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RESEARCH PAPER

Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter

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BACKGROUND AND PURPOSE

Bath salts is the street name for drug combinations that contain synthetic cathinone analogues, among them possibly mephedrone (MEPH) and certainly methylenedioxypyrovalerone (MDPV). In animal studies, cathinone and certain cathinone analogues release dopamine (DA), similar to the action of amphetamine (AMPH) and methamphetamine (METH). AMPH and METH act on the human DA transporter (hDAT); thus, we investigated MEPH and MDPV acting at hDAT.

EXPERIMENTAL APPROACH

We recorded electrical currents mediated by hDAT expressed in *Xenopus laevis* oocytes and exposed to: DA, METH, a known hDAT stimulant and DA releaser, MEPH, MDPV, MEPH + MDPV, or cocaine, a known hDAT inhibitor.

KEY RESULTS

DA, METH and MEPH induce an inward current (depolarizing) when the oocyte is held near the resting potential (–60 mV), therefore acting as excitatory hDAT substrates. Structurally analogous MDPV induces an outward (hyperpolarizing) current similar to cocaine, therefore acting as an inhibitory non-substrate blocker.

CONCLUSIONS AND IMPLICATIONS

Two components of *bath salts*, MEPH and MDPV, produce opposite effects at hDAT that are comparable with METH and cocaine, respectively. In our assay, MEPH is nearly as potent as METH; however, MDPV is much more potent than cocaine and its effect is longer lasting. When applied in combination, MEPH exhibits faster kinetics than MDPV, viz., the MEPH depolarizing current occurs seconds before the slower MDPV hyperpolarizing current. *Bath salts* containing MEPH (or a similar drug) and MDPV might then be expected initially to release DA and subsequently prevent its reuptake via hDAT. Such combined action possibly underlies some of the reported effects of *bath salts* abuse.

Introduction

Amphetamine (AMPH) and AMPH analogues, including methamphetamine (METH), methylenedioxymethamphetamine (MDMA, ecstasy), and cathinones are catecholamine releasers. These drugs exert profound effects on mental function and behaviour and are implicated in drug abuse and addiction (Sulzer *et al.*, 2005; Han and Gu, 2006; Kelly, 2011; Sulzer, 2012). The behavioural effects associated with these agents are closely linked to enhanced dopaminergic activity (Howell and Kimmel, 2007; Howell *et al.*, 2007). More specifically, the rewarding and hyperlocomotor effects of AMPH are

related to dopamine (DA) release in the nucleus accumbens (Sellings and Clarke, 2003), which activates DA receptors resulting in the behavioural effects associated with AMPH and related drugs (Kalix, 1992; Rothman and Baumann, 2006; Williams and Galli, 2006; Negus *et al.*, 2007; Banks *et al.*, 2011; Sulzer, 2012).

The DA transporter is encoded by the gene *SLC6A3* and is a member of the solute carrier (SLC) family, which includes symporters that allow the concentration gradient of one solute to move a second solute across a membrane against its own gradient. Transporters in the SLC family may also express the electrogenic properties of ion channels (Alexander *et al.*, 2011). We previously demonstrated that R(-)AMPH or S(+)AMPH produce an inward current at –60 mV when they are applied individually to the human DA transporter (hDAT) expressed in *Xenopus laevis* oocytes (Rodriguez-Menchaca *et al.*, 2011). Both AMPH isomers produce an electrical effect much like DA itself. However, only the S(+)AMPH isomer generates an *induced* persistent leak current that lasts more than 30 min after removal of the drug from the extracellular milieu (distinct from the *endogenous* leak current). The inward current and the persistent leak current may have significance for dopaminergic neurons because they would depolarize the presynaptic terminal and increase excitability (Ingram *et al.*, 2002; Carvelli *et al.*, 2008). Depolarization of the terminal would be conducive to DA release either through vesicular fusion or non-vesicular mechanisms including reverse transport (Kahlig *et al.*, 2005; Sulzer *et al.*, 2005; Sulzer, 2012). The extent to which such depolarization currents would be significant depends on the synaptic physiology in question, the details of which are usually unknown. Where it has been studied, however, a significant contribution of transporter currents to synaptic transmission exists (Bruns *et al.*, 1993; Ingram *et al.*, 2002; Carvelli *et al.*, 2008).

