Effects of MDMA on blood glucose levels and brain glucose metabolism

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Abstract
Purpose This study was designed to assess changes in glucose metabolism in rats administered single or repeated doses of MDMA.
Methods Two different experiments were performed: (1) A single-dose study with four groups receiving 20 mg/kg, 40 mg/kg, saline or heat, and (2) a repeated-dose study with two groups receiving three doses, at intervals of 2 h, of 5 mg/kg or saline. Rats were imaged using a dedicated small-animal PET scanner 1 h after single-dose administration or 7 days after repeated doses. Glucose metabolism was measured in 12 cerebral regions of interest. Rectal temperature and blood glucose were monitored.
Results Peak body temperature was reached 1 h after MDMA administration. Blood glucose levels decreased significantly after MDMA administration. In the single-dose experiment, brain glucose metabolism showed hyperactivation in cerebellum and hypo-activation in the hippocampus, amygdala and auditory cortex. In the repeated-dose experiment, brain glucose metabolism did not show any significant change at day 7.
Conclusion These results are the first to indicate that MDMA has the potential to produce significant hypoglycaemia. In addition, they show that MDMA alters glucose metabolism in components of the motor, limbic and somatosensory systems acutely but not on a long-term basis.

Keywords MDMA · PET · FDG · Brain metabolism

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Introduction

The recreational drug 3,4-methylenedioxymethamphetamine (MDMA), also known as "ecstasy", is a synthetic analogue of amphetamine. MDMA produces a characteristic set of psychoactive effects and has the potential to produce untoward neuropsychiatric sequelae in some individuals [1, 2]. Numerous studies also indicate that MDMA has serotonin neurotoxic potential.

Acutely, MDMA acts mainly by increasing the release of serotonin (5-HT), dopamine and noradrenaline from brain monoamine-containing presynaptic terminals [3–5]. It also prolongs the presence of these neurotransmitters in the synaptic cleft by inhibiting monoaminergic reuptake mechanisms and monoamine oxidase activity [6–8]. MDMA abuse has been linked with cognitive and psychopathological disorders, as well as memory impairment [9, 10]. As alluded to above, in a variety of animal species, MDMA produces persistent reductions in presynaptic 5-HT neural markers, including tryptophan hydroxylase, 5-HT itself and its metabolite, 5-hydroxyindoleacetic acid [2, 11–16], and the 5-HT transporter SERT [17]. Although increased monoaminergic release and the neurotoxic effects of MDMA have been widely reported [1, 11, 18–20], relatively few imaging studies of the effect of ecstasy on brain function have been published [9, 21–23].

Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) permit imaging of the serotonergic and dopaminergic systems, as well as blood flow and metabolism, and are ideally suited for studying the potential neurotoxic effects of MDMA in the living brain. Several markers for these systems (SERT, 5-HT_{1A}, 5-HT_{2A}) are currently being investigated [24, 25]. Buchert et al. have proposed that MDMA-induced functional alterations of the serotonergic system may affect the glucose metabolism of cortical and subcortical structures [26]. However, possible effects of MDMA on regional brain glucose metabolism and blood glucose levels have received relatively little attention.

The acute and long-term effects of MDMA on blood glucose levels and cerebral glucose metabolism in vivo in rats have not been previously described. In the present study, two groups of animals were imaged at different times: 1 h after a single MDMA dose, to study brain metabolism during the acute and rapid release of monoamines, and 7 days after MDMA administration, to study the effect of MDMA withdrawal on brain glucose metabolism at a time when a 50% loss in 5-HT and its metabolites has been observed in rats [27]. A preliminary version of these results has been reported [28].

Materials and methods

Animals, drug administration and experimental protocol

Adult female Dark Agouti (DA) rats (Harlan Iberica, Barcelona, Spain) weighing 138–185 g were housed in individual cages at a constant temperature (24±0.5°C) with a 12-h light/dark cycle, and fed with commercial rodent laboratory chow (Letica) and water ad libitum. The animals were deprived of food but allowed free access to water for more than 6 h before the PET scan, as in protocols with humans. All animal procedures were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Institutional Animal Care and Use Committee of the Hospital.

Racemic MDMA, obtained from the National Institute on Drug Abuse (NIDA) (Research Triangle Park, NC, USA), was dissolved in saline (0.9% NaCl) and administered intraperitoneally (i.p.).

