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Stimulant mechanisms of cathinones — Effects of mephedrone and other cathinones on basal and electrically evoked dopamine efflux in rat accumbens brain slices

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ABSTRACT

Mephedrone, an erstwhile "legal high", and some non-abused cathinones (ethcathinone, diethylpropion and bupropion) were tested for stimulant effects *in vitro*, through assessing their abilities to increase basal and electrically evoked dopamine efflux in rat accumbens brain slices, and compared with cocaine and amphetamine. We also tested mephedrone against cocaine in a dopamine transporter binding study.

Dopamine efflux was electrically evoked and recorded using voltammetry in the rat accumbens core. We constructed concentration response curves for these cathinones for effects on basal dopamine levels; peak efflux after local electrical stimulation and the time-constant of the dopamine decay phase, an index of dopamine reuptake. We also examined competition between mephedrone or cocaine and [^{125}I]RTI121 at the dopamine transporter.

Mephedrone was less **potent** than cocaine at displacing [^{125}I]RTI121. Mephedrone and amphetamine increased basal levels of dopamine in the absence of electrical stimulation. Cocaine, bupropion, diethylpropion and ethcathinone all increased the peak dopamine efflux after electrical stimulation and slowed dopamine reuptake. Cocaine was more potent than bupropion and ethcathinone, while diethylpropion was least potent. Notably, cocaine had the fastest onset of action.

These data suggest that, with respect to dopamine efflux, mephedrone is more similar to amphetamine than cocaine. These findings also show that cocaine was more potent than bupropion and ethcathinone while diethylpropion was least potent. Mephedrone's binding to the dopamine transporter is consistent with stimulant effects but its **potency** was lower than that of cocaine. These findings confirm and further characterize stimulant properties of mephedrone and other cathinones in adolescent rat brain.

1. Introduction

Mephedrone (4-methylmethcathinone) is a synthetic cathinone made primarily in China and South East Asia (Wood and Dargan, 2012), and a former "legal high" or "research chemical". The recreational use of mephedrone (also known as MCAT, bubbles and miaow miaow) peaked in 2010 (Deluca et al., 2012) and was controlled by the UK government in April 2010 after a review of this drug by the Advisory Council on the Misuse of Drugs (ACMD). In their report "Consideration of the cathinones" the ACMD, focused on mephedrone as the most commonly encountered drug amongst all the cathinone derivatives identified in the UK, and equated the harms associated with cathinones to be similar to class B compounds **e.g. amphetamine, cannabis, codeine and methylphenidate** (ACMD, 2010). Mephedrone is still commonly found in pills or powders, which are no longer "legal highs" but more accurately described as new or novel psychoactive substances, and has led to a number of medical emergencies (Wood and Dargan, 2012). Thus despite its UK ban, it is still abused in the UK, Europe and the USA (Spiller et al., 2011).

Although mephedrone was synthesized for the first time almost 100 years ago, there is limited information on its pharmacokinetics and pharmacodynamics. It is known that mephedrone causes sympathomimetic effects such as dilated pupils, tachycardia and hypertension in human abusers (Sivagnanam et al., 2013; Wood and Dargan, 2012; Wood et al., 2010) who also experience the "desired" effects of euphoria and general stimulation (Kapitány-Fövény et al., 2013), consistent with stimulant properties. Following the recent media attention regarding the abuse of this drug and suspected mephedrone-related deaths (Schifano et al., 2012), there have been a few studies on the neurochemistry of

Abbreviations: aCSF, artificial cerebrospinal fluid; MDMA, 3,4-methylenedioxy-*N*-methylamphetamine; HEK, human embryonic kidney; ACMD, Advisory Council on the Misuse of Drugs; FCV, fast cyclic voltammetry; ANOVA, analysis of variance; DAT, dopamine transporter; NET, noradrenaline transporter; SERT, serotonin transporter.

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mephedrone. In one of them, where microdialysis was used to measure dopamine and 5-HT in the nucleus accumbens of freely moving rats, it was found that mephedrone increased both dopamine and 5-HT levels, having a greater effect on 5-HT (Kehr et al., 2011). The authors concluded that mephedrone had similar effects to MDMA (ecstasy), but with some amphetamine-like effects. In another report (Baumann et al., 2012) microdialysis was again used to measure dopamine and 5-HT **efflux** in the accumbens, although on this occasion mephedrone had a larger effect on dopamine when compared with 5-HT. Although there is a growing interest in behavioural pharmacology of cathinones (Gregg and Rawls, 2013; Gregg et al., 2013) when it comes to their neurochemistry, apart from the above in *vivo* microdialysis approaches to mephedrone (Baumann et al., 2012; Kehr et al., 2011), cathinones tend to be studied for their actions not in brain tissue but in cell lines (e.g. Arias et al., 2009; Iversen et al., 2013; Simmler et al., 2013) or *Xenopus* oocytes (Cameron et al., 2013) which are not native of brain. Hence there is a need for more understanding of cathinone mechanisms of action using functional neurochemistry in brain tissue; a balance which the present paper aims to help to redress.