The question arose whether the peak current or the persistent current associated with AMPH–hDAT interactions, which we expect to be significant with regard to DA release, is a general phenomenon common to abused stimulants that are structurally similar to AMPH. Thus, we have extended our earlier studies to other AMPH-like, phenylisopropylamine stimulants, and, in particular, to two synthetic cathinones commonly found in *bath salts* (Iversen, 2010).

In mice, the ability of butylone, mephedrone (MEPH), or methylone to inhibit plasmalemmal and vesicular monoamine transporters and 5-HT or DA receptors was studied. Butylone and methylone induced hyperlocomotion, and they inhibited 5-HT and DA uptake. MEPH induced only moderate increase in hyperlocomotion; however, in humans, MEPH induced psychostimulant effects 15–45 min after oral ingestion and lasts for 2–3 h. Furthermore, the potency of MEPH in inhibiting NE uptake has suggested a sympathetic component (López-Arnau *et al.*, 2012). Since this paper has been submitted, a pharmacological characterization of synthetic cathinones has appeared (Simmler *et al.*, 2012).

Methods

Expression of hDAT in Xenopus *oocytes*

Oocytes were harvested and prepared from adult female *Xenopus laevis* following standard procedures (Machaca and Hartzell, 1998; Iwamoto *et al.*, 2006). We selected stages V–VI oocytes for cRNA injection within 24 h of isolation. cRNA was transcribed in the p oocyte transcription vector (gift of Mark Sonders, Columbia University) using Ambion mMessage Machine T7 kit (Ambion Inc., Austin, TX, USA). Each oocyte was injected with 40 nL of 1 μg μL^{-1} hDAT cRNA (final amount 40 ng) (Nanoject AutoOocyteInjector, Drummond Scientific Co., Broomall, PA, USA) and incubated at 18°C for 6–10 days in Ringers solution supplemented with NaPyruvate (550 μg mL^{-1}), streptomycin (100 μg mL^{-1}), tetracycline (50 μg mL^{-1}) and 5% dialyzed horse serum.

HEK293 cells

Maintenance of stably expressing hDAT (hDAT–HEK) cells. Cells were prepared in DMEM supplemented with 10% FBS, 2 mM L-glutamine, penicillin (100 units mL^{-1}), streptomycin (100 μg mL^{-1}) and G418. hDAT-HEK cells in DMEM suspension are counted and 200 000 cells are put in individual Eppendorf tubes, and incubated for 10 min at room temperature with mixtures of 3% [^{3}H]-DA and the compounds tested (cocaine (COC), MDPV, MEPH) at a broad range of concentrations. Ice-cold (4°C) PBS is added to the samples, cells are centrifuged for 1 min at 2000 rpm, supernatant is removed, and cells are washed twice with PBS addition, centrifugation and removal of supernatant. Cells are solubilized with ice-cold Ecoscint H (National Diagnostics, Atlanta, GA, USA) and [^{3}H]-DA remaining is measured using a scintillation counter.

Two-electrode voltage clamp

Electrodes had resistances from 1 to 5 MΩ. *Xenopus* oocytes expressing hDAT are voltage-clamped to –60 mV (unless otherwise noted), and buffer was gently perfused until a stable baseline was obtained, then the experimental substrates were perfused until stable currents were obtained, or for time periods indicated. The voltage clamp apparatus used for these experiments was a Gene Clamp 500 Amplifier and a 16 bit A/D converter (Digidata 1320A, Axon Instruments, Sunnyvale, CA, USA). Data were sampled at 5 kHz and digitally stored for off line analysis using Clampfit 10.2 software and 1-kHz filtering. Inward and outward currents are compared with holding currents required for voltage clamp at –60 mV, which were from 100 to 0 nA in oocytes with –20 to –60 mV resting potentials. To measure drug-induced I(V) curves, we first generated an I(V) curve for buffer without drug, which we then subtract from I(V)s for buffer with drug, as indicated in Figure 3B. Buffer subtracted I(V) curves for 10 mM DA, MEPH-peak, MDPV and COC were generated under voltage clamp between –100 and +20 mV. To normalize I(V) curves, we set the 10 μM DA-induced current to 100 at –100 mV in each oocyte. See also (Rodriguez-Menchaca *et al.*, 2011).