We conducted two different experiments:

1. Single-dose: 22 animals were divided into four groups. Five received 20 mg/kg, six received 40 mg/kg and four received saline. A separate group of seven animals was heated to 38.5–39.0°C for 1 h to evaluate a possible contribution of hyperthermia to brain glucose metabolism as compared to those animals with MDMA-induced hyperthermia. PET scans were performed 1 h after MDMA injection.
2. Repeated-dose: Nine animals were divided into two groups. Six received three doses of MDMA (5 mg/kg) every 2 h and three received three doses of saline at the same time points. PET scans were performed 7 days after administration.

In all cases, rectal temperature (°C) was recorded using a digital thermometer coupled to a lubricated rectal probe. In the heated group, body temperature was continuously monitored and maintained at 38.5–39.0°C by a thermostatically controlled heating lamp and an electric blanket. The time required to reach the temperature setting point averaged ~50 min.

Blood glucose (mg/dl) was measured with Gluco-Touch strips at −30, 0, 10, 20, 30 and 60 min in the single-dose experiment. In the repeated-dose experiment, blood glucose was also measured at 2, 3, 4, 5, 6 and 7 h after the first drug administration.

Figure 1 summarises the experimental protocol.

FDG-PET study

Animals were scanned for 90 min in a dedicated small animal PET scanner, 35 min after administering [^{18}F] fluorodeoxyglucose (FDG) (66.19±6.25 MBq) via the tail vein. Since the study was not longitudinal, we sacrificed the animals by cervical dislocation before imaging, as proposed by the Institutional Animal Care and Use Committee of the hospital.

Imaging was performed with the piPET system [29], whose detectors consist of 26×22 arrays of BGO crystals coupled with optical grease to the face of a Hamamatsu R3941 position-sensitive photomultiplier tube (PSPMT).

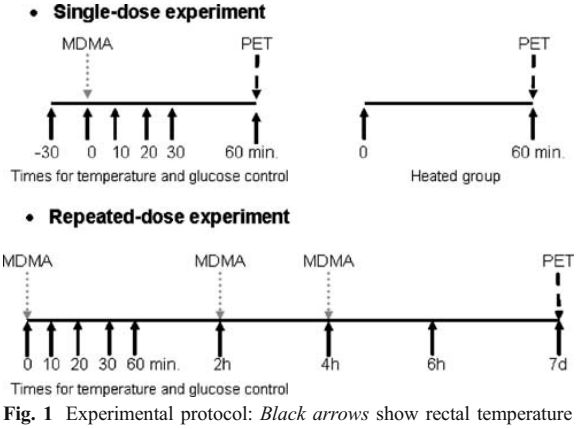

Fig. 1 Experimental protocol: *Black arrows* show rectal temperature and blood glucose measurements. *Dotted and dashed arrows* indicate MDMA administration and FDG PET scan, respectively

Each crystal is 2 mm×2 mm×10 mm long, polished on five sides and finely ground on its entrance end. The detectors were 18 cm apart. Tomographic images were reconstructed using a 3D ordered subsets expectation maximisation algorithm (20 iterations, 5 subsets), creating 43 102×102 tomographic images that spanned the 55 mm diameter×45 mm high imaging volume of the scanner [30]. The voxel size is 0.55×0.55×1.1 mm^3, and the spatial resolution in these images is 1.65 mm FWHM isotropic. The energy window was 300–650 keV, and decay and deadtime corrections were applied. The usefulness of the piPET imaging device has been previously reported [31].

The PET study in the repeated-dose experiment was performed in seven animals instead of nine owing to the death of two rats after the third dose of MDMA. In the single-dose experiment, PET study could be performed in all the animals.

Regions of interest (ROIs) were chosen according to ROIs described in humans [26, 32]. Twelve ROIs were manually drawn on coronal sections: cerebellum, brain stem, thalamus, hippocampus, caudate-putamen, frontal cortex, amygdala, somatosensory cortex, visual cortex, auditory cortex, superior colliculus and whole brain. Because of the low anatomical resolution of PET images, ROIs were placed by identifying the 3D coordinates of each structure in a rat brain atlas [33] and locating the corresponding position in the PET image (Fig. 2).

A background ROI was also drawn outside the brain area. The activity of this ROI was very low in all the PET studies, indicating a small contribution from random coincidence events and from scatter. To normalise FDG uptake, tissue activity was corrected by subtracting background and dividing by whole brain activity. Results are expressed as a percentage (%) ([FDG uptake=(study ROI/whole brain)×100].

Statistical analysis

In both experiments, glucose brain metabolism data were analysed by means of one-way analysis of variance (ANOVA) followed by contrast tests. Data normality and homoscedasticity were previously assessed by Kolmogorov-Smirnov and Levene's tests. Body temperature and blood glucose data were studied by means of Student's *t* tests.

