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ORIGINAL INVESTIGATION

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Acute methylenedioxymethamphetamine administration: effects on local cerebral blood flow and glucose utilisation in the dark agouti rat

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Abstract *Rationale:* Clinical reports indicate that acute exposure to 3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy") may induce pathological cerebrovascular responses in human users of the drug, however, the mechanism by which MDMA might effect these pathological changes is not clear. *Objectives:* To examine the effects of acute MDMA administration on the relationship between local cerebral blood flow (LCBF) and local cerebral glucose utilisation (LCMRglu); to determine the effect, if any, acute exposure to MDMA has on the cerebral circulation, independently of alterations in cerebral metabolic demand. *Methods:* Dark Agouti rats were injected with 15 mg.kg^{-1} i.p. MDMA or saline equivalent. LCBF and LCMRglu were measured in 50 brain areas using the fully quantitative [^{14}C]iodoantipyrine and [^{14}C]2-deoxyglucose autoradiographic techniques, respectively. *Results:* MDMA produced significant increases in LCMRglu in 23 brain areas, most markedly in the motor system (globus pallidus; +82%; medial striatum; +71%). In contrast, significant decreases in LCBF were observed in 28 brain areas, most markedly in primary sensory nuclei (superior colliculus; −32%) and limbic areas (anterior thalamus; −34%). Global analysis revealed a close correlation (r=0.87) between LCMRglu and LCBF with a ratio of 1.53 in controls. Despite the divergence of LCMRglu (increases) and LCBF (decreases) in MDMA-treated groups, there was a similar close correlation (r=0.84), but the ratio was decreased to 1.22. *Conclusions:* This study provides clear evidence that acute exposure to MDMA results in cerebrovascular dysfunction. The uncoupling of LCBF from underlying metabolic demand, possibly due to the vasoconstrictor action of 5-HT, could provide the basis for oligaemia-induced pathological changes in the brain.

Keywords Ecstasy · 5-Hydroxytryptamine · Cerebrovascular accident · Flow-metabolism coupling · Oligaemia

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Introduction

3,4,-Methylenedioxymethamphetamine (MDMA; "Ecstasy") is a ring-substituted analogue of amphetamine and, possessing both stimulatory and hallucinogenic properties (Nichols and Oberlender 1990), is one of a novel class of drugs known as entactogens (Nichols 1986; Nichols and Oberlender 1990; O'Loinsigh et al. 2001). The negative consequences of human MDMA exposure, although widely reported, remain controversial. Although Ecstasy-associated fatalities have been documented, these are relatively few in proportion to the size of the user population. Of greater concern is the potential for MDMA-induced morbidity, particularly in the brain, but the fact that this morbidity may remain occult, adds to the controversy (Cole et al. 2002). A greater appreciation of the risks involved in exposure to MDMA may arise from a fuller understanding of the interaction between the pharmacological profile of MDMA and the physiological responses which could lead to pathophysiological consequences.

Parenteral MDMA administration induces a biphasic response in experimental animals. The acute effects are short lived, lasting approximately 24 h (Schmidt et al. 1986; Schmidt 1987, 1989; Stone 1987; McKenna and Peroutka 1990), while long-term effects, including those secondary to neurotoxicity, may extend up to 12 months or more (Battaglia et al. 1988, Scanzello et al. 1993). Acutely, MDMA increases extracellular 5-hydroxytryptamine (5-HT), dopamine and noradrenaline (NA) in the brain by initiating the rapid release of these monoamines from pre-synaptic terminals (Schmidt et al. 1986; Schmidt 1987; Stone 1987; Rudnick and Wall 1992; Wichems et

al. 1995; Koch and Galloway 1997; Rothman et al. 2001). MDMA also prolongs and increases the synaptic availability of extracellular monoamines by inhibiting monoaminergic reuptake mechanisms (Green et al. 1995; White et al. 1996) and monoamine oxidase (MAO) activity (Stone et al. 1986; Kokotos Leonardi and Azmitia 1994).

The effects of widespread monoamine release and the direct toxic effects of MDMA or its metabolites on brain are manifold and have been widely reported (Li et al. 1989; Wilkerson and London 1989; Frederick and Paule 1997); but, in parallel to their effects on neuronal function, the monoamines also have the potential to act upon cerebrovascular receptors, resulting in vasoconstriction (NA), vasodilatation (dopamine) or both (5-HT). Certainly, the link that has emerged between cerebrovascular accident (CVA) and acute exposure to MDMA (Harries and De Silva 1992; McEvoy et al. 2000) might suggest that an interaction between the drug and the blood vessels of the brain represents an important component to the actions of the drug in the human brain. However, in the intact animal, cerebral blood flow is finely regulated to match the metabolic demands of underlying neuronal tissue, and there is little evidence that either dopaminergic or noradrenergic innervation plays a role in regulating cerebrovascular resistance (Edvinsson and MacKenzie 1977; McCulloch et al. 1982). Only intrinsic 5-HT systems appear to exert a tonic influence upon the cerebral circulation (McBean et al. 1990, 1991), but even here the evidence is not unequivocal (McBean et al. 1999).