Solutions and drugs

Extracellular (in mM): 120 NaCl, 7.5 HEPES, 5.4 Kgluconate, 1.2 Ca^{2+} gluconate, pH 7.4 with KOH. Intracellular electrode: 3 M KCl. Racemic METH, MEPH and MDPV were prepared as hydrochloride salts. The speed of solution exchange was 4 mL min^{-1}. No drug used in this study had any effect in non-injected oocytes (data not shown). See also (Rodriguez-Menchaca *et al.*, 2011).

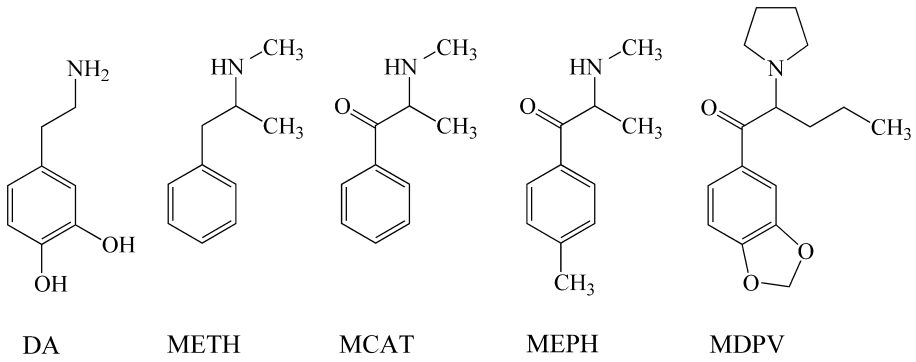

Figure 1

Structures. Chemical compositions of DA, METH, MCAT, MEPH and MDPV showing their structural similarity. The latter two compounds are potential ingredients in the drug combination known as '*bath salts*'.

The nomenclature in this article conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2011).

Results

Figure 1 compares five compounds, DA, METH, methcathinone (MCAT), MEPH and 3,4-methylenedioxypyrovalerone (MDPV). The latter three are synthetic versions of the naturally occurring cathinone, a monoamine alkaloid found in the shrub *Catha edulis* (khat) responsible for the stimulant effect of *khat* (Glennon and Showalter, 1981; Kalix, 1986; 1992; Glennon, 1993). MCAT is included for comparison with METH, with which it differs in a ketone functional group on the β position of the side chain (Glennon *et al.*, 1987). Note that MDPV bears structural similarity to MDMA (3,4-methylenedioxy-*N*-methylamphetamine) in the methylenedioxy functional group. Components commonly found in *bath salts* include MEPH and certainly MDPV, but *bath salts* ingredients can vary widely (Iversen, 2010; Drug Enforcement, 2011; Symposium, 2012).

Figure 2 compares currents generated by four of the compounds shown in Figure 1 and by COC. The hDAT expressing oocytes from which these data were generated are voltage clamped at –60 mV, near the resting potential of cells, and drug applications in each case are for 60 s at 10 μM. As previously reported, the DA response under these conditions is a relatively large inward current that returns to baseline after DA is removed from the external milieu (Figure 2A). METH generates a similar inward current, but in addition, it has an induced leak current that persists long after METH is removed (Figure 2B). The persistent current observed with METH is analogous to a similar persistent current observed with AMPH (Rodriguez-Menchaca *et al.*, 2011). The MEPH-induced current is qualitatively similar to METH (Figure 2C). Although the MEPH-induced peak current is smaller on an absolute scale than the METH-induced peak current, the persistent current relative to peak is larger in MEPH than in METH. Figure 2 is composed of data from different oocytes with similar DA-induced peak amplitudes. Surprisingly, MDPV,

