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Effect of repeated ('binge') dosing of MDMA to rats housed at normal and high temperature on neurotoxic damage to cerebral 5-HT and dopamine neurones

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Abstract

The technique of 'binge' dosing (several doses in one session) by recreational users of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) requires evaluation in terms of its consequences on the acute hyperthermic response and long-term neurotoxicity. We examined the neurotoxic effects of this dosing schedule on 5-HT and dopamine neurones in the rat brain. When repeated (three) doses of MDMA (2, 4 and 6 mg/kg i.p.) were given 3 h apart to rats housed at 19 °C, a dose-dependent acute hyperthermia and long-term loss of 5-HT was observed in several brain regions (hippocampus, cortex and striatum), with an approximate 50% loss following 3 × 4 mg/kg and 65% decrease following 3 × 6 mg/kg. No decrease in striatal dopamine content was detected. When MDMA (4 mg/kg i.p.) was given repeatedly to rats housed at 30 °C, a larger acute hyperthermic response than that observed in rats treated at 19 °C environment was seen (maximum response 2.6 ± 0.1 °C versus 1.3 ± 0.2 °C). A long-term cerebral 5-HT loss of approximately 65% was also detected in both the cortex and hippocampus, but no loss in striatal dopamine content occurred. These data emphasize the increased acute hyperthermic response and neurotoxicity which occurs when MDMA is administered in a hot room environment compared to normal room temperature conditions, and support the view that MDMA is a selective 5-HT neurotoxin, even when a binge dosing schedule is employed and the rats are present in a hot environment.

Keywords

binge dosing, dopamine, ecstasy, 5-hydroxytryptamine, hyperthermia, 3,4-methylenedioxymethamphetamine, room temperature

Introduction

There is a widely held view that 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a selective 5-HT neurotoxin. This view has been generated by data obtained in both rats and primates (Green *et al*., 2003). Nevertheless there have been a few studies suggesting that MDMA may have a modest effect on the integrity of dopamine nerve endings in the brains of rats when more 'extreme' dosing conditions are applied (e.g. very high doses; 20 mg/kg every 12 h for 4 days) (Commins *et al*., 1987) or administration of the drug at the high ambient room temperature of 27 °C (Yuan *et al*., 2002).

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A practice sometimes employed by recreational users of ecstasy is 'binge' dosing, comprising the ingestion of several doses on a single occasion (Parrott, 2002; Weir, 2000). This type of dosing regime has been modelled in a recent preclinical study (Badon *et al*., 2002) and was utilized by us to examine how such a regime influences the effect of MDMA on acute hyperthermia in rats (Green *et al*., 2004). We report on the consequences of such dosing schedules on the longer-term neurotoxic effects of MDMA when it is administered to rats housed either at 19 °C or in a room kept at 30 °C to simulate the way the drug is sometimes taken by human recreational users in a hot, dance club environment. We examined whether binge dosing and a hot environment would result in MDMA producing a greater loss in cerebral 5-HT content than that produced in animals dosed in normal room temperature conditions, and also whether it would produce neurotoxic damage to dopamine nerve endings.

Methods

Animals and drug administration

Male Dark Agouti (DA) rats, weighing 200–260 g, were used in all experiments (Harlan UK Ltd, Bicester, Oxon, UK and Interfauna, Barcelona, Spain). The animals were housed in groups, at an ambient temperature of 20 ± 2 °C and on a 12 : 12 h light/dark cycle (lights on 07.30 h). Both food and water were available *ad libitum*.

All procedures were carried out following approval by the University Experimental Ethics Committee and in accordance with the guidelines of the United Kingdom Home Office and the Animal Welfare Committee of the Universidad Complutense (following DC86/609/EU), which both ensure the humane and proper care of research animals. The studies in normothermic room conditions were conducted at Universidad Complutense de Madrid, Spain, and the studies using high ambient room conditions were performed at De Montfort University, Leicester, UK.

(±)-MDMA obtained from either Dr P. Guiry, University College, Dublin or NIDA (Research Triangle Park, NC, USA) was dissolved in 0.9% w/v saline and injected intraperitoneally (i.p.). The acute hyperthermic response and the long-term neurotoxic effect of MDMA injection examined doses of 2, 4 or 6 mg/kg. In the study on the effect of MDMA in rats housed at high ambient temperature, a dose of 4 mg/kg was chosen because previous experience (Green *et al*., 2004) suggested this to be the highest dose possible to administer without fatality. The results obtained indicate the accuracy of this decision. All studies were conducted with groups of rats (six per group), both to simulate crowded dance club conditions and because amphetamines produce more marked behavioural effects in grouped animals (Chance, 1947; Morton *et al*., 2001; Fantegrossi *et al*., 2003). Ambient room temperature of the procedures room was maintained at 19–21 °C (subsequently referred to as 19 °C in the text) or at 30–32 °C (referred to as 30 °C) during studies on the hyperthermic effect of MDMA administration. Animals were housed in these conditions for 1 h before the first drug or vehicle administration, and for 7.5 h thereafter. Animals were then returned to the holding room. Rats were only ever used once in the investigations. The rectal temperature data for the study on animals housed at 19 °C were published as part of a previous report (Green *et al*., 2004). Here, we report on the biochemical studies performed on these rats together with further investigations on the temperature and biochemical consequences of administering MDMA at high dose to animals housed at 30 °C.