The acute effects of MDMA on local cerebral glucose utilisation in rat have been reported previously (Wilkerson and London 1989). However, in order to determine whether acute exposure to MDMA does indeed have an effect on the cerebral circulation, independent of alterations in cerebral metabolic demand, we have measured the effects of MDMA on local cerebral blood flow (LCBF) and local cerebral glucose utilisation (LCMRglu) in parallel groups of Dark Agouti rats, and the effect that the drug has on the normal relationship between the two. Dark Agouti rats are deficient in the enzyme involved in demethylation of MDMA (debrisoquine hydroxylase, coded by the gene CYP2D2), and as a consequence are more susceptible to the effects of the drug (Colado et al. 1995; O'Shea et al. 1998; Malpass et al. 1999). An analogous gene (CYP2D6) is present in man (Tucker et al. 1994; Parrot 2001) and the Dark Agouti rat may therefore provide a good model of a genetically defined human sub-population in which clinical complications are more likely to occur.

Methods

The principles of laboratory animal care were followed throughout all experiments. All animal procedures were performed with local ethics approval and in accordance with the United Kingdom Animals (Scientific Procedures Act, 1986) and associated guidelines. A total of 19 male Dark Agouti rats weighing between 230 g and 300 g were used in this study. Animals were group-housed under a 12-h/12-h light/dark cycle with access to food and water ad libitum until the day of the experiment. Room temperature in the animal house was maintained at approximately 21°C.

Animal preparation and drug treatment

On the day of the experiment animals were anaesthetised with halothane (maintained at 1% in a mixture of 70% nitrous oxide and 30% oxygen), and polythene cannulae were inserted into both femoral arteries, to allow sampling of arterial blood and monitoring of arterial blood pressure, and both femoral veins, for the injection of radiolabelled tracers and barbiturate at the time of killing. An additional cannula was inserted intraperitoneally via a trocar and held in place with a fine suture. A loose-fitting, individually moulded plaster cast was applied around the hindquarters and pelvis and secured to a weighted platform. Thus lightly restrained and supported, a rectal temperature probe was inserted and anaesthesia was discontinued. Animals were allowed to recover from anaesthesia for at least 2 h prior to further experimental manipulation.

Blood gases were recorded prior to any experimental procedures, and mean arterial blood pressure (MABP) and rectal temperature were monitored throughout. At the end of the recovery period, animals were injected with either MDMA ((±)-3,4-methyelenedioxymethamphetamine hydrochloride; Ultrafine Ltd., Manchester, U.K.) at a dose of 15 $mg.kg^{-1}$ (n=10) or an equivalent volume of saline (0.5 ml; n=9) via the indwelling intraperitoneal cannula. The choice of dose was based on previously published dose–response relationships (Wilkerson and London 1989; O'Shea et al. 1998).

The measurement of LCMRglu (n=9) was initiated at 15 min after the i.p. injection of MDMA (or saline), at which time the behavioural response was fully established and physiological effects were manifest. Although the measurement of LCMRglu covers a 45-min period, the median point of integrated specific 2-deoxyglucose in the brain, from which LCMRglu is derived, is around 10 min after a bolus injection of the tracer (Sokoloff et al. 1977). Thus, the measurement of LCBF (n=10), which has a time scale of less than 1 min, was initiated at 25 min after the i.p. injection of MDMA (or saline), so that it coincided with the median point of LCMRglu measurement.

Measurement of local cerebral glucose utilisation

LCMRglu was measured in five rats treated with MDMA and four rats treated with saline using the quantitative [^{14}C]-2-deoxyglucose autoradiographic technique (Sokoloff et al. 1977). An intravenous injection of [^{14}C]-2-deoxyglucose (1.3 MBq per rat in 0.75 ml saline) was administered at a constant rate over the first 30 s of the experiment. A series of 14 timed arterial blood samples were collected at pre-determined intervals over the subsequent 45 min. Samples were centrifuged in order to separate the plasma, aliquots of which were removed for the determination of plasma glucose (10 μl) and ^{14}C concentrations (20 μl) by semi-automated glucose oxidase assay (Beckman) and liquid scintillation analysis (Packard), respectively. At 45 min, the animals were killed by rapid intravenous injection of sodium pentobarbitone, and the brains were immediately dissected out intact and rapidly frozen in pre-cooled 2-methylbutane (–45°C). Frozen brains were mounted onto specimen holders with embedding medium (Lipshaw) and stored at −70°C until sectioned.