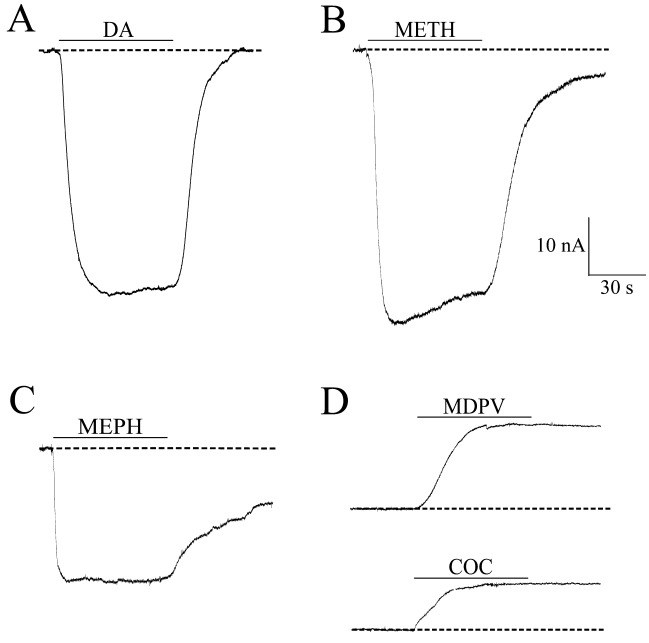

Figure 2

Current through hDAT elicited by 60 s, 10 μM external drug application at V = –60 mV. (A) DA induces a relatively large inward 'peak' current that returns to baseline when DA is removed. (B) METH induces a similar inward peak current; however, induced persistent leak currents remain long after METH is removed. (C) MEPH induces a similar inward current and persistent leak current after MEPH is removed. Although the MEPH-induced peak current is smaller than the METH-induced peak, the persistent current is proportionally larger in MEPH than in METH. (D) Top trace MDPV exposure produces an outward, hyperpolarizing current similar to COC (bottom trace), which is a known reuptake inhibitor. Both MDPV- and COC-induced traces reveal the presence of an endogenous leak current. Traces A through D are from different oocytes that respond the same to DA.

which is structurally analogous to MEPH (Figure 1), gives a completely different response than MEPH under the same conditions. Whereas MEPH generates an inward depolarizing current through hDAT held at –60 mV, MDPV generates an outward hyperpolarizing current compared with baseline that lasts more than 30 min, even after the drug has been removed. This outward current is comparable with the response that is generated by COC (Figure 2D). We interpret the outward current as the blockade of an inherent inward current in hDAT that is present even in the absence of substrate (Sonders *et al*., 1997; Rodriguez-Menchaca *et al*., 2011).

The similarity in the actions of MDPV and COC are further explored in Figure 3, which compares the drugs in two ways. Figure 3A shows a classic uptake experiment in which we measure DA transport into HEK-hDAT cells in the presence of MDPV or COC at various concentrations. In this assay, MDPV has an IC50 value of 28 nM whereas COC has an IC50 value of 995 nM – 35× less potent than MDPV as an uptake inhibitor. MEPH also diminishes the accumulation of DA into cells with an IC50 value of 3950 nM, 4× less potent than COC. Second (Figure 3B), we measured the I(V) curves in hDAT expressing oocytes that were induced by a fixed concentration of 10 μM DA, MDPV, COC, or MEPH. The currents generated under voltage clamp between –100 and +20 mV support the interpretation that MDPV is a more potent hDAT inhibitor than COC, which is verified in Figure 3A; namely, at a particular voltage, MDPV blocks more of the endogenous leak current than COC. The MEPH-induced I(V) curve is similar to the DA-induced curve except at a particular voltage MEPH generates less current. MEPH is approximately half as potent as DA at blocking DA uptake [$IC50_{DA} = 2.1 \pm 0.3$ μM (Guptaroy *et al*., 2011)]. Together, Figure 3A and 3B suggest that MDPV functions as a non-substrate blocker of DA uptake and that MEPH is an hDAT substrate that reduces DA uptake, consistent with Simmler *et al*. (2012).

Unlike COC, the inhibitory effect of MDPV persists for more than 30 min. Figure 4A–B shows raw traces of MDPV block compared with COC block. In the example shown, the application of either blocker occurred during the induced persistent leak current phase; the effect is comparable when MDPV or COC are applied during the peak current, namely, MDPV block lasts longer than COC. The COC blockade of the inherent leak lasts longer (Figure 2), but even in that case MDPV is more resistant to washout than COC. Figure 4C shows examples of DA-induced current recovery after COC block and the longer-lasting effect of MDPV block. At V = –60 mV, after an initial 30-s pulse of 10 μM DA (bar), we block hDAT endogenous leak currents with 1 min exposure to 10 μM COC or MDPV (thick bars), and test recovery at different times by subsequently pulsing 10 μM DA. Figure 4D summarizes 4C data by plotting the percent recovery of the initial DA response: after 10 and 30 μM COC or after 1, 3, and 10 μM MDPV. After exposure to 10 μM COC (saturating), the DA response recovers immediately; on the other hand, after exposure to 10 μM MDPV (saturating), the DA response recovers by only 20% after 30 min. Figure 3C shows that COC is 35× less potent than MDPV; after exposure to 30 μM COC, the DA response recovers immediately, whereas after 1 μM MDPV (saturating), recovery is less than 50% after 30 min washout. The long-lasting blockade of hDAT summarized in Figure 4D corrects for the diminished response with repeated pulses of DA alone.

