Neuroscience 146 (2007) 321–329

THE EFFECTS OF CO-ADMINISTRATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE ("ECSTASY") OR PARA-METHOXYAMPHETAMINE AND MOCLOBEMIDE AT ELEVATED AMBIENT TEMPERATURES ON STRIATAL 5-HT, BODY TEMPERATURE AND BEHAVIOR IN RATS

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Abstract—We have recently demonstrated that co-administration of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") with the reversible monoamine oxidase type A (MAO-A) inhibitor moclobemide at an ambient temperature of 22 °C significantly increases striatal 5-HT outflow and 5-HT-mediated behaviors. In the present study, using microdialysis, we examined the effects of co-administration of MDMA or para-methoxyamphetamine (PMA) with moclobemide on striatal 5-HT outflow at the elevated ambient temperatures of 30 °C. Samples were collected every 30 min for 4 h and analyzed by high-performance liquid chromatography assay with electrochemical detection (HPLC-ED). 5-HT-mediated effects on body temperature and behavior were also recorded. Rats were treated with either saline or 20 mg/kg (i.p.) moclobemide, followed by 10 mg/kg (i.p.) MDMA, 10 mg/kg (i.p.) PMA or saline 60 min later. Both MDMA and PMA produced significant increases in 5-HT outflow (370% peak and 309% peak, respectively, $P<0.05$). MDMA and PMA significantly increased body temperature (+2.0 °C and +2.1 °C, respectively, $P<0.01$) and drug-related behaviors ($P<0.05$). When MDMA or PMA was co-administered with moclobemide, additional significant increases were seen in 5-HT outflow (850% peak, $P<0.01$ and 1450% peak, $P<0.001$, respectively) and only MDMA showed additional significant increase in body temperature (+5.0 °C, $P<0.001$). No additional increases were seen in behavioral activity. When moclobemide was co-administered with MDMA, sustained increases in body temperature were recorded that were significantly higher than with MDMA alone and such increases were not observed in our previous study at normal room temperature. Our results suggest greater risk of MDMA-induced adverse effects on body temperature regulation, compared with PMA, when used in combination with moclobemide at elevated ambient temperatures.

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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; AUC, area under the curve; DA, dopamine; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; MAO-A, monoamine oxidase type A; MDMA, 3,4-methylenedioxymethamphetamine; Moc, moclobemide; PMA, para-methoxyamphetamine; Sal, saline; S.E.M., standard error of mean; 5-HIAA, 5-hydroxyindoleacetic acid.

Key words: hyperthermia, neurotoxicity, 3;4-methylenedioxymethamphetamine, para-methoxyamphetamine, monoamine oxidase-A inhibitor.

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a widely used drug among teenagers and young adults especially by those who attend dance parties and nightclubs. In Europe, typically 80–96% of clubbers have used "ecstasy" at least once (Parrott, 2004) and one of five Australian teenagers report "ecstasy" use within the last week (Degenhardt et al., 2004). Para-methoxyamphetamine (PMA), a drug related to MDMA, has been responsible for many fatalities in Australia and is taken by people who believe they are administering ecstasy. PMA is associated with greater incidence of adverse effects and death compared with MDMA (Cimbura, 1974; Ling et al., 2001; Caldicott et al., 2003).

Both MDMA and PMA increase 5-HT outflow (Gudelsky and Nash, 1996; Gough et al., 2002; Freezer et al., 2005), block its re-uptake (Daws et al., 2000; Callaghan et al., 2005) and disrupt physiological and behavioral thermoregulation (Gordon et al., 1991; Blessing et al., 2003; Jaehne et al., 2005). These drugs can also cause hyperthermia in rats (Gordon et al., 1991; Mechan et al., 2002; Brown and Kiyatkin, 2004; Saadat et al., 2005) and humans (Henry et al., 1992; Ling et al., 2001; Gowing et al., 2002; Caldicott et al., 2003; Freedman et al., 2005). Hyperthermia, associated with elevated 5-HT outflow, is thought to be the main contributor to the adverse effects and subsequent fatalities attributed to these drugs (Lin et al., 1998; Shankaran and Gudelsky, 1999).

