5-HT transporters and receptors, suggesting a pharmacological profile similar to other psychoactive drugs such as the amphetamine-like compounds.

Butylone particularly induces intense hyperlocomotion through an activation of 5-HT $_{2A}$ receptors and an increase in extra-cellular dopamine concentration. The activation of 5-HT $_{1B}$ receptors also contributes to this psychostimulant effect. This cathinone inhibits 5-HT and dopamine uptake as a consequence of a competition with the substrate. At high doses, its effect lasts for 4 h after its administration.

The locomotor profile of methylone, similar to that of butylone, points to a saturation of its metabolism. *In vitro*, this cathinone derivative is the most potent 5-HT and dopamine uptake inhibitor. This inhibition partially persists after its withdrawal.

Finally, mephedrone also induced endogenous 5-HT-dependent hyperlocomotion, which was prevented by 5-HT_{2A} and dopamine D₂ receptor antagonists. Vesicular content plays a key role in the effect of mephedrone, especially in the 5-HT uptake. Its potency in inhibiting noradrenaline uptake is suggestive of a significant sympathetic effect of this drug.

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Conflicts of interest

None.

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