In an effort to shed more light on the neurochemical properties of mephedrone and other cathinones, in particular their effects on dopamine efflux as a hallmark of psychostimulant action (e.g. Berridge, 2006), we have examined the effects of mephedrone and selected cathinones on basal and electrically stimulated dopamine efflux in the rat accumbens brain slice and compared them with cocaine and amphetamine. We have used the technique of fast cyclic voltammetry, which allows us to measure dopamine release and reuptake in real-time with sub-second time resolution. Thus we are able to examine the effects of these drugs with greater spatial and temporal resolution than in the microdialysis studies. This approach allows us to examine dopamine efflux in the accumbens core as a sub-region of the striatum directly involved in responses to drugs of abuse (Cardinal et al., 2001) and to assess the effects not only on basal levels of dopamine, but also on electrically evoked dopamine, which is similar to the dopamine release triggered by action potentials during short bursts of midbrain dopamine neurons (Wanat et al., 2009).

Amongst the cathinones there are some not typically abused, for example ethcathinone, diethylpropion and bupropion, which we selected for the present paper. Bupropion (Wellbutrin, Zyban) is clinically used, originally developed as an antidepressant, but now used mostly in smoking cessation (Grandi et al., 2013). Its neurochemistry has been previously examined (Santamaria and Arias, 2010), as has its **reinforcement and discriminative stimulus properties** in animal studies (Lamb and Griffiths, 1990). Diethylpropion (Anorex) is a cathinone used as an appetite suppressant (Cercato et al., 2009) with low abuse potential although instances of abuse do exist (Cohen, 1977; Jasinski and Krishnan, 2009). Ethcathinone, (ethylpropion, *N*-ethylcathinone, 2-ethylaminopropiophenone and *N*-ethylaminopropiophenone (ACMD report, 2010; Rothman and Baumann, 2006) is a cathinone derivative. It is a drug that very little is known about, although it has been sold as an MDMA substitute "legal high" under the names "Neodove" and "Sub Coca" (Camilleri et al., 2010; Prosser and Nelson, 2012) and is a metabolite of diethylpropion.

This study, designed to provide an insight into the mechanisms of the stimulant effects of mephedrone, also intended to establish to what extent the other cathinones affected dopamine release. In particular we examined the effects on basal and evoked dopamine, in the nucleus accumbens core as a brain region directly involved in responses to stimulants.

2. Methods and Materials.

2.1. Animals

We used 8 week old male Wistar (Charles River, UK) rats kept on a 12/12 h light dark cycle (lights on at 7 AM). Food (standard diet RM1) and water were freely available and rats were housed 6 per cage. Temperature and humidity ranges were 18–22 °C and 55 ± 15% respectively. Rats were treated in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and UK Home Office regulations, and killed by cervical dislocation with no anaesthesic. Male adolescent rats were used as human drug-taking typically starts in adolescence; this is especially true for the "legal highs" (new psychoactive drugs) which appear to be taken mostly by adolescent males, at least in the case of mephedrone (Carhart-Harris et al., 2011; Vardakou et al., 2011).

2.2. Radioligand binding study at the dopamine transporter

Rat brains were rapidly removed, frozen at −40 °C and stored at −80 °C. Frozen brains were cut into 20 μm coronal sections in an anterior–posterior direction to harvest the nucleus accumbens according to Paxinos and Watson (2005). Serial accumbal 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, brain sections were incubated with 20 pM [^{125}I]RTI121 in NaPB pH 7.4 with increasing concentrations of mephedrone or cocaine (0–30 μM) for 60 min at room temperature. Non-specific binding was assessed in the presence of 200 μM nomifensine ('block'). 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 accumbens core regions of interest were sampled for relative optical density; left and right accumbens values were averaged and their means were calculated to assess the specific binding. Brain sections of inferior quality were excluded from quantitative analysis and therefore n = 5 **rats** for mephedrone and n = 6 **rats** for cocaine in Fig. 1.

2.3. Brain slice preparation for voltammetry studies

The brain was rapidly removed to produce a block containing the accumbens (from approximately −5 to +3 mm versus bregma (Paxinos and Watson, 2005) which was attached to the chuck of a vibratome, using cyanoacrylate glue, and immersed in the bath containing 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 cut and transferred to a slice saver, comprised of a plastic mesh suspended in a 500 ml container of aCSF continuously bubbled with 95% O_2/5% CO_2. Once cut, slices were left to equilibrate for 1–4 h at room temperature (22 ± 1 °C), to allow recovery from any trauma associated with slicing. Following equilibration, slices were transferred to the brain slice chamber, which was heated by a thermostatically controlled circulating water bath (Grant, Cambridgeshire, UK). The slice chamber was superfused continuously with aCSF (100 ml/h) and the temperature in the slice chamber was measured before and after each experiment with a CEM DT-613 digital thermometer with K-type thermocouple probe. The temperature in the brain slice chamber was 32.5 ± 0.5 °C. The slice was equilibrated for 45 min prior to electrical stimulation or application of amphetamine or mephedrone (non-stimulated experiments, see Fig. 3). The composition of aCSF was: (mM): NaCl (126.0), KCl (2.0), KH_2PO_4 (1.4), $MgSO_4$ (2.0), $NaHCO_3$ (26.0), $CaCl_2$ (2.4), (+)glucose (10.0), bubbled for at least 60 min with 95% O_2/5% CO_2.

2.4. Fast cyclic voltammetry (FCV)

Carbon fibre electrodes were made by inserting a 7 μm diameter carbon fibre into a 10 cm length borosilicate glass capillary (o.d., 2.0 mm; i.d., 1.16 mm: Harvard Apparatus LTD, Kent, UK), which was pulled to a fine tip using an electrode puller (Model P-30, Sutter Instruments Co, USA), such that the carbon fibre protruded from the pulled tip. The

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