It has also been found that PMA is 100 times more potent than MDMA *in vitro* as an inhibitor of monoamine oxidase type A (MAO-A) (Scorza et al., 1997; Gallardo-Godoy et al., 2005), the enzyme responsible for the breakdown of 5-HT, leading to the formation of 5-hydroxyindoleacetic acid (5-HIAA). This effect of PMA may contribute to the elevated 5-HT outflow and may partially explain why PMA has a greater *in vivo* toxicity than MDMA (Daws et al., 2000). This proposed mechanism is supported by Vuori et al. (2003) who reported four deaths in Finland which were attributed to the co-administration of MDMA with the antidepressant moclobemide (Moc), a potent reversible MAO-A inhibitor. Our own data indicate that the combined Moc and "ecstasy" use in humans is not con-

0306-4522/07$30.00+0.00
doi:10.1016/j.neuroscience.2007.01.012

fined to Finland (Irvine et al., 2006) and may be quite widespread.

We have recently investigated whether co-administration of MDMA or PMA with Moc significantly increases striatal 5-HT outflow and 5-HT-mediated behaviors in conscious rats at an ambient temperature of 22 °C (Freezer et al., 2005). Although both PMA and MDMA significantly increased 5-HT outflow, only MDMA was capable of producing additional significant increases in 5-HT outflow when co-administered with Moc (Freezer et al., 2005). However, we did not find significant changes in rat body temperature in any treatment group. Examination of the current literature shows there is conflicting evidence as to the effects of these drugs on body temperature but it is known that the effects are dependent on ambient temperatures (Malberg and Seiden, 1998; Gordon et al., 1991; O'Shea et al., 2005). In the present study we measured changes in 5-HT and 5-HIAA outflow in rat striatum after co-administration of Moc with MDMA or PMA at increased ambient temperature of 30 °C. Animal behavior was simultaneously observed and core body temperature was measured at 30 min intervals. Experiments were conducted at ambient temperature of 30 °C in order to mimic the hot conditions that drug users may be exposed to at nightclubs (Parrott, 2004, 2006).

It was hypothesized that at an elevated ambient temperature of 30 °C, both MDMA and PMA will significantly increase striatal 5-HT outflow and 5-HT-mediated behavior and body temperature. Co-administration with Moc was expected to increase 5-HT outflow and 5-HT-mediated behavior and body temperature to an even greater extent.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats were used, weighing 250–300 g and kept on a 12- h light/dark cycle (lights on at 07:00 h, temperature maintained at 22 °C) with free access to food and water. All animals were supplied by the Adelaide University Laboratory Animal Service (Adelaide, SA, Australia). Ethical approval was given by the University of Adelaide Ethics Committee, and all procedures were in strict accordance with the National Health and Medical Council of Australia Guidelines for the Care and Use of Laboratory Animals. All experiments conformed to international guidelines on the ethical use of animals and were designed to minimize the number of animals used and their suffering.

Brain microdialysis

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p. injection) in 0.9% saline (Sal) and placed on a water-heated pad (38 °C) to maintain core body temperature. Once fully anesthetized, the animal's head was secured in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA), the skull was exposed, bregma located and an intracerebral cannula and guide stylet (BAS MD-2251, Bioanalytical Systems Inc., West Lafayette, IN, USA) was implanted into the striatum. The coordinates of the striatum (caudate putamen) are A: +1.2 mm and L: +2.2 mm relative to bregma and V: −5.5 mm below the skull. These coordinates were referenced from a rat brain atlas (Paxinos and Watson, 1986). The guide cannula (BAS MD-2251) was implanted and held in place with dental cement (Vertex, Dentimex BV HJ, Zeist, Netherlands). Following guide cannula implantation, animals were allowed to recover on the water-heated pad until regaining consciousness and then placed in a clear Perspex observation bowl. A further 24 h recovery was allowed in a separate room before commencement of the microdialysis sampling and during this period animals were handled and habituated to the microdialysis testing environment. A recovery period of 24 h is considered as satisfactory for microdialysis studies, as neurotransmitter levels are stabilized and interference due to surgery and anesthesia is minimal (Westerink, 2000; Esteban et al., 2001; O'Shea et al., 2005).