Finally, we investigated the effect of MEPH and MDPV together, as might be expected for *bath salts* abuse. Figure 5 shows the induced current in hDAT expressing oocytes held at V = –60 mV for continuous exposure to different ratios of MEPH/MDPV (in μM). From top to bottom the ratios are MEPH/MDPV = 1/19, 5/15, and 15/5. In mixtures composed predominantly of MEPH, the effect is a relatively long-lasting excitatory current. Contrariwise, in mixtures composed predominantly of the non-substrate inhibitor, the effect is a long-lasting inhibitory current. Thus, when both compounds have equal access to the transporter, it appears that the kinetics of MEPH are more rapid than the kinetics of MDPV in terms of their ability either to elicit or block current through the hDAT transporter.

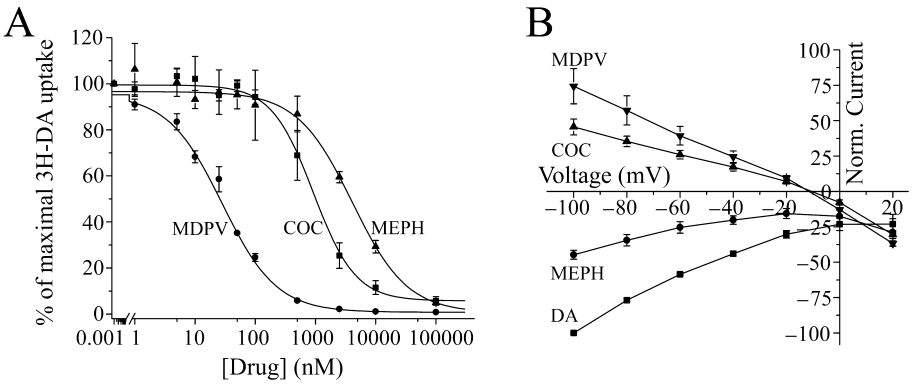

Figure 3

DA uptake assay and I(V) curves. (A) hDAT-HEK cells in various concentrations of MDPV, COC or MEPH (see Methods). Experiment is performed in triplicates and data is fitted to the Hill equation to obtain IC50 values. MDPV IC50 is 28 nM, COC IC50 is 995 nM, and MEPH IC50 is 3950 nM. (B) Buffer subtracted I(V) curves for 10 μM DA, MEPH peak, MDPV or COC generated under voltage clamp between –100 and +20 mV. The MEPH I(V) curve is similar in shape to the DA I(V) curve, which suggests that MEPH is an hDAT substrate. The COC I(V) curve is similar in shape to the MDPV I(V) curve, which suggests that MDPV is a non-substrate hDAT uptake inhibitor. Recordings were normalized such that the current in DA was set equal to 100 at –100 mV ($n = 3$). All I(V) curves are generated at 10 μM. Unlike panel A, the data in B are collected under voltage clamp.