Results

Effects of a single dose of MDMA

Effects on behaviour

The animals exhibited stereotypical behaviour 15–20 min after MDMA administration in both the 20 and the 40 mg/kg group, reaching a maximum after 30–45 min. This involved head weaving, salivation, pilo-erection and increased locomotor activity. Animals in the saline group did

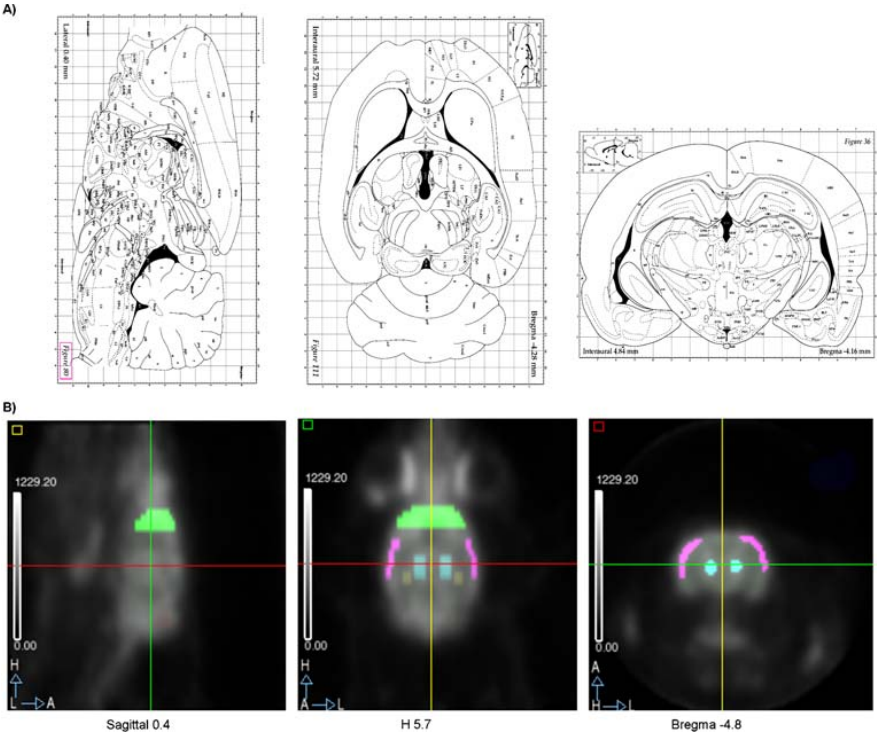

Fig. 2 Rat brain sagittal, coronal and axial sections corresponding to the same slice of the rat brain atlas (**a**) and the PET image (**b**). ROIs were placed by identifying the 3D coordinates of each structure on the rat brain atlas and locating the corresponding position in the PET image (*green*, frontal cortex; *pink*, cerebral cortex; *blue*, thalamus; *yellow*, hippocampus). Atlas images have been reprinted from Paxinos G, Watson C. The rat brain in stereotaxic coordinates, 4th ed. San Diego, CA: Academic Press; 1998, with permission from Elsevier

not show any of these changes. In contrast, animals in the heated group showed a decrease in motor activity.

Two rats from the single-dose group (one receiving 40 mg/kg and another receiving 20 mg/kg) died 1 h after MDMA administration. Although these rats died 60 min after the MDMA injection, we were still able to perform the FDG-PET scan.

Effects on body temperature

Following single-dose MDMA administration, animals showed an increase in rectal temperature ranging from 1.99±0.17°C for the 20 mg/kg dose group to 2.56±0.164°C for the 40 mg/kg dose group ($p<0.05$, compared with the saline group). In all animals, maximum temperature was reached 60 min after MDMA administration (Fig. 3). Saline had no effect on rectal temperature. Heated animals maintained their temperature at between 38.5° and 39.0°C for 60 min, similar to that observed in MDMA-treated animals Fig. 4.

Effects on blood glucose level

Blood glucose decreased significantly 1 h after a single dose of MDMA, showing changes of −26.0±9.8 and −54.4±25.3 mg/dl for the 20 mg/kg and 40 mg/kg MDMA doses, respectively (mean±SEM, $p<0.05$, compared with the saline group) (Fig. 5). Blood glucose levels also decreased in the heated group as compared with the saline group (increase of −17.67±5.45, $p<0.05$, compared with the saline group). Bivariate correlation showed an association between the degree of hypoglycaemia and higher temperature at 60 min ($p<0.01$).