Measurement of local cerebral blood flow

LCBF was measured in equal numbers of rats from each treatment group (n=5) using the quantitative [^{14}C]-iodoantipyrine autoradiographic technique (Sakurada et al. 1978). The [^{14}C]-iodoantipyrine (1.5 MBq per rat in 0.6 ml saline) was infused intravenously at a constantly increasing rate over 45 s, at the end of which the animals

were killed by decapitation, and the brains dissected out and prepared as in the LCMRglu experiments. During the measurement, a maximum of 18 timed arterial blood samples were collected onto pre-weighed filter paper discs from the free-flowing femoral arterial cannula. The discs were re-weighed at the end of the measurement period to determine the weight of each sample, from which the volume of blood was calculated, assuming a specific gravity of 1.01, and prepared for liquid scintillation analysis.

Preparation and analysis of autoradiograms

Frozen brains were serially sectioned (20 μm) in the coronal plane. A series of three sections were retained from every 200 μm, thaw mounted onto a glass coverslip and rapidly dried on a hot plate (75°C). Autoradiograms were prepared by applying these sections, together with a series of eight precalibrated ^{14}C-standards (40–1069 nCi/g tissue equivalents: Amersham International, U.K.), to X-ray film (Kodak, SB-5). Analysis of the autoradiograms was performed using a computer-based image analysis system (MCID/M5+). Local tissue isotope concentrations were derived from the optical density of autoradiographic brain images relative to the [^{14}C]-standards, and LCBF and LCMRglu were calculated from tissue tracer concentrations and arterial tracer profiles using the appropriate operational equations. Measurements were made in a total of 50 anatomically distinct and functionally diverse brain regions.

Statistical analysis

In keeping with previous studies of similar experimental design, anatomically discrete brain regions were assumed to represent variables that can be analysed independently within each measure (LCMRglu or LCBF, McCulloch et al. 1982) and no correction was applied for multiple comparisons. Data were evaluated statistically using student's *t*-test for grouped variables, with acceptable levels of significance set at $P<0.05$. To determine whether there were any differences in the overall relationship between LCBF and LCMRglu between the two groups, Mann-Whitney *U* test was applied to the ratios of mean LCBF to mean LCMRglu for each of the brain areas. All data are presented as mean ±SEM.

Results

Behavioural observations and physiological parameters

MDMA produced stereotypical behaviour (head weaving, forepaw treading, rearing and salivation) which began 2–3 min after the administration of the drug. Pilo-erection was also observed in the MDMA-treated animals. There were no significant differences in physiological parameters between any of the groups prior to treatment, but MDMA produced prolonged increases in both rectal temperature (+1.8°C) and MABP (+30%) when compared with saline-treated controls. Saline had no effect on either parameter (Fig. 1 and Fig. 2).

Local cerebral glucose utilisation

MDMA produced a tendency towards increased LCMRglu in the majority of brain areas analysed, and in 23 areas the increases were statistically significant. However, LCMRglu was also significantly decreased in two brain regions, posterior cingulate cortex (−21%) and the superficial layers of the superior colliculus (−18%). Whilst the most marked increases were observed in components of the motor system (cerebellar vermis; +69%; globus pallidus; +82%; and caudate nucleus; +71%), discernible increases were also found in elements of other functional systems in the brain including the limbic system (e.g. nucleus accumbens, +37%), and somatosensory system (trigeminal nucleus, +30%) (Table 1; Fig. 3, Fig. 4, and Fig. 5). The increases observed in sensorimotor cortex were arranged in a columnar pattern which could be identified from visual inspection of the autoradiographic images. Quantification of the autoradiograms revealed that the columnar appearance in somatosensory cortex resulted from columns of increased

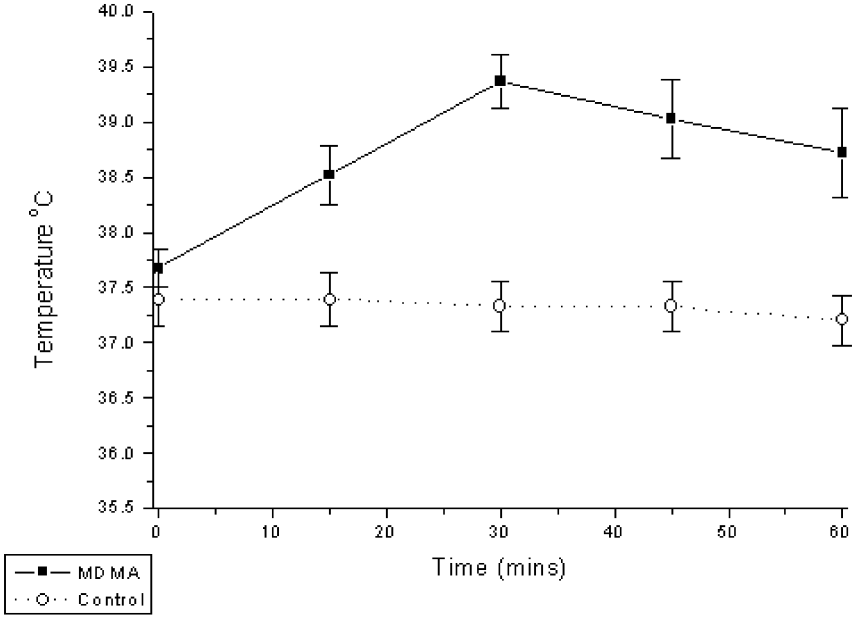

Fig. 1 Time course of rectal temperature changes in rats following the administration of a single dose of either saline or 3,4-methylenedioxymethamphetamine (MDMA) (15 mg·kg^{-1}) at time zero. Data points represent mean±SEM. values for MDMA (*n*=5) or saline (*n*=4) treated animals