Temperature measurement

Temperature measurement was performed using an MC 8700 thermometer, with digital readout, and a H-RB3 rectal temperature probe (EXACON A/S, Roskilde, Denmark) lubricated with lanolin hand cream. Each rat was lightly restrained by hand for approximately 20 s while the probe was inserted approximately 2.5 cm into its rectum and a steady reading was obtained.

Measurement of monoamines and their metabolites in cerebral tissue

Seven days after MDMA administration, animals were killed by cervical dislocation and decapitation, the brains rapidly removed and cortex, hippocampus and striatum dissected out on ice. Tissue was homogenized and 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) measured by high-performance liquid chromatography (HPLC). Catechol concentration was only determined in the striatum because the majority of monoaminergic terminals are dopaminergic in this area. Briefly, the mobile phase consisted of KH_2PO_4 (0.05 M), octanesulfonic acid (0.16 mM), ethylenediaminetetraacetic acid (0.1 mM) and methanol (16%), and was adjusted to pH 3 with phosphoric acid, filtered and degassed. The flow rate was 1 ml/min and the working electrode potential was set at +0.8 V.

The HPLC system consisted of a pump (Waters 510) linked to an automatic sample injector (Loop 200 µl, Waters 717 plus autosampler), a stainless steel reversed-phase column (Spherisorb ODS2, 5 µm, 150 × 4.6 mm) with a precolumn and a coulometric detector (Coulochem II, Esa Inc, Chelmsford, MA, USA). The current produced was monitored by using integration software (Unipoint, Gilson, Middleton, WI, USA).

Analysis of data

All studies measured rectal temperature in groups of rats. Rats were injected with saline or MDMA and the temperature was measured at various times thereafter. The rectal temperature of the MDMA treated rat was subtracted from the mean value of the temperature of the saline-injected control group at each time point thereby providing a mean ± SEM value of the MDMA-induced temperature change. Statistical analyses of the temperature measurements were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Cork, Eire). Data were analysed by analysis of variance (ANOVA) with an

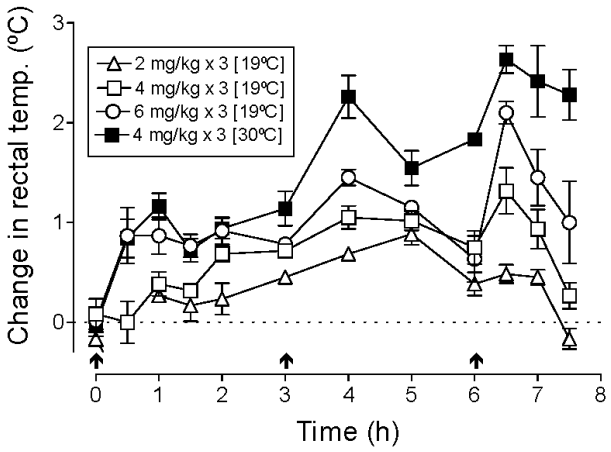

Figure 1 The rectal temperature response of rats given MDMA at the doses shown at 3-h intervals (marked by arrows on graph) when housed at either 19 °C or 30 °C. The rectal temperature data on animals housed at 19 °C was previously published in Green *et al.* (2004)

unbalanced repeated measures (program 5V). The statistical analysis was carried out by an initial two-way repeated measures ANOVA (time × treatment group) on the four treatment groups to determine an overall effect of treatment. Subsequently, separate two-way repeated measures ANOVAs (time × treatment group) were carried out between pairs of treatments to determine individual treatment differences.

Data from the biochemical results were analysed using one-way ANOVA plus a Bonferroni corrected *t*-test.

Results

Effects of repeated doses of MDMA on rectal temperature

The difference in rectal temperature of rats given three doses of MDMA (2, 4 or 6 mg/kg) when housed in 19 °C and MDMA (3× 4 mg/kg) when housed at 30 °C is shown in Fig. 1. There was an overall effect of treatment [$F(3,19) = 153.75$, $p < 0.001$]. Repeated doses of MDMA of 4 mg/kg produced a hyperthermic response which was greater than that produced by 2 mg/kg [$F(1,10) = 26.24$, $p < 0.01$]. The hyperthermia following the dose of 4 mg/kg was enhanced at the dose of 6 mg/kg [$F(1,10) = 7.56$, $p < 0.01$]. Administration of repeated doses of MDMA (4 mg/kg × 3) to rats housed at 30 °C produced a severe hyperthermic response that was greater than that seen in animals housed at 19 °C and administered repeated doses of either 4 mg/kg [$F(1,9) = 67.24$, $p < 0.001$] or 6 mg/kg [$F(1,9) = 10.35$, $p < 0.01$] (Fig. 1).