Effects on brain glucose metabolism

The ANOVA analysis revealed significant differences between groups in the single-dose experiment (1 h after MDMA administration) in the cerebellum ($F=5.989$; $p=0.001$), hippocampus ($F=3.212$; $p=0.026$), amygdala ($F=4.919$; $p=0.004$) and auditory cortex ($F=2.856$; $p=0.04$). Percent uptake differences between the different groups are shown in Table 1.

Figure 6 shows sagittal, coronal and axial sections of the PET scan of a representative MDMA rat (40 mg/kg), where the higher uptake of FDG in the cerebellum is noticeable. Figure 7 shows axial sections of the PET scan of the same MDMA rat (40 mg/kg).

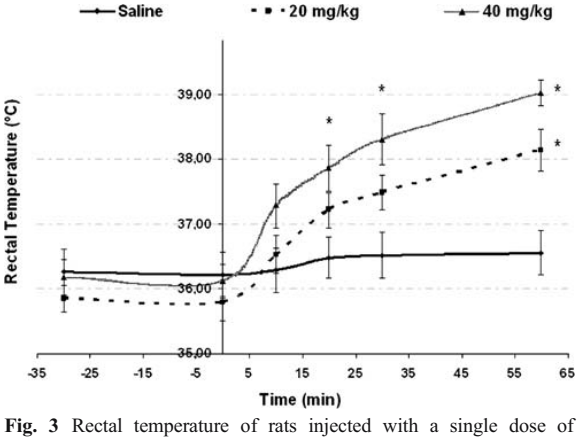

Fig. 3 Rectal temperature of rats injected with a single dose of MDMA (20 or 40 mg/kg) or saline. Values are expressed as mean±SEM (*$p<0.05$, compared with saline group)

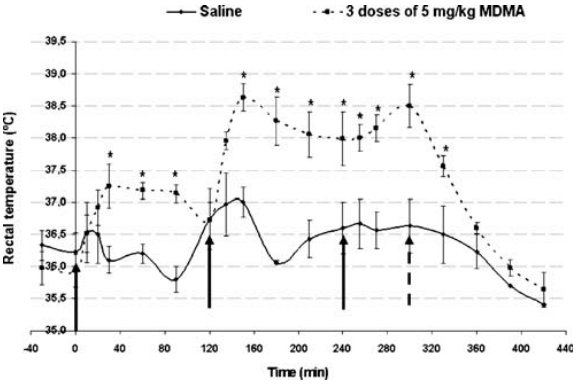

Fig. 4 Rectal temperature of rats injected with three doses of MDMA (5 mg/kg) or saline. Values are expressed as mean±SEM (*$p<0.05$, compared with the saline group). *Solid arrows* indicate MDMA or saline administration. *Dashed arrow* indicates glucose saline solution administration (1.5 mg/kg) in MDMA treated rats

Effects of repeated doses of MDMA

Effects on behaviour

As in the previous experiment, animals showed salivation, pilo-erection and increased locomotor activity. Locomotor stimulant effects were more apparent as additional doses were administered.

Two rats from the repeated-dose group died after the third MDMA dose.

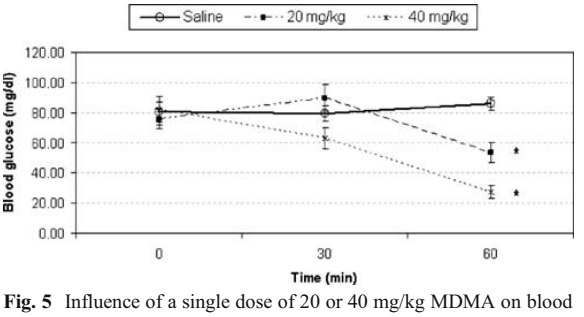

Fig. 5 Influence of a single dose of 20 or 40 mg/kg MDMA on blood glucose. Values correspond to mean±SEM (*$p<0.05$, compared with the saline group)

Table 1 ROI analysis in the single dose experiment: tissue activity has been normalised to whole brain activity and results are showed as a percentage