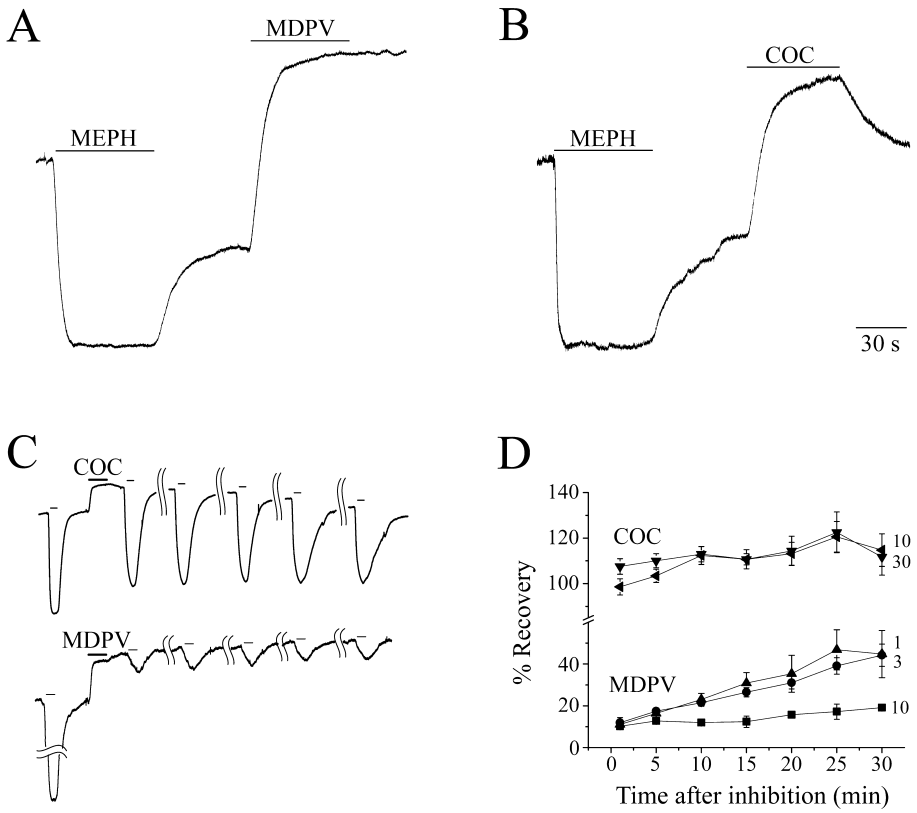

Figure 4

MDPV reversibility. Panels (A) and (B) show raw traces of 30-s blocker application (MDPV and COC, 10 μM) during the persistent leak current induced by 60 s MEPH external application (10 μM) at V = –60 mV. The data in A and B are from different oocytes normalized to the same MEPH peak current. (C) At V = –60 mV, 30 s exposure of 10 μM DA (dash) followed by 60 s application of 10 μM COC (above) or MDPV (below) block and short 30 s pulses of DA following block at time in min: 1, 5, 10, 15, 20, 25, 30 (25 and 30 min not shown). The 10 μM DA-induced peak currents in panel C have the value of 60 nA. (D) Cumulative data from experiments similar to (C) n = 3. Recovery of DA-induced current after treatment with COC or MDPV plotted on the y-axis. One minute after COC treatment (10 and 30 μM), the DA-induced current recovers completely (and even exceeds initial peak). One minute after MDPV treatment (1, 3, 10 μM), the DA-induced current recovers only 10% of its initial value. At 30 min, recovery from MDPV is 50, 50 and 20%, respectively.

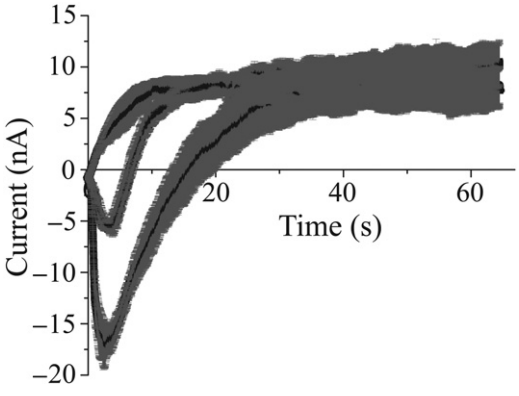

Figure 5

Bath salts current. Currents induced by continuous exposure of different proportions of *bath salts* mixtures at V = –60 mV: MEPH/MDPV (substrate/blocker) (in μM): 1/19, 5/15 and 15/5 (n = 3, grey colour represent SEM).

Discussion

Despite their growing illegal status, cathinone-related compounds such as those found in *bath salts* are likely to prevail as drugs of abuse for the foreseeable future (Iversen, 2010; Kelly, 2011). Over 30 different synthetic cathinones have been reported as potential compounds for abuse (Spiller *et al.*, 2011; Symposium, 2012). *Bath salts* consist of heterogeneous mixtures of various psychoactive substances, including synthetic cathinones, with each preparation having its own distinct effects. MEPH and especially MDPV in combination with other drugs are falsely marketed as 'research chemicals', 'plant food', or 'bath salts' and sold at smoke shops, convenience stores, adult book stores, gas stations, or from the Internet and mailed through regular services. According to drug surveys, the amount of synthetic cathinones used per dose ranges from 25 to 250 mg. Drug surveys and law enforcement accounts reveal 'bath salts' products containing MEPH, MDPV, other synthetic cathinones (methylone, butylone and flephedrone), other abused substances (AMPH, MDMA, COC), and lidocaine, caffeine, or benzocaine. Furthermore,