Following the 24 h recovery period, animals were lightly restrained by hand and the microdialysis probe was inserted through the guide cannula into the striatum. The animals were placed in a room where the ambient temperature was maintained at 30±1 °C. Relative humidity was 27±6% over the period of the experiment, as measured using a data-logging humidity meter (Tinytag, Gemini Data Loggers UK Ltd., Chichester, UK). The probe was perfused at a flow rate of 1.5 μl/min using a BAS pump with artificial cerebrospinal fluid (aCSF) (in mM): NaCl 125, KCl 2.5, $MgCl_2.6H_2O$ 1.18, Na_2HPO_4 2 and $CaCl_2.2H_2O$ 1.2 and adjusted to pH 7.4 with 2 M HCl and 0.1 M NaOH. Samples were collected every 30 min into 1.5 ml Eppendorf tubes containing 10 μl of 2% acetic acid for 4.5 h. The first 60 min samples following probe insertion were discarded and the next two 30 min samples were used as baseline samples and defined as 100%. Changes in 5-HT and 5-HIAA concentrations following Sal, MDMA or PMA administration were expressed as percentages of the mean pre-drug baseline values. To determine the *in vitro* recovery of the probe, it was placed into a solution of 10 ng/ml 5-HT and 5-HIAA and perfused with aCSF at 1.5 μl/min.

HPLC with electrochemical detection

The samples were immediately analyzed for 5-HT and 5-HIAA using a high-performance liquid chromatography (HPLC) assay with electrochemical detection (HPLC-ED) as previously described (Freezer et al., 2005). Briefly, the system consisted of a controller (Shimadzu LC-10AD; Shimadzu, Kyoto, Japan) degasser (Shimadzu DGU-14AD) and BAS LC-4B (Bioanalytical Systems) fitted with a working electrode potential set at 0.7 V with a range of 0.1 nA. The mobile phase was composed of (in mM): NaH_2PO_4 100, octanesulfonic acid 1, EDTA 0.1 and 12% methanol and was adjusted to pH 2.9 with phosphoric acid. The mobile phase was filtered, degassed and delivered at a flow rate of 0.07 ml/min. Compounds of interest were separated using 100×1.0 AD Luna 3 μ c^{18} column (Phenomenex, Torrance, CA, USA) and sampling was recorded using an ICI DP800 Chromatograph Data station (Version 2.5; ICI Instruments, Melbourne, Australia).

Drug treatments

MDMA, PMA and Moc were dissolved in 0.9% Sal solution and administered i.p. At 60 min post-microdialysis probe insertion, animals were injected with either 20 mg/kg Moc or Sal and at 120 min after probe insertion received 10 mg/kg MDMA, 10 mg/kg PMA or Sal. Doses of MDMA and PMA used in our study are based on previous multiple dose microdialysis studies in our laboratory and by other workers (Gough et al., 2002; Freezer et al., 2005) demonstrating similar changes in 5-HT and 5-HIAA outflow and reliable changes in body temperature (Dafters and Lynch, 1998; Malpass et al., 1999: Daws et al., 2000; Freezer et al., 2005). In addition, the dose of MDMA used in this study has been shown to result in similar plasma concentrations of the drug as has been reported in human cases of hyperthermia (Colado et al., 1995; Chu et al., 1996; Brown and Kiyatkin, 2004). The dose of Moc used in our study and 1 h pretreatment period prior to MDMA and PMA administration were based on its reported half-life in the rat and effects on 5-HIAA concentrations (Da Prada et al., 1989; Colzi et al., 1992; Oie et al., 1992; Iurlo et al., 2001).