	Saline (n=4)	Heated (n=7)	20 mg/kg MDMA (n=5)	40 mg/kg MDMA (n=4)	p value[a]
Cerebellum	109.11±4.96	111.3±1.54	127.78±3.39* (+16%)	133.64±8.07* (25%)	0.001
Thalamus	114.2±2.07	118.64±1.94	115.99±3.15	111.73±0.70	0.196
Brain stem	110.49±0.82	115.96±2.88	118.35±3.38	125.02±7.27	0.348
Hippocampus	98.62±4.47	107.53±2.78	95.91±3.01	99.71±3.36	0.026
Frontal cortex	89.04±5.98	88.74±1.74	96.28±3.84	93.81±5.51	0.095
Caudate putamen	103.13±3.40	106.06±1.77	106.71±1.73	105.51±6.76	0.397
Visual cortex	106.87±4.44	104.6±2.48	97.37±1.88	93.61±4.56* (13%)	0.062
Superior colliculus	132.16±3.43	126.68±1.80	126.31±1.29	135.03±8.88	0.512
Amygdala	94.43±2.69	85.08±2.47* (9%)	85.7±2.53* (9%)	86.4±3.53	0.004
Somatosensory cortex	92.18±4.29	89.77±1.33	96.17±5.14	94.4±5.76	0.150
Auditory cortex	99.15±7.53	100.9±1.46	93.87±4.16	84.68±3.10* (15%)	0.040

n represents the number of animals in each group. Values are expressed as mean±SEM. Percent uptake differences are shown in parentheses.
*p<0.05 versus saline group
[a] p value obtained by one way ANOVA

Effects on body temperature

Figure 4 shows the effect of repeated doses of 5 mg/kg MDMA on rectal temperature. After the first dose, rectal temperature increased by 0.98±0.13°C (p<0.005, compared with the saline group), followed by a decrease over the next hour. After the second MDMA dose (at 120 min), the temperature rose again by 2.56±0.22°C compared with the saline control group (p<0.005). The third dose produced a less evident temperature increase (1.87 ±0.33°C) (p<0.005, compared with the saline group).

Effects on blood glucose level

As in the previous experiment, blood glucose levels decreased in the repeated-dose group, reaching a minimum (56.50±21.14 mg/dl) 1 h after the second dose. With the third MDMA dose, blood glucose decreased rapidly to 30.5±23.04 mg/dl 60 min later. Owing to severe hypoglycaemia, two animals received a glucose–saline solution (i.p.) (1.5 mg/kg) 5 h after the first MDMA dose. Control rats in the saline group did not require glucose–saline infusion.

Effects on brain glucose metabolism

The ANOVA of data corresponding to the repeated-dose experiment (7 days after MDMA administration) did not reveal significant hyper- or hypo-activation areas (Table 2).

Discussion

To the best of our knowledge, this is the first report to demonstrate significant hypoglycaemia shortly after MDMA. In addition, this is the first study to examine

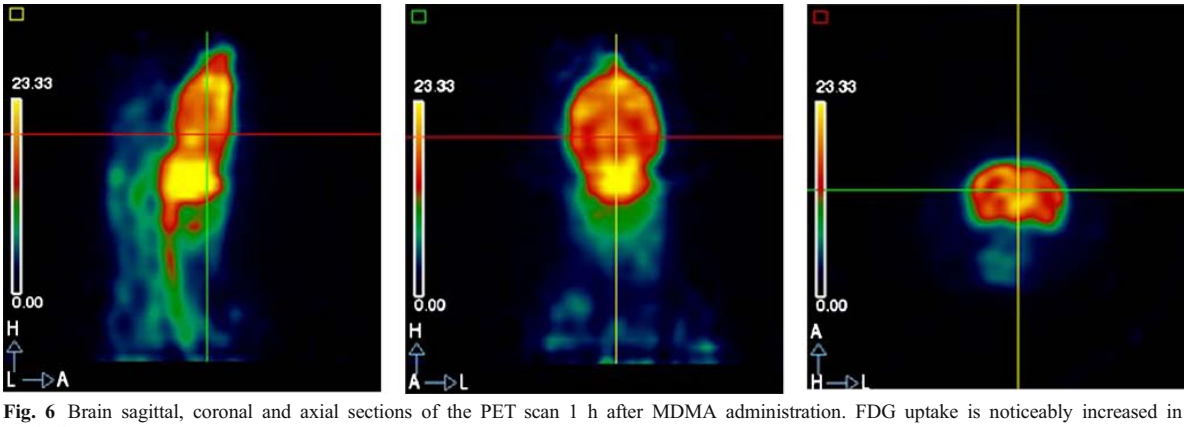

Fig. 6 Brain sagittal, coronal and axial sections of the PET scan 1 h after MDMA administration. FDG uptake is noticeably increased in cerebellum