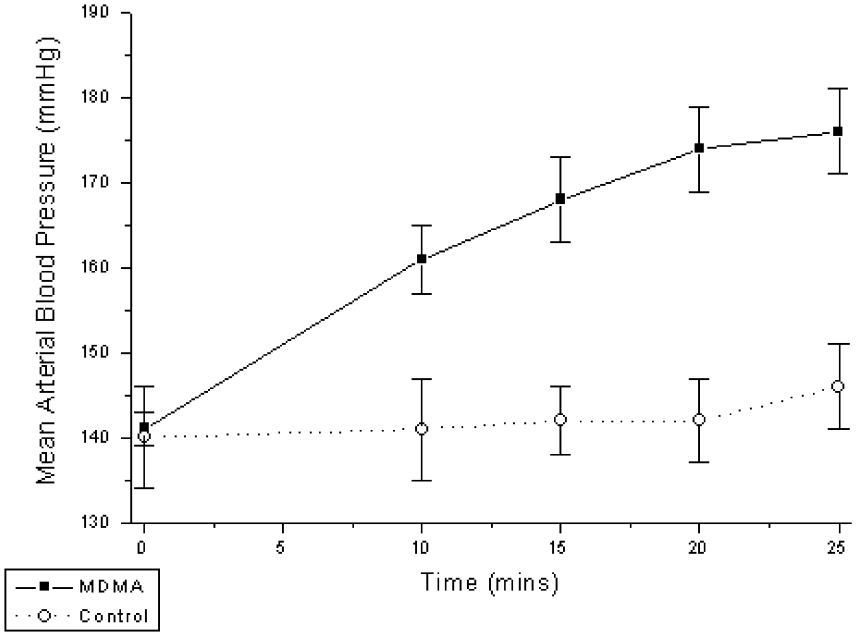

Fig. 2 Time course of mean arterial blood pressure changes in rats following the administration of a single dose of either saline or 3,4-methylenedioxymethamphetamine (MDMA) (15 mg·kg^{-1}) at time zero. Data points represent mean±SEM. values for MDMA (n=5) or saline (n=4) treated animals

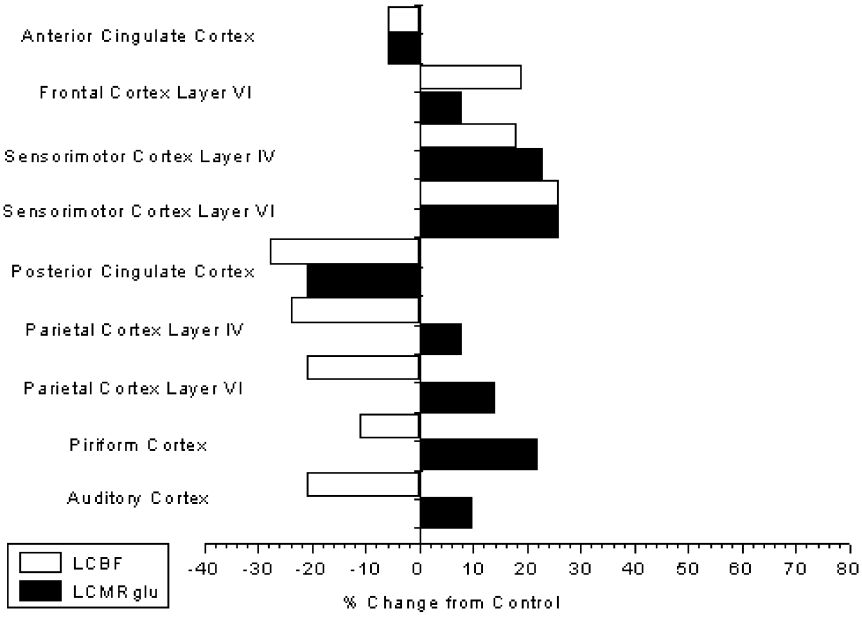

Fig. 3 The effect of a single acute administration of 3,4-methylenedioxymethamphetamine on local cerebral blood flow and local cerebral glucose utilisation: data points represent mean percentage change from saline control animals in cortical brain areas

LCMRglu (+20%) being found against a general pattern of near-normal LCMRglu (+2%). No significant effects of MDMA upon LCMRglu were observed in the raphé nuclei. Although we did not correct for multiple comparisons across 50 brain areas, the large number of regions that showed effects of MDMA exceeded the small number (three regions) expected to show significant effects by chance alone.

