Progress in Neuro-Psychopharmacology & Biological Psychiatry 75 (2017) 1–9

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Combined in vitro and in silico approaches to the assessment of stimulant properties of novel psychoactive substances – The case of the benzofuran 5-MAPB

Michelle A. Sahai [a,1], Colin Davidson [b,c,1], George Khelashvili [d], Vincenzo Barrese [b], Neelakshi Dutta [b], Harel Weinstein [d,e], Jolanta Opacka-Juffry [a,*]

[a] University of Roehampton, London SW15 4JD, UK
[b] St George's University of London, Cranmer Terrace, London SW17 0RE, UK
[c] Pharmacy & Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, UK
[d] Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University (WCMC), New York, NY, 10065, USA
[e] HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute of Computational Biomedicine, Weill Cornell Medical College of Cornell University, New York, NY, 10065, USA

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 19 November 2016
Accepted 21 November 2016
Available online 24 November 2016

Keywords:
Addiction
Autoradiography
Dopamine transporter
Legal highs
Molecular modelling
Voltammetry

ABSTRACT

Novel psychoactive substances (NPS) are increasingly prevalent world-wide although their pharmacological characteristics are largely unknown; those with stimulant properties, due to interactions with the dopamine transporter (DAT), have addictive potential which their users may not realise.

We evaluated the binding of 1-(1-benzofuran-5-yl)-*N*-methylpropan-2-amine (5-MAPB) to rat striatal DAT by means of quantitative autoradiography with [^{125}I]RTI-121, and the effects of 5-MAPB on electrically-evoked dopamine efflux by fast-cyclic voltammetry in rat brain slices. 5-MAPB displaced [^{125}I]RTI-121 in a concentration-dependent manner, with significant effects at 10 and 30 μM. The voltammetry data suggest that 5-MAPB reduces the rate of dopamine reuptake; while the peak dopamine efflux was not increased, the area under the curve was augmented. 5-MAPB can also cause reverse dopamine transport consistent with stimulant properties, more similar to amphetamine than cocaine. Molecular modelling and docking studies compared the binding site of DAT in complex with 5-MAPB to dopamine, amphetamine, 5-APB, MDMA, cocaine and RTI-121. This structural comparison reveals a binding mode for 5-MAPB found in the primary binding (S1) site, central to transmembrane domains 1, 3, 6 and 8, which overlaps with the binding modes of dopamine, cocaine and its analogues. Atomistic molecular dynamics simulations further show that, when in complex with 5-MAPB, DAT can exhibit conformational transitions that spontaneously isomerize the transporter into inward-facing state, similarly to that observed in dopamine-bound DAT.

These novel insights, offered by the combination of computational methods of biophysics with neurobiological procedures, provide structural context for NPS at DAT and relate them with their functional properties at DAT as the molecular target of stimulants.

1. Introduction

Among novel psychoactive substances (NPS), benzofurans have a prominent presence in Europe and elsewhere. They are phenethylamine derivatives and related to methylenedioxyphenethylamines such as 3,4-methylenedioxy-methamphetamine (MDMA, Ecstasy) and 3.4-methylenedioxy-amphetamine (MDA), both classified as Class A drugs in the U.K. Benzofuran analogues of MDA were first synthesised in the early 1990s (Monte et al., 1993) and became prevalent as recreational drugs, also known as 'legal highs', nearly two decades later. They were permanently banned as Class B substances in the U.K. in 2014 (The Misuse of Drugs Act 1971 (Ketamine etc.) (Amendment) Order 2014, 2014).