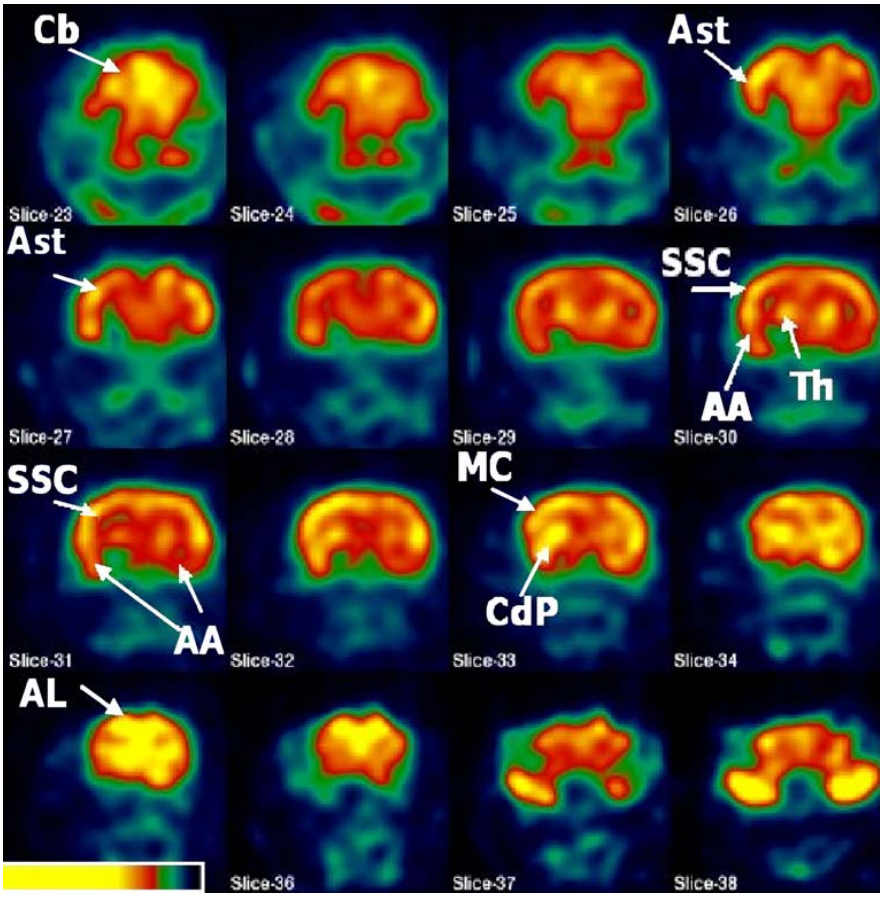

Fig. 7 Brain axial sections of the PET scan 1 h after MDMA administration (40 mg/kg). *Th* thalamus, *MC* motor cortex, *SSC* somatosensory cortex, *Ast* visual cortex, *CdP* striatum, *AA* amygdala, *AL* anterior limbic area, *Cb* cerebellum

glucose metabolism in the rat brain in vivo using FDG-PET following MDMA administration.

Female Dark Agouti rats were used as they are deficient in the enzyme involved in demethylenation of MDMA (debrisoquine hydroxylase, coded by the gene CYP2D2) and thus provide a good model of the human CYP2D6 poor metaboliser phenotype, in which clinical complications are more likely to occur [20, 34, 35]. Vincent-Viry et al.

Table 2 ROI analysis in the repeated dose experiment: tissue activity has been normalised to whole brain activity and results are showed as a percentage

	3 doses of saline (n=3)	3 doses of 5 mg/kg MDMA (n=4)	p value[a]
Cerebellum	122.55±2.63	112.75±3.23	0.077
Thalamus	121.41±6.57	112.39±1.74	0.185
Brain stem	118.04±9.23	112.85±2.60	0.560
Hippocampus	91.92±2.54	95.22±2.57	0.414
Frontal cortex	104.48±4.44	99.6±3.54	0.423
Caudate putamen	101.16±0.72	98.2±1.71	0.220
Visual cortex	100.47±2.37	105.81±3.47	0.294
Superior colliculus	129.49±5.24	134.55±4.50	0.496
Amygdala	98.59±4.42	95.44±1.45	0.475
Somatosensory cortex	108.29±7.86	101.89±6.73	0.563
Auditory cortex	104.04±3.96	95.52±1.92	0.087

n represents the number of animals in each group. Values expressed as mean±SEM

[a] p value obtained by one way ANOVA

demonstrated that the liver of the female Dark Agouti rat cleared debrisoquine at a significantly slower rate than that of the male [36]. This suggests that females have lower debrisoquine hydroxylase activity, which is consistent with the evidence that liver microsomes in female Dark Agouti rats have lower debrisoquine oxidising activity than those in males [37]. Low activity of the debrisoquine hydroxylase enzyme will result in a lower rate of demethylenation, higher plasma levels of MDMA and therefore a greater risk of acute toxicity [38].