Local cerebral blood flow

In only two areas of the brain were any significant increases in LCBF measured following MDMA (frontal cortex, +19%; globus pallidus, +16%), but significant decreases in LCBF were observed in 28 of the 50 brain areas analysed. Once again, the large number of regions that showed effects of MDMA exceeded the small number (three regions) expected to show significant effects by chance alone. The most marked decreases were found in primary sensory nuclei (superior colliculus, −32%; medial geniculate, −25%) and limbic areas (anterior thalamus, −34%; dorsal subiculum, −30%) (Table 1; Fig. 3, Fig. 4, and Fig. 5). There was no evidence that these decreases in blood flow were organised according to the vascular territories of the principal cerebral arteries, although, in the cortex at least, perfusion in brain regions

Table 1 Effects of acute 3,4-methylenedioxymethamphetamine (MDMA) on local cerebral blood flow (LCBF) and local cerebral glucose utilisation (LCMRglu). All data are presented as mean ±SEM. *Significantly different from appropriate control group, $P<0.05$, students *t*-test for grouped variables

Brain area	LCMRglu (μmol/100 g/min)		LCBF (mg/100 g/min)		Ratio LCBF/LCMRglu	
	Saline	MDMA	Saline	MDMA	Saline	MDMA
Neocor tex	(*n*=4)	(*n*=5)	(*n*=5)	(*n*=5)		
Auditory	133±5	146±7	245±18	195±5*	1.8	1.3
Piriform	54±4	65±2*	103±8	92±5	1.9	1.4
Parietal, layer IV	121±6	137±10	250±21	196±6*	2.1	1.4
Layer VI	100±9	107±5	206±14	157±7*	2.1	1.5
Sensorimotor, Layer IV	114±4	144±5*	174±5	220±20	1.5	1.5
Layer VI	88±3	109±3*	151±5	179±14	1.7	1.6
Frontal, Layer VI	90±4	97±5	145±8	172±10*	1.6	1.8
Anterior cingulate	119±3	112±6	162±5	152±7	1.4	1.4
Posterior cingulate	117±5	92±7*	171±8	123±9*	1.5	1.3
Cerebellum						
Vermis	85±1	135±6*	150±8	169±6	1.8	1.3
Paramedian lobule	52±1	65±2*	103±6	101±4	2.0	1.6
Copula pyramis	60±2	92±4*	128±8	140±5	2.1	1.5
White matter	25±1	30±1*	42±4	32±2*	1.7	1.1
Inferior olive	79±1	101±5*	167±8	154±8	2.1	1.5
Red nucleus	70±2	80±3*	140±6	122±5*	2.0	1.5
Substantia nigra, reticulata	53±3	89±10*	105±4	100±5	2.0	1.1
Compacta	64±2	91±5*	125±7	112±5	1.9	1.2
Lateral habenula	109±10	89±7	185±9	153±9*	1.7	1.7
Medial habenula	78±3	71±3	145±8	112±6*	1.9	1.6
Subthalamic nucleus	80±3	88±4	161±14	157±9	2.0	1.8
Ventrolateral thalamus	92±4	104±4	143±10	134±8	1.6	1.3
Globus pallidus	47±2	75±5*	67±4	78±3*	1.4	1.1
Medial striatum	103±4	126±7*	123±5	131±6	1.3	0.9
Lateral striatum	93±3	152±9*	136±5	138±8	1.3	1.1
Hippocampus						
Molecular layer	87±8	95±6	128±7	108±5*	1.5	1.1
Dorsal subiculum	76±5	85±4	127±6	90±3*	1.7	1.1
Dentate PO	56±2	60±5	105±7	87±4*	1.9	1.5
Dorsal CA1	58±2	76±5*	96±5	84±4	1.7	1.1
Ventral CA1	65±3	73±4	139±10	105±6*	2.1	1.5
Ventral subiculum	64±5	67±5	140±11	99±6*	2.2	1.5
CA3	67±2	68±5	119±7	94±5*	1.8	1.4
Ventral tegmental area	45±2	57±3	89±5	74±4*	2.0	1.3
Mamillary body	100±4	99±5	202±12	155±6*	2.0	1.6
Lateral amygdala	75±2	90±3*	108±5	109±3	1.4	1.2
Anterior thalamus	105±5	95±6	168±5	117±2*	1.6	1.2
Septal nucleus	61±4	78±4*	106±7	96±4	1.7	1.2
Nucleus accumbens	87±3	119±7*	163±9	153±7	1.9	1.3
Superior colliculus superficial	109±5	97±8*	191±8	137±6*	1.7	1.4
Superior colliculus deep	86±3	103±8	176±9	127±6*	2.0	1.2
Medial geniculate	95±3	102±10	180±8	138±4*	1.9	1.4
Lateral geniculate	92±3	92±9	167±8	135±8*	1.8	1.5
Trigeminal nucleus	61±1	83±5*	143±5	144±15	2.3	1.7
Superior olive	113±4	109±6	207±8	180±8*	1.8	1.6
Nucleus ambiguus	62±1	85±3*	150±8	128±5*	2.4	1.5
Dorsal tegmental nucleus	98±3	89±4	179±6	152±6*	1.8	1.7
Dorsal raphé	81±1	81±3	168±7	137±7*	2.1	1.7
Median raphé	90±2	83±2	165±7	145±6	1.8	1.8
Paramedian raphé	85±2	86±3	154±7	136±3*	1.8	1.6
Pontine reticular formation	53±1	60±2*	112±7	101±8	2.1	1.7
Internal capsule	26±1	32±2*	47±3	40±3	1.8	1.3

supplied by the anterior (anterior cingulate) and middle cerebral arteries were either unchanged (somatosensory and piriform cotex) or increased (frontal cortex).