Benzofurans, similarly to MDA and MDMA, evoke both empathogenic (entactogenic) and stimulant effects in users, according to anecdotal reports. While entactogenic properties are those which users value for the type of drug-related experience, the stimulant element associates with addiction liability that the user may not realise. Stimulant drugs normally increase dopamine availability in the brain, including the dorsal and ventral striatum, the latter being part of the mesolimbic pathway implicated in the reward-related behaviour (Di

* Corresponding author at: Department of Life Sciences, University of Roehampton, London SW15 4JD, UK.
E-mail address: j.opacka-juffry@roehampton.ac.uk (J. Opacka-Juffry).
[1] Joint first authors.

http://dx.doi.org/10.1016/j.pnpbp.2016.11.004
0278-5846/

Chiara and Bassareo, 2007). It has been convincingly demonstrated that dopamine release in the nucleus accumbens of this pathway associates with the perception of pleasure and reward; it also contributes to the biological underpinnings of drug dependence (Di Chiara and Imperato, 1988). The dopamine transporter (DAT) is the molecular target for stimulants, responsible for their dopamine-enhancing effects, as originally proposed for cocaine that binds at DAT and inhibits dopamine re-uptake thus potentiating dopaminergic neurotransmission in mesolimbic/mesocortical pathways (Kuhar et al., 1991).

We have previously examined a benzofuran, 5-APB (5-(2-aminopropyl)benzofuran, 1-benzofuran-5-ylpropan-2-amine, benzofury), and found it to bind to the DAT as shown through a displacement of [^{125}I]RTI-121 from the DAT in the striatal and cortical areas of rat brain, apart from its activity at 5-HT$_{2A}$ receptors (Dawson et al., 2014). 5-APB also caused an increase in electrically evoked dopamine efflux, and at high concentrations caused reverse transport of dopamine in rat accumbens brain slices (Dawson et al., 2014). Another study using cell lines has demonstrated that 5-APB binds to other members of the neurotransmitter: sodium symporter (NSS) family of monoamine transporters, with greater affinity at the DAT > serotonin transporter (SERT) > norepinephrine transporter (NET) (Iversen et al., 2013). In contrast, 5-APB has been found to have a higher preference to the SERT than DAT in human embryonic kidney cells (Rickli et al., 2015). The pKi of 5-APB and MDMA at human cloned monoamine transporters have been determined: 5-APB: 6.33; 6.30; 5.78, (−)MDMA: 5.50; 5.21; 5.17, (+)MDMA: 5.70; 5.62; 5.56 for the NET, DAT and SERT respectively (Iversen et al., 2013). To our knowledge the pKi for 5-MAPB at monoamine transporters has not yet been determined although this drug has been shown to displace monoamine radioligands: at 10 μM 5-MAPB displaced 33% 3H-nisoxetine (NET), 73% 3H-BTCP (DAT) and 75% 3H-imipramine (SERT) (Shimshoni et al., 2016).

For the present study, we have chosen 1-(1-benzofuran-5-yl)-*N*-methylpropan-2-amine (5-MAPB) which is a *N*-methylated version of 5-APB, structurally related to MDMA (Welter et al., 2015a, 2015b). Online drug user fora suggest that 5-MAPB, despite its illegality, is still being used today. Its psychoactive effects include euphoria, visual distortions and reduced inhibitions. Inexperienced users appear to feel that 5-MAPB is similar to MDMA, while experienced users feel that it is quite unique, with less empathy than one might associate with MDMA and reduced sexual dysfunction versus MDMA. Unwanted effects include hyperthermia, tachycardia, restlessness, inability to sleep, nystagmus, pupil dilation and jaw tension. Some users suggest taking 5-MAPB with a psychedelic dissociative or alcohol to enhance the psychoactive effects, or a benzodiazepine to reduce unwanted side-effects and help with sleep (Erowid; Bluelight; The Trip Report). On firmer scientific grounds, the National Program on Substance Abuse Deaths (np_SAD report, 2014) in the UK has reported nine instances of benzofurans found in post mortem toxicology in the UK in 2012 (the latest figures) with benzofurans being cited as the actual cause of death in seven of these cases (np_SAD report, 2014).