The experimental design was chosen in accordance with the literature. The delay of 1 h before performing the PET study is appropriate given the acute and rapid release of monoamines during the peak period after MDMA administration [39, 40]. The delay of 7 days is based on the 50% loss of 5-HT and its metabolites observed after a single dose of MDMA [27]. To rule out any possible confounding effect of hyperthermia on brain glucose metabolism, we also performed PET scans in a group of rats heated for 1 h, the same period as was used in the single-dose experiment.

Physiological changes

Following MDMA administration, several physiological changes were observed, including hyperthermia, tachycardia and increased locomotor activity. This pattern of physiological responses and stereotypical behaviours is indicative of serotonergic effects of MDMA [19, 20].

In the present study, MDMA induced an increase in rectal temperature of approximately 2.56°C 1 h after drug injection at the highest dose (40 mg/kg), which is in agreement with previous studies using the same dose [27, 41, 42]. Fifty minutes after the drug administration, two rats (one receiving 20 mg/kg and another receiving 40 mg/kg) suffered from tremor, muscular rigidity, bruxism and tachycardia. Finally, these animals experienced severe respiratory depression and died. Similar effects have been observed in humans suffering acute adverse responses to MDMA [43, 44].

To our knowledge, there is no either previous report on the effect of MDMA on peripheral blood glucose levels in rats. We observed a severe decrease in the peripheral blood glucose level after MDMA administration. Moreover, we showed an association between the degree of hypoglycaemia and higher temperatures. Malignant hyperthermia has been previously associated with hyperglycaemia. Several works regarding hepatic metabolism during porcine malignant hyperthermia have shown a large release of glucose and potassium from the liver [45, 46]. The hypoglycaemia detected in our study may have been due to severe acute hepatotoxicity induced by MDMA, suggesting that the liver could be more sensitive to MDMA at higher temperatures. This effect increased when MDMA action was prolonged by repeated dosing. MDMA has been reported to be a cause of severe acute hepatotoxicity that can be followed by acute hepatic failure in humans [47]. Jaundice, a high level of serum transaminase activity, hypoglycaemia and low prothrombin activity have been reported in a relatively high number of cases of liver damage in young people after taking ecstasy [48]. We observed significant hypoglycaemia in those animals treated with MDMA, not only 1 h after taking the drug, but also 7 h later. This effect may have been due to an increased use of glucose, higher release of insulin or liver damage caused by MDMA. Several mechanisms have been proposed for the liver damage after MDMA, including influence of MDMA on body temperature regulation, direct toxic effects of the drug on liver cells or the genetic vulnerability of some individuals to amphetamines and amphetamine derivatives [49]. Although hepatotoxicity in association with MDMA is becoming increasingly recognised, the fact that continued supplementation was required to maintain blood glucose levels suggests that acute hepatic failure was the cause. However, more studies are necessary to understand the underlying mechanism of the liver damage produced by MDMA.

Short-term effects on brain glucose metabolism

The ROI analysis showed an increase in FDG uptake in the cerebellum in the single-dose experiment. This increase may reflect an acute and rapid release of 5-HT, dopamine and other neurotransmitters and/or inhibited re-uptake, widely reported in humans and small animals in the cortex, hippocampus and striatum [15, 43]. We also detected hypo-activation in the amygdala, similar to that reported by Obrocki et al., which may be associated with discomfort or unpleasant stimuli [32], and in components of the somatosensory cortex, similar to that described by Gamma et al. [21]. Two previous studies have evaluated the effects of a single dose of MDMA (1.7 mg/kg) on regional cerebral blood flow in MDMA-naïve healthy human subjects, measured with $H_2{}^{15}O$-PET. MDMA has been reported to produce changes in regional blood flow: increases in the ventromedial frontal and occipital cortex, inferior temporal lobe and cerebellum, and decreases in the motor and somatosensory cortex, temporal lobe including left amygdala, cingulate cortex, insula and thalamus [21]. In addition, these changes were associated with pronounced mood enhancement, increased extroversion and intensification of sensory perception [50]. An autoradiographic 2-deoxy-D[^{14}C]glucose (2DG) study [19] showed marked 2-DG uptake in components of the motor system of the rat similar to that observed in our study, which indicates a rise in glucose metabolism and brain activity. Our data indicate that a dedicated small animal PET scanner could be suitable for studies of drug dependence, since we obtained results similar to those shown by autoradiographic 2DG techniques.