Relationship between LCBF and LCMRglu

In saline-treated animals, the relationship between LCBF and LCMRglu was, as expected, closely coupled. A global analysis encompassing all 50 brain areas (Fig. 6), revealed a close correlation (r=0.87) between the two parameters, with an overall ratio (slope) of 1.53.

In all but four of the brain areas analysed, (subthalamic nucleus; anterior cingulate cortex; ventrolateral thalamus; median raphé), either LCMRglu or LCBF (or both) were affected by exposure to MDMA. However, despite the normal close physiological relationship between LCMRglu and LCBF, in only three regions were changes

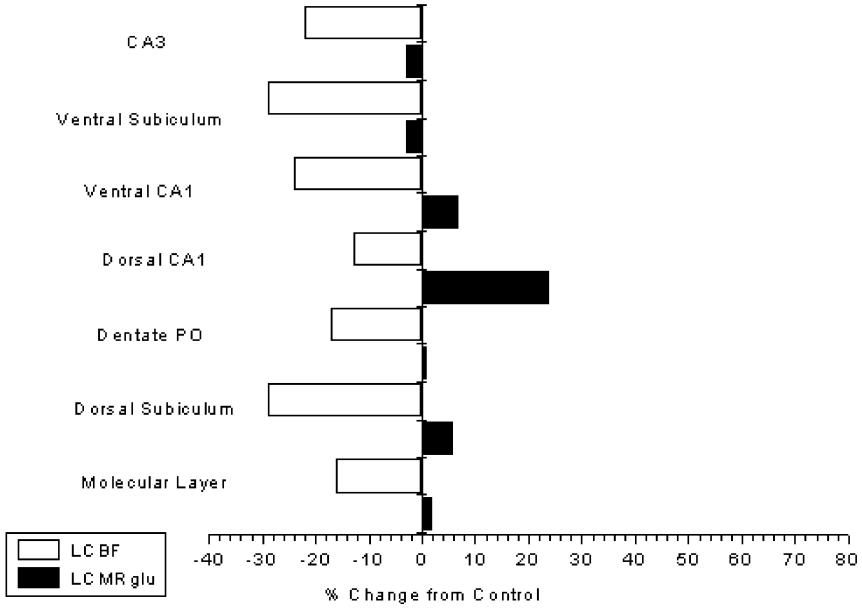

Fig. 4 The effect of a single acute administration of 3,4-methylenedioxymethamphetamine on local cerebral blood flow and local cerebral glucose utilisation: data points represent percentage change from saline control animals in hippocampus

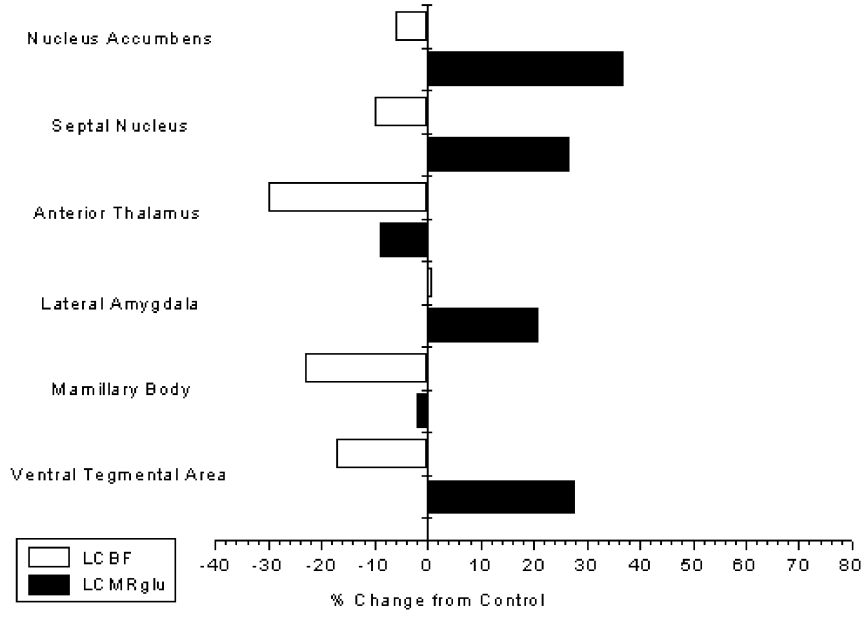

Fig. 5 The effect of a single acute administration of 3,4-methylenedioxymethamphetamine on local cerebral blood flow and local cerebral glucose utilisation: data points represent percentage change from saline control animals in limbic brain areas