the amount of these highly heterogeneous mixtures that are used per session can be as high as 5 g depending on the particular combination, duration of intake and route of administration (Drug Enforcement, 2011). In a retrospective case study of patients exposed to *bath salts*, 13 of 17 live patients had 186 nM MDPV in their blood and 2.75 μM in their urine. Four samples with no detected drug reported last use of *bath salts* ~20 h prior to presentation. Post-mortem samples detected MDPV in blood at 545 nM and in urine at 4.5 μM. No other synthetic cathinones were detected in this study (Spiller *et al.*, 2011). It is essential that the actions of individual compounds are understood as well as combinations of compounds. In the present study, we have concentrated on MEPH and MDPV applied individually or together in varying proportions.

It is known from studies on synaptosomes isolated from the cortex or striatum of rats that MEPH has a greater affinity for DA transporters than for serotonin transporters (Glennon *et al.*, 1987; Martínez-Clemente *et al.*, 2011). MEPH is, however, relatively nonselective for monoamine transporters by *in vitro* release assays in rat brain synaptosomes and is similar in potency and selectivity to MDMA. MEPH produces a dose-dependent increase in both extracellular DA and 5-HT in the rat nucleus accumbens and is a weak motor stimulant compared with METH. Repeated administrations of MEPH cause hyperthermia, but no chronic change in cortical or striatal amines; while similar treatment with MDMA also evoked hyperthermia, no persistent depletion of cortical and striatal 5-HT was observed. Thus, MEPH has transmitter-releasing activity comparable with MDMA (Baumann *et al.*, 2012). From our studies MEPH induces a stimulatory (depolarizing) current with a relatively large persistent leak current that remains long after MEPH is removed (Figure 2C). Thus, MEPH is similar to METH, but potentially stronger as a DA-releasing agent than METH (Baumann *et al.*, 2012), perhaps because of the relatively larger persistent current we observe relative to the peak current. Hadlock *et al.* (2011), on the other hand, showed that MEPH produces persistent serotonergic deficits, but not dopaminergic deficits, pointing to similarities between MEPH and MDMA.

Meltzer and colleagues have described the synthesis and biological activity of a wide class of 2-aminopentanophenones (Meltzer *et al.*, 2006). The lead compound for their study was 1-(4-methylphenyl)-2-pyrrolidin-1-ylpentan-1-one (pyrovalerone), which is close in structure to MDPV. Pyrovalerone is a potent inhibitor of β-carbomethoxy-3β-(4-iodophenyl)tropane binding with a Ki value of 21.4 nM, 20× more potent than COC in the same assay. It is also an inhibitor of DA uptake, with an IC50 value of 52 nM, about 9× more potent than COC. In addition, pyrovalerone is a strong inhibitor of norepinephrine transporter (Ki = 195 nM) and norepinephrine uptake (IC50 = 28.3 nM), and it is > 10× more effective than COC in the same assay. There was little effect of pyrovalerone on serotonin transporters or a variety of monoamine receptors (Meltzer *et al.*, 2006). MDPV is 3,4-methylenedioxypyrovalerone. The clinical, pharmacological and toxicological information about this relatively new drug, which is a major component of *bath salts*, is scarce. MDPV is a catecholamine reuptake inhibitor derived from pyrovalerone, and it is originally classified as a research chemical. Some reports have suggested that its stimulant action could be more potent than COC. The literature and Internet information have suggested that there is a high risk of cardiovascular and CNS toxicity of MDPV related to the powerful stimulation of the catecholaminergic system. MDPV stimulation of the dopaminergic reward system could explain withdrawal symptoms reported by users (Coppola and Mondola, 2012). From our electrophysiological studies, we infer that MDPV is similar to COC in eliciting an inhibitory (hyperpolarizing) current (Figure 2D). The hyperpolarizing current induced by MDPV and COC is the blockade of an endogenous leak conductance present in hDAT (Sonders *et al.*, 1997; Rodriguez-Menchaca *et al.*, 2011). Note that after the removal of MDPV or COC, the blockade of the endogenous leak conductance persists. To explore the blockade of DA uptake that is reported for pyrovalerone (Meltzer *et al.*, 2006), we directly measured DA uptake in HEK-hDAT cells (Figure 3A). In this assay, MDPV is 35× more potent than COC as an uptake inhibitor. The more than 100× potency difference of MDPV over MEPH in Figure 3A may reflect differences in drug dissociation rates, which are perhaps influenced by lipophilic partitioning. Figure 5 suggests that the two drugs have comparable association rates; however, the actual association and dissociation rates of MDPV are unknown. Nevertheless, we may speculate that either MDPV is still present and available after removal (e.g. through lipid partitioning), or MDPV induces a long-lived conformational state in hDAT.