Hyperthermia

MDMA-induced 5-HT neurotoxicity can be separated from any possible confounding effects of MDMA-induced hyperthermia. We observed no significant changes in cerebral glucose metabolism when rats underwent hyperthermia (38.5–39°C) as compared with the saline control group, except for a slight reduction in FDG uptake in the amygdala, which may have been related to the discomfort secondary to the use of the heat lamp and electric blanket. Similar results were previously obtained using quantitative 2DG autoradiography in hyperthermic rats (40.2±0.3°C) [51]. These authors reported glucose utilisation to be minimally altered or slightly higher in the thalamus and neocortex. Changes in regional cerebral metabolism have also been studied with FDG-PET in humans during steady-state hyperthermia [52]. This study reported a significant increase in FDG in the hypothalamus, thalamus, corpus callosum, cingulated gyrus and cerebellum, as well a significant decrease in the caudate, putamen, insula and posterior cingulum. We did not observe this precise constellation of changes in glucose metabolism in rats. However, differences in MDMA dose and both cognitive and sweating abilities between humans and animals should be considered before comparing the results.

In our study, animals which were administered a single dose of MDMA showed significant changes in FDG uptake in several brain areas, as compared with the heated group. We found changes in components of the motor (cerebellum), limbic (hippocampus and amygdala) and somatosensory system (visual and auditory cortex). These differences suggest that the changes in brain glucose metabolism observed in our study were not due to hyperthermia per se, but were a direct effect of MDMA. Furthermore, these results agree with the metabolic changes in different brain regions of the rat reported by Wilkerson and London using quantitative autoradiography of 2DG [19]. These metabolic changes could reflect the action of MDMA on the serotonergic and dopaminergic systems that, if extrapolated to humans, could be related to persistent neuropsychiatric syndromes after chronic exposure to MDMA, such as psychosis, mood disturbance, anxiety disorders and cognitive deficits [53].

Long-term effects on brain glucose metabolism

Regarding long-term effects, the present study showed no changes in brain glucose metabolism persisting after 1 week. Using high-performance liquid chromatography, O'Shea et al. showed that a single dose of MDMA produces an important decrease in 5-HT and 5-HIAA in the cortex, hippocampus and striatum in rats, which is still present 7 days later [27]. However, we were unable to find equivalent changes in glucose metabolism after 1 week.

To our knowledge, only three reports have used FDG-PET to study the specific neurotoxicity of chronic use of ecstasy in humans [32]. The glucose metabolic uptake of the ecstasy user group was reduced in the amygdala and hippocampus (a brain region known to be consistently affected by MDMA in animals treated with this drug) and striatum. The reduction in the striatum and amygdala of ecstasy users was significantly higher in those who initiated consumption before the age of 18 years. However, no significant differences were found when cumulative doses of ecstasy and glucose metabolism were compared [32]. Although we found no changes in brain glucose metabolism in rats after 1 week, it must be pointed out that our study used adult rats and that these results could be different in adolescent rats, which may show more pronounced MDMA effects on glucose metabolism, as highlighted by Obrocki et al. [32].

Limitations of the study

Our study has several limitations. The first is the low anatomical resolution of the PET images. Measurements of metabolic activity by this technique in small regions may not be entirely accurate, since ROI activity could be contaminated by that of surrounding brain regions. In future studies, metabolic activity could be improved if partial volume effects were to be corrected by defining the ROI on a registered MRI scan of the same animal.

Second, the sample size was relatively small owing to the extremely labour intensive nature of FDG-PET studies. No control animals died due to the protocol. Despite the low sample size, the number of animals in each group proved sufficient to detect significant changes in glucose metabolism.

Third, the dosage regimens used in our animals can be considered fairly high for one single injection, and may not be comparable to those used by most humans. However, the doses presently employed are comparable to those used by other investigators studying the effects of MDMA in small laboratory animals, and there are humans who use high repeated doses.

Fourth, the study was performed in female rats. Little is known about gender differences in the effects of MDMA. We did not check the rat oestrous cycle although we are conscious that ovarian hormones may be an important factor in the modulation of the sensitivity and reactivity of the serotonergic neurotransmission system [54]. However, liver metabolism may make a greater contribution to the differences in the responsiveness to MDMA [55]. Indeed, the liver of the female Dark Agouti rat cleared debrisoquine hydroxylase at a significantly slower rate than that of the male, and resulting in a lower rate of demethylenation, higher plasma levels of MDMA and therefore a greater risk of acute toxicity [36].

Conclusion

In summary, we observed significant hypoglycaemia after MDMA administration and, by using a dedicated small animal PET scanner, we were able to detect changes in components of the motor, limbic and somatosensory systems 1 h after MDMA administration but not after 1 week, thus indicating possible alteration of brain glucose metabolism immediately after administration.

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