Effect of repeated doses of MDMA on brain monoamine concentrations

MDMA given to rats housed at 19 °C produced a dose-dependent decrease in the content of 5-HT in all three brain regions examined with a similar profile in the loss of the major metabolite 5-HIAA (Table 1). No change was seen in the concentration of striatal dopamine or its metabolites (Table 1).

MDMA (4 mg/kg) given repeatedly to rats housed at 30 °C produced a similar loss in 5-HT concentration to that seen following MDMA (6 mg/kg) given to rats housed at 19 °C in both the cortex and hippocampus. The striatal 5-HT loss was modest. Again, no alteration in the concentration of striatal dopamine or its metabolites was observed (Table 1).

Table 1 The percentage of concentration of 5-HT, dopamine and their metabolites in MDMA-treated rats compared to an appropriate saline-injected control group

			Percent of concentration of compound in control group			
		Room temperature:	19 °C			30 °C
Brain region	Measured	MDMA dose (3 × mg/kg)	2	4	6	4
Cortex	5-HT		82 ± 5	54 ± 10*	38 ± 2**	36 ± 11**
	5-HIAA		93 ± 5	64 ± 10*	42 ± 3**	62 ± 11*
Hippocampus	5-HT		61 ± 5**	40 ± 2**	28 ± 3**	29 ± 2**
	5-HIAA		92 ± 4	47 ± 5**	26 ± 3**	37 ± 8**
Striatum	5-HT		83 ± 3	53 ± 4**	35 ± 4**	81 ± 16
	5-HIAA		93 ± 3	68 ± 6*	47 ± 3**	73 ± 13
	Dopamine		109 ± 2	107 ± 5	117 ± 5	99 ± 8
	HVA		104 ± 4	108 ± 7	112 ± 7	126 ± 13
	DOPAC		118 ± 4	115 ± 5	111 ± 8	108 ± 12

All data are reported as a percentage of the concentration of monoamine or metabolite in a group of MDMA-treated rats (n = 4–6) compared to the mean value found in appropriate saline-injected animals. Data are reported as mean ± SEM. Statistical evaluation was performed on absolute monoamine concentrations before conversion to percentage values. The results are shown as the difference compared to saline-injected control group: *$p < 0.01$, **$p < 0.001$.

Discussion

These data confirm previous observations indicating that MDMA administration to rats housed in high ambient temperature conditions produces an acute hyperthermic response larger than that seen in rats housed at normal room temperatures (Dafters, 1995; Malberg and Seiden, 1998; Green *et al*., 2004). The hyperthermic response of animals housed at 30 °C and given repeated doses of MDMA (4 mg/kg) was greater than the response induced by repeated doses of either 4 and 6 mg/kg to animals housed at 19 °C.

It has been proposed that the severity of the MDMA-induced acute hyperthermic response in rats is a major determinant of the size of the subsequent long-term neurotoxic damage to 5-HT nerve endings (Broening *et al*., 1995; O'Shea *et al*., 1998; Malberg and Seiden, 1998) and this proposal is supported by the current study. In rats kept in a 19 °C room temperature, the repeated 2 mg/kg dose of MDMA induced a modest hyperthermic response and only produced a significant 5-HT loss in the hippocampus. However, a dose of either 4 and 6 mg/kg produced both a significant acute hyperthermic response and neurotoxicity. Although the loss of 5-HT and 5-HIAA in both the cortex and hippocampus of animals given MDMA (4 mg/kg) when housed at 30 °C was apparently larger than that seen in these brain regions of rats given a dose of 4 mg/kg when housed at 19 °C, this change was not statistically significant. However, it is worth noting that the loss of 5-HT and 5-HIAA in both regions in animals given MDMA (4 mg/kg) when housed at 30 °C was very similar to the loss produced by MDMA (6 mg/kg) given to rats housed at 19 °C. Why the striatal loss was small in the animals housed at high temperature is unknown, but this may be an artefact of this particular experiment given the substantial loss seen in all brain regions in the animals dosed in 19 °C room conditions.

This investigation was initiated in part because of the previous study by Ricaurte *et al*. (2002) suggesting that 'binge' dosing of primates produced damage to dopamine nerve terminals. We wished to determine whether extreme conditions ('binge' dosing and high ambient temperature) would produce a similar change in rats. Following the start of our study, the primate data were retracted following the discovery that there had been an error in the drug supply and that the monkeys had actually been dosed with methamphetamine (Ricaurte *et al*., 2003). Consequently, there is still no evidence that MDMA produces dopamine neurotoxicity in primates.

Our study further demonstrates that even repeated dosing of rats with MDMA when they are housed at high temperature fails to produce dopamine neurotoxicity. This emphasizes that MDMA is also a selective 5-HT neurotoxin in rats and can be added to a recent demonstration of the 5-HT selective neurotoxicity of MDMA in guinea-pigs (Saadat *et al*., 2004). The results further emphasize the point that the MDMA-induced neurotoxicity to dopamine neurones, which has been found to occur in the mouse brain (Stone *et al*., 1987; Logan *et al*., 1988; O'Shea *et al*., 2001), has yet to be demonstrated in any other species.

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