qualitatively similar in the two parameters; both were decreased in posterior cingulate cortex and superficial layers of the superior colliculus, while both were increased in globus pallidus. However, in keeping with most of the brain, even in these areas the ratio of LCBF to LCMRglu was decreased. A global analysis encompassing all 50 brain areas (Fig. 6) in MDMA-treated rats revealed a close correlation between the two parameters ($r=0.84$), similar to control, but with a reduced overall ratio (slope) of 1.22. Statistical analysis of the two ratio data sets (Mann-Whitney *U* test) revealed that, over the 50 brain areas taken together, the difference in ratios between saline and MDMA-treated animals was highly significant ($P<0.001$).

In only one area, frontal cortex, was the ratio found to increase. This was the only area in the brain in which blood flow increased in the absence of any change in LCMRglu (cf. globus pallidus in which both LCMRglu and LCBF increased), representing a clear hyperaemia.

Discussion

In recent years, an increased incidence of cerebrovascular accident (CVA) in young people has run parallel to an increased use of recreational drugs, including MDMA (Kaku et al. 1990; Hanyu et al. 1995; Petitti et al. 1998; Perez et al. 1999; Auer et al. 2002; Miranda and O'Neill

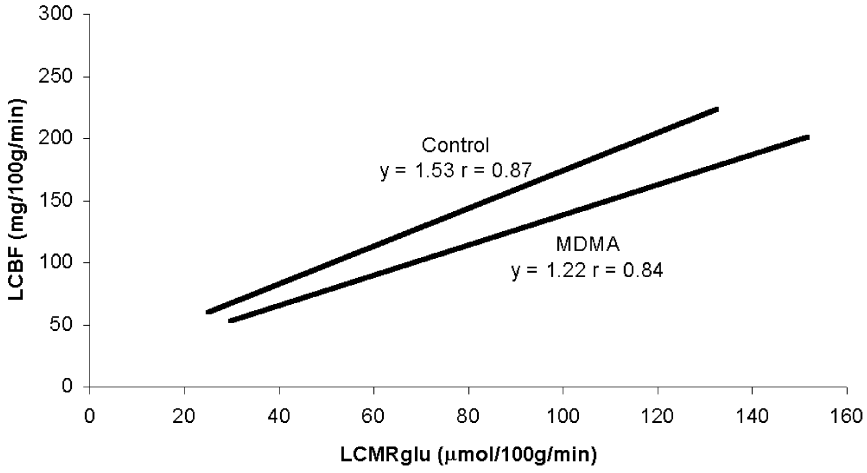

Fig. 6 The relationship between local cerebral blood flow and local cerebral glucose utilisation in 50 brain areas of saline and 3,4-methylenedioxymethamphetamine-treated rats. Data points represent mean LCBF and mean LCMRglu. The best fitting straight line is shown for each treatment group

2002). Although a known association is less frequently reported (Harries and De Silva 1992; McEvoy 2000), MDMA is now considered a risk factor which should be considered as a causative factor when otherwise healthy young individuals present with CVA (Perez et al. 1999; Miranda and O'Neill 2002). However, cerebrovascular dysfunction associated with CVA is a complex, multifactorial, and evolving process, which is mechanistically ill defined, but is known to include frank structural damage to the blood vessels. How MDMA exposure might cause these pathological changes is not clear, particularly when it appears that they may result from the acute effects of the drug (Harries and De Silva 1992).

The acute response to MDMA involves all central monoaminergic systems to some extent, but behavioural responses and the pattern of metabolic activation found in the 2-deoxyglucose studies strongly suggest an involvement of dopaminergic systems in the brain (Wilkerson and London 1989). Certainly the activation that was found most markedly in striatal efferent pathways could result from the inhibition of GABAergic neurones projecting from the caudate with subsequent disinhibition at the terminal sites. Such disinhibition can be produced by lesions of the striatum (Kelly et al. 1982) or by direct pharmacological intervention (Kelly and McCulloch 1984), both of which result in patterns of metabolic activity similar to that reported here. However, it is not possible to distinguish between changes in LCMRglu induced directly by the effects of the treatment, and those that arise due to feedback from drug-induced behaviours. A typical example of the latter was found in somatosensory cortex, where columnar activation is most likely due to proprioceptive feedback from whisker movement rather than any heterogeneity in monoamine receptors.

