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Neurochemical profiles of some novel psychoactive substances

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ABSTRACT

Fourteen substances from the class of drugs sometimes known as "legal highs" were screened against a battery of human receptors in binding assays, and their potencies as inhibitors of monoamine uptake determined in functional in vitro assays. Thirteen of the test substances acted as inhibitors of monoamine uptake at submicromolar concentrations, including 9 potent inhibitors of the dopamine transporter (DAT), 12 potent inhibitors of the norepinephrine transporter (NET) and 4 potent inhibitors of the serotonin transporter (SERT). Seven compounds acted as submicromolar inhibitors of both DAT and NET, and three substances 1-(benzofuran-5-yl)propan-2-amine (5-APB), 1-naphthalen-2-yl-2-pyrrolidin-1-ylpentan-1-one hydrochloride ("naphyrone") and 1-naphthalen-1-yl-2-pyrrolidin-1-ylpentan-1-one hydrochloride ("1-naphyrone") were submicromolar inhibitors of all three monoamine transporters. There was a lack of correlation between results of functional uptake experiments and in vitro binding assays for the monoamine transporters. There was also no correlation between the human behavioral effects of the substances and the results of bindings assays for a range of receptor targets, although 1-(benzofuran-5-yl)propan-2-amine (5-APB), 1-(benzofuran-6-yl)propan-2-amine hydrochloride (6-APB) and 5-iodo-2,3-dihydro-1H-inden-2-amine hydrochloride (5-iodo-aminoindane) exhibited <100 nM affinities for $5HT_{2B}$ and α_{2C} receptors. Functional assays revealed that 5-APB and 6-APB were potent full agonists at $5HT_{2B}$ receptors.

1. Introduction

The recent emergence of novel synthetic psychoactive drugs and their sale through internet sites has raised concerns about the potential harms associated with compounds for which formal toxicology profiles are lacking (Corazzo et al., 2012; Schifano et al., 2011; Winstock et al., 2011; ACMD, 2011). Among the novel psychoactive substances that have emerged in recent years are a range of phenethylamine analogs designed to mimic the psychostimulant properties of amphetamine and/or ecstasy (3,4-methylenedioxymethamphetamine, "MDMA"). These include cathinone derivatives (e.g. mephedrone) (Measham et al., 2010), aminoindanes, aminotetralins and benzofurans (e.g. 1-(benzofuran-5-yl)propan-2-amine (5-APB) and1-(benzofuran-6-yl)propan-2-amine hydrochloride (6-APB)—"benzo-fury"). The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) monitors the emergence of these psychoactive substances, reporting 42 new compounds during 2011 (EMCDDA Annual Reports, 2010, 2011). These substances are sold without any safety data and have been described as "plant food", "bath salts", "fish food" or simply as "research chemicals". The use of untested novel chemical substances presents clear potential hazards.

Pharmacologically, the psychostimulant actions of amphetamine and ecstasy are thought to be related to their ability to promote the release of dopamine, norepinephrine and/or serotonin from nerve terminals in the brain (Iversen, 2008). These drugs act not only as competitive inhibitors of the respective monoamine transporters (DAT for dopamine), (NET for norepinephrine) and (SERT for serotonin), but also as substrates for these uptake systems acting to displace monoamines from their vesicular storage sites in the synaptoplasm (Rothman and Baumann, 2003).

Studies of the pharmacological profiles of novel psychoactive compounds have revealed their interactions with monoamine transporters. Nagai et al. (2007) reported that a variety of synthetic amphetamine and tryptamine analogs inhibited monoamine uptake and release in rat brain synaptosomes. Simmler et al. (2012) recently reported detailed studies on a range of cathinone derivatives, showing many of them to be potent inhibitors of monoamine transporters, using human targets, with a correlation between these properties and their behavioral stimulant effects in vivo. Martinez-Clemente et al. (2012) reported that mephedrone inhibited dopamine and serotonin uptake in rat brain synaptosomes at submicromolar concentrations, and had appreciable affinity for

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displacing selective DAT and SERT radioligands in vitro. Mephedrone also had appreciable affinity for $5HT_2$ and dopamine D2 receptors. Lopez-Arnau et al., 2012 compared the neuropharmacological profile of mephedrone with the related cathinones butylone and methylone, finding that all three compounds inhibited monoamine uptake via DAT, NET and SERT. In addition mephedrone had appreciable affinity for the vesicular monoamine transporter VMAT2 (Lopez-Arnau et al., 2012). In vivo the compounds also displayed amphetamine- and MDMA-like behavioral stimulant activity in rats. Baumann et al. (2012) and Kehr et al. (2011) found that mephedrone and methylone increased the release of dopamine and serotonin in rat brain using in vivo microdialysis techniques.

In the present study the resources of the National Institute of Mental Health Psychoactive Drug Screening Program were used to obtain neurochemical profiles for 14 novel synthetic psychoactive substances and to compare these profiles with those of amphetamine, MDMA ('ecstasy'), and other reference compounds. Unlike some previous studies, the present results were largely obtained using human receptor and transporter targets.

2. Materials and methods

2.1. Chemicals

A sample of 2-methylamino-1-(4-methylphenyl)propan-1-one hydrochloride "mephedrone" was acquired from an internet supplier. The full spectral data and elemental analysis were published (Gibbons and Zloh, 2010) and were in close agreement with previously published data (Camilleri et al., 2010).