Figure 3B further shows that MDPV and COC have similar I(V) relationships in a voltage clamp experiment on hDAT expressing oocytes. MDPV has a larger inhibitory current than COC at negative voltages; however, both I(V)s reverse near –10 mV suggesting that they block the same pathway. It is worth repeating that the *inhibitory current* induced by COC or MDPV is actually the drug-induced diminution of an endogenous leak current that is inwardly directed (depolarizing) at –60 mV (Sonders *et al.*, 1997).

It is known that both MEPH and MDPV enhance DA neurotransmission. However, behavioural studies in rats show categorical distinctions for these drugs indicating that MDPV produces prototypical locomotor stimulant effects, whereas MEPH is more like MDMA (Huang *et al.*, 2012). This study warns against assuming that all cathinone derivatives share neuropharmacological or behavioural properties; some derivatives are similar to prototypical stimulants, some are similar to MDMA, and some combinations may have unique effects. MEPH has powerful stimulant effects, causes locomotor hyperactivity resembling METH and MDMA, and activates mesolimbic regions as determined using Fos immunohistochemistry. These results may provide an empirical basis to user reports that MEPH subjectively resembles a MDMA/METH combination (Motbey *et al.*, 2011). Acute administration of MEPH elevates DA in the nucleus accumbens of awake rats to a greater extent than MDMA; however, the corresponding increase in 5-HT was much greater for MEPH as well as MDMA. Thus the neurochemical and functional properties of MEPH resemble those of MDMA; however, MEPH also shows an AMPH-like effect in that it evokes a rapid release and elimination of DA in the brain reward system (Kehr *et al.*, 2011). On the other hand, MEPH produces persistent serotonergic deficits, but not comparable dopaminergic deficits, attesting to the similarities between

MEPH and MDMA (Hadlock *et al.*, 2011). Furthermore, METH is more potent and selective as a DA-releasing agent than MEPH, and MEPH increases extracellular 5-HT more than extracellular DA. In this sense, MEPH is more similar to MDMA than METH (Baumann *et al.*, 2012).

Whereas *bath salts* are not well defined as a mixture, samples include MEPH, MDPV, methylone and other compounds (Drug Enforcement, 2011; Spiller *et al.*, 2011; Symposium, 2012). Figure 5 shows how two synthetic cathinones act together when applied directly to the DA transporter: excluding pharmacokinetics in actual tissue, MEPH acts more quickly than MDPV. Thus, DA release would precede DA uptake blockade in a mixture of *bath salts* containing MEPH (or a comparable drug) and MDPV. Furthermore, the blockade of hDAT by MDPV is longer lasting than the blockade by COC, as shown in Figure 4. Some reports suggest re-dosing in a single session occurs because MDPV has a short duration of action; thus, the slow kinetics of MDPV we report may not reflect *in vivo* pharmacokinetics (Coppola and Mondola, 2012). A tissue culture model shows that MDPV is apparently more penetrant to the blood–brain barrier than other synthetic cathinones (Simmler *et al.*, 2012). The data in Figure 5 may reflect competition between two drugs with equal access to the transporter, for example if MEPH and MDPV had similar association rates, but dissimilar dissociation rates. However, the molecular mechanisms behind MEPH–hDAT and MDPV–hDAT interactions are unknown. Likewise, in neurons the ability of MEPH to affect DA release may depend on its direct action on vesicular monoamine transporter (Sulzer, 2012), but again not enough is known at present.

These results have led us to a simple hypothesis regarding the action of *bath salts*: the DA transporter is a primary target for synthetic cathinones found in *bath salts*, MEPH (or a similar drug) and MDPV, which first stimulate DA release and subsequently inhibit DA reuptake. This synergistic combination may be especially dangerous to the user, for whom the combined action of releasing drugs and blocking drugs is greater than the sum of their individual effects.

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Conflict of interest

The authors declare no conflict of interest of this work with any other work or organization.

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