This problem has been recognised previously (Wilkerson and London 1989) but these authors found no difference in patterns of LCMRglu between unanaesthetised and anaesthetised animal preparations, despite the fact that behavioural changes were not able to be expressed in the latter (Wilkerson and London 1989). In general, Wilkerson and London found patterns of LCMRglu that were anatomically less widespread but, with some exceptions such as neocortex, were similar to those reported here. These authors legitimately interpreted their results, and particularly the activation of extrapyramidal motor areas, in terms of MDMA-induced dopamine release. However, the complex pattern of physiological responses (hyperthermia and myoclonus) and stereotypical behaviours (forepaw treading, piloerection, ataxia and hind-limb abduction) that they described, and that we also found, are indicative of serotonin effects (Li et al. 1989; Wilkerson and London 1989; Green et al. 1995; Frederick and Paule 1997). Thus, both dopamine and 5-HT may be involved in the altered cerebral function induced acutely by MDMA in this study, and this might explain the more widespread changes in LCMRglu which we find in functionally diverse brain areas.

Under normal circumstances, LCBF is closely coupled to the metabolic demands of the brain tissues which are being perfused (Lou et al. 1987). The complementary autoradiographic techniques for measuring LCMRglu and LCBF used in this study have been applied on numerous occasions previously to show that this dynamic relationship remains intact in the face of a variety of physiological and pharmacological challenges (Edvinsson et al. 1983). This approach has proved extremely useful in furthering our understanding of cerebrovascular control mechanisms, and indeed is the only approach that currently allows us to differentiate between direct effects of an experimental intervention on cerebral blood vessels (via specifically vascular effector mechanisms) and indirect effects via changes to neuronal metabolic demand. However, the very different time scales over which the two measures are made, 45 min for LCMRglu and 45 s for LCBF, raises methodological complications that must be accommodated within the experimental design if the two methods are to capture the same events. The dynamics of the 2-deoxyglucose technique are such that the median point of integrated specific 2-deoxyglucose in the brain, from which LCMRglu is derived, is found at around 10 min after the i.v. bolus delivery of the tracer. Thus, an experimental design that incorporates a 10-min delay

between the initiation of LCMRglu and LCBF measurements will, to a large extent, take account of these methodological considerations. In this study, the two measurement parameters were initiated at 15 min (LCMRglu) after MDMA treatment, and 10 min later at 25 min (LCBF) and consequently both methods measure events at a time of peak MDMA effect.

Given the widespread increases in LCMRglu observed in this study following MDMA treatment, one might expect LCBF to increase in parallel. In fact, with only two exceptions (frontal cortex and globus pallidus), LCBF was found to decrease in response to the drug. However, in the globus pallidus the increase in LCBF did not match the increase in LCMRglu so that even here the ratio of mean LCBF to LCMRglu was found to decrease. It would appear, therefore, that there is a direct cerebrovascular response to MDMA, which is independent of changes in metabolic demand, most probably mediated via vascular monoaminergic receptors. Whilst many of the effects of MDMA on neuronal function may be explained by the release of dopamine, this cannot explain the vascular effects. Dopamine generally mediates vasodilatation in cerebral vessels in vitro (Edvinsson et al. 1985) which, if transposed into the intact animal, would result in increased blood flow. There is little evidence that central noradrenergic systems act upon resistance arterioles in the cerebrovascular bed in vivo to alter cerebral blood flow, although the activation of sympathetic innervation of more proximal arteries does have a role to play in pressure autoregulation (Edvinsson and MacKenzie 1977). Only 5-HT appears to provide a mechanism by which MDMA exposure might result in altered cerebrovascular perfusion. The data from this present study would be consistent with MDMA-induced release of 5-HT eliciting cerebrovascular constriction and decreased blood flow. It is interesting to note that inhibition of 5-HT reuptake by citalopram does not affect the normal flow-metabolism relationship (McBean et al. 1999), suggesting that tonic release of 5-HT at normal physiological levels is insufficient to produce the cerebrovascular vasoconstriction induced by MDMA.

In this study, we have provided clear evidence that acute exposure to MDMA results in cerebrovascular dysfunction, from which oligaemic conditions arise. The uncoupling of cerebral blood flow from underlying metabolic demand could contribute to feelings of confusion associated with acute exposure to the drug, but may also promote CVA. The relative oligaemia in response to the drug that we have described here, will result in an insufficiency in supply of blood-borne glucose to the tissues of the brain and, if it is prolonged, could conceivably result in irreversible brain damage. It is, however, unlikely that this situation would progress to that extent a build-up of local metabolites would cause dilatation (Kuschinsky and Wahl 1978). It is much more likely that it is this uncontrolled loss of constrictor tone, possibly together with sustained hypertension, which is directly responsible for MDMA-induced cerebrovascular accident (Kelly et al. 1995). There is some evidence that such a mechanism may be apparent in our MDMA-treated rats; in frontal cortex an increased blood flow was measured, with no change in metabolism, which might suggest localised vasodilatation and it is interesting to note that it is in this area of the brain that intracerebral haemorrhage has been reported clinically (Harries and DeSilva 1992).

It is clear that not all users of the drug succumb to CVA, and it is possible that MDMA merely contributes to pre-existing conditions, such as congenital abnormalities in vascular structure. In otherwise healthy young adults, these may remain occult until presented with the pharmacological and physiological challenges induced by the drug. Our results are relevant in providing a greater understanding of MDMA-induced CVA.

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