Here, we aim to assess the stimulant properties of 5-MAPB through in vitro and in silico tests that include the evaluation of drug binding to rat striatal DAT by means of quantitative autoradiography with [^{125}I]RTI-121 as a selective DAT ligand (Hume et al., 1996). We also use a functional test measuring electrically evoked dopamine efflux in the nucleus accumbens, as the brain region involved in reinforcement and addiction, by means of fast cycling voltammetry. We augment our ligand binding and functional data with structural results from computational molecular modelling, docking studies and atomistic molecular dynamics simulations, which have been previously shown to be robust methods for predicting the binding sites of various psychostimulants at human DAT (hDAT) in relation to the substrate binding site (Beuming et al., 2008; Bisgaard et al., 2011). We present here, on the basis of the crystal structure from *Drosophila melanogaster* dopamine transporter (dDAT) (PDB ID: 4XP1) (Wang et al., 2015), a molecular model of 5-MAPB bound to rat DAT (rDAT), in comparison with that of dopamine, amphetamine, 5-APB, MDMA, cocaine and RTI-121 also bound to rDAT. Our studies compare the structural changes produced by various ligands binding to rDAT to the already ongoing work in hDAT models (Hamilton et al., 2013; Hansen et al., 2014; Khelashvili et al., 2015a, 2015b) since it is the primary animal model used in functional studies and pre-clinical drug abuse testing.

We propose here that such a structural analysis is important when creating a stimulant profile of NPS as it adds to the insights of binding and functional data derived from the so-far used neurobiological techniques, autoradiography and voltammetry, applied in animal models. It is important to understand the molecular determinants of stimulant NPS actions, which may underlie their distinct pharmacological effects at DAT.

2. Methods

2.1. Animals

Eight week old male Wistar rats (Charles River, UK) were kept on a 12/12 h light/dark cycle (lights on at 7 AM). Food and water were freely available and rats were housed 6 per cage. Temperature and humidity were 18–22 °C and 55 ± 15% respectively. Rats were treated in accordance with the U.K. Animals (Scientific Procedures) Act 1986 (related to the 1986 EU Directive 86/609/EEC) and sacrificed by cervical dislocation with no anaesthesia. In the ligand binding studies we used 6 rats. For the voltammetry studies we used approximately 20 rats to ensure that each concentration of 5-MAPB was tested in a bran slice from a different rat.

2.2. Chemicals

All chemicals used were supplied by Sigma Chemicals (Poole, UK) except 5-MAPB which was a gift from John Ramsey (TICTAC Communications Ltd., UK). The radioligand for the dopamine transporter, [^{125}I]RTI-121 (specific activity 81.4TBq/mmol) was purchased from Perkin Elmer (Beaconsfield, U.K.).

2.3. Radioligand binding study at the dopamine transporter

Brains were removed and frozen at −40 °C in a mixture of methanol and dry ice, then stored at −80 °C. Frozen brains were cut into 20 μm coronal sections to harvest the striatum at +1.7 mm to −0.3 mm versus bregma (Paxinos and G Watson, 2007). Serial striatal sections were collected onto polysine-coated slides and stored at −80 °C prior to autoradiography. The autoradiography procedure was conducted according to Strazielle et al., 1998. After preincubation in 0.05 M NaPB pH 7.4, sections were incubated with 20 pM [^{125}I]RTI-121 in NaPB pH 7.4 with increasing concentrations of 5-MAPB (0–30 μM) for 60 min at room temperature. Non-specific binding was assessed in the presence of 200 μM nomifensine ('block' - control). Rinsed and air-dried slides were apposed to Kodak BioMax MR films for 3 days; autoradiograms were analysed using MCID™, Version 7.0, Imaging Research Inc., Interfocus Ltd., U.K. Flat-field correction was applied. The caudate regions of interest were sampled in duplicates for relative optical density; left and right caudate values were averaged and their means were calculated to assess the specific binding.

2.4. Brain slice preparation for functional neurochemistry studies

The brain was removed and cut to produce a block containing the accumbens (−5 to +3 mm vs bregma (Paxinos and G Watson, 2007)) which was attached to the chuck of a vibratome, using cyanoacrylate glue, then immersed in ice-cold artificial cerebro-spinal fluid (aCSF; pH 7.4). Coronal slices (400 μm) at the level of the accumbens (+1.0 to +2.2 mm versus bregma) were transferred to a slice saver where the slices were immersed in aCSF continuously bubbled with 95% O_2/