The remaining thirteen chemicals were supplied as reference materials by LGC Standards, as listed below:

1-(benzofuran-5-yl)propan-2-amine hydrochloride, "5-APB", "benzofury"
1-(benzofuran-6-yl)propan-2-amine hydrochloride, "6-APB", "benzofury"
2-(benzylamino)-1-(4-methylphenyl)propan-1-one hydrochloride, ("benzedrone")
2-benzhydrylpiperidine hydrochloride, ("desoxypipradrol")
6,7-dihydro-5 H-cyclopenta[f][1,3]benzodioxol-6-amine, ("methylenedioxyaminoindane, MDAI")
2-ethylamino-1-(4-methylphenyl)propan-1-one hydrochloride, ("4-methylethcathinone")
5-iodo-2,3-dihydro-1 H-inden-2-amine hydrochloride ("5-iodo-2-aminoindane")
4-methylhexan-2-amine hydrochloride, ("dimethylamylamine")
methyl[(1-(2-thienyl)]propan-2-amine hydrochloride, ("methiopropamine")
2-(benzylamino)-1-(3,4-methylenedioxyphenyl)propan-1-one, ("methylenedioxybenzedrone, BMDP")
1-naphthalen-1-yl-2-pyrrolidin-1-ylpentan-1-one hydrochloride, ("1-naphyrone")
1-naphthalen-2-yl-2-pyrrolidin-1-ylpentan-1-one hydrochloride, ("naphyrone")
5,6,7,8-tetrahydrobenzo[f][1,3]benzodioxol-6-amine hydrochloride, ("methylenedioxyaminotetralin, MDAT")

Certified purities ranged from 95.2% to 99.9% (full Certificates of Analysis for each material are available from the LGC Standards website, www.logical-standards.com).

Production of six of these reference materials (5-APB, 6-APB, benzedrone, methylenedioxybenzedrone, 5-iodo-2-aminoindane and methiopropamine) was supported by the UK's Forensic Early Warning System (FEWS) and we thank the UK Home Office's Centre for Applied Science and Technology (CAST) for permission to use these materials in this study.

Table 1
Primary and secondary radioligand binding assays.

Serotonin receptors	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7
Dopamine receptors	D_1, D_2, D_3, D_4, D_5
Glutamate receptors	rNMDA receptor (MK-801 binding site), mGluR$_5$
GABA receptors	rGABA-A, rGABA-B, central and peripheral benzodiazepine sites
Biogenic amine transporters	SERT, NET, DAT
Adrenoceptors	α_{2A}, α_{2B}, α_{2C} β_1, β_2, β_3, α_{1A}, α_{1B}, $\alpha_{1\Delta}$
Cannibinoid	CB_1, CB_2
Muscarinic receptors	M_1, M_2, M_3, M_4, M5
Opioid receptors	MOR, KOR, DOR
Sigma receptors	r$Sigma_1$, r$Sigma_2$
Histamine receptors	H_1, H_2, H_3, H_4

Shown are the human cloned neurotransmitter receptors, ion channels and transporters assayed in this study except where noted; r=Rat.

2.2. Assays

Ki determinations, receptor binding profiles and functional assays were provided by the National Institute of Mental Health's Psychoactive Drug Screening Program essentially as previously described (Roth et al., 2002; Setola et al., 2003; Zolkowska et al., 2009; Jensen et al., 2008). A total of 14 compounds (mephedrone, methiopropamine, methylenedioxy-N-benzylcathinone, 5-APB, 6-APB, 5-Iodo-aminoindane, benzedrone, desoxypipradrol, dimethylamylamine, methylenedioxyaminotetralin, methylenedioxyaminoindane, 1-naphyrone, naphyrone and methylethcathinone) were screened against a total of 49 molecular targets listed in Table 1 initially in quadruplicate at 10 μM concentration (N=686 screening assays). Compounds which yielded inhibition of binding >50% were subjected to Ki determinations via 12-point concentration–response studies in triplicate as described previously (Jensen et al., 2008). Functional assays of potencies of compounds as inhibitors of monoamine uptake at human cloned transporters were determined using the Molecular Devices Neurotransmitter Assay Kit (NET=norepinephrine transporter; DAT=dopamine transporter; SERT=serotonin transporter). h5-HT_{2B} serotonin receptor functional assays were performed as described using a FLIPR^{TETRA} in the 384-well format (Jensen et al., 2008).

3. Results

In keeping with the amphetamine/MDMA-like profiles of these compounds as subjectively reported by human users thirteen of the fourteen compounds tested were active at submicromolar concentrations as inhibitors of substrate uptake by at least one monoamine transporter (the exception being benzedrone—NET Ki=1222 nM; DAT Ki=1411 nM) (Table 2). Overall the compounds were more potent inhibitors of NET and DAT than of SERT. Seven compounds were active at submicromolar concentrations on DAT and NET; nine were active at submicromolar concentrations on DAT, and twelve were active at submicromolar concentrations on NET. Only three compounds (5-APB, 5-iodo-aminoindane and naphyrone) acted as submicromolar inhibitors of SERT. Some of the most potent inhibitors had Ki values <100 nM for DAT inhibition (desoxypipradrol, naphyrone and 1-naphyrone).