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Short communication

Hyperaemia in rat neocortex produced by acute exposure to methylenedioxymethamphetamine

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Abstract

Cerebral blood flow and glucose utilization were measured in rat neocortex, hippocampus and striatum following methylenedioxymethamphetamine injection (5 mg/kg, i.v.), using the tracers [^{14}C]iodoantipyrine and [^{14}C]2-deoxyglucose, respectively. In control rats, blood flow was coupled to glucose metabolism, but in methylenedioxymethamphetamine-treated rats, marked hyperperfusion was measured in frontal and parietal cortex with no change in glucose use. This suggests that methylenedioxymethamphetamine has the potential to disrupt cerebrovascular control.

Keywords: Amphetamine abuse; Methylenedioxymethamphetamine; Local cerebral blood flow; Cerebrovascular control; Cerebrovascular accident

Methylenedioxymethamphetamine (MDMA; 'Ecstasy') is a psychostimulant drug of abuse which is used regularly by a growing number of young adults in particular. Despite evidence to the contrary from animal studies [2,12], MDMA is widely perceived as a 'safe' recreational drug. However, a recent report has suggested that as with other amphetamines [4], a link exists between exposure to MDMA and the occurrence of intracerebral haemorrhage in otherwise healthy young adults [9].

Although these clinical observations suggest that MDMA might in some way promote cerebrovascular accident, uncertainty remains as to whether it is the amphetamine derivative itself, or some contaminant in the illicit 'ecstasy' available on the streets, which is responsible for the apparent pathological disturbances in cerebrovascular physiology. Moreover, abusers who have presented with cerebrovascular accident may also have been using other substances which might bear primary responsibility for the condition, or at least provide a contributory factor [9]. In this investigation, we have examined whether a single exposure to MDMA in its pure form has the potential to disrupt cerebrovascular regulatory processes in the laboratory rat, and if so, to provide some insight into the possible mechanisms which might be involved in the aetiology of cerebrovascular accident in human users.

Male Sprague–Dawley rats were anaesthetized with halothane in a mixture of oxygen (30%) and nitrous oxide (70%) and cannulae were inserted into both femoral arteries (for the measurement of arterial blood pressure and the sampling of arterial blood), and both femoral veins (for the injection of drugs and radiolabelled tracers). All surgery was performed by fully trained and experienced personnel and complied with local ethical codes and UK government regulations. A loose-fitting plaster cast was applied around the lower abdomen and pelvis, with care being taken to ensure that respiratory movement of the thorax was not compromised. Thus restrained, and supported on blocks, the rats were allowed to recover from anaesthesia for at least two hours before further manipulation. Arterial blood pressure and rectal temperature were monitored continuously, and blood gases were measured before the drug treatment and again immediately before the initiation of the measurement procedures for cerebral glucose utilization or blood flow.

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3,4-Methylenedioxymethamphetamine hydrochloride (MDMA; Sigma) was dissolved in saline (5 mg/ml), and rats were injected i.v. with 5 mg/kg made up to a final volume of 0.6 ml with excess saline ($n = 12$) or with the same volume of saline alone ($n = 12$). The measurement of local cerebral glucose utilization (LCMRglu) was initiated five minutes after the injection using the glucose analogue [^{14}C]2-deoxyglucose according to the methodology of Sokoloff et al. [16]. Local cerebral blood flow (LCBF) was measured 15 min after the injection of MDMA, using the freely-diffusible tracer [^{14}C]iodoantipyrine according to methodology derived by Sakurada et al. [13]. The experimental procedures were performed in exact accordance with the methodolgy as originally published and as reported in detail from this laboratory previously [14].

At the end of the measurement periods, the animals were killed by intravenous injection of barbiturate and the brains rapidly dissected intact from the skull. Freshly dissected tissue samples from 3 areas of neocortex (parietal, occipital, and frontal including cingulate cortex up to the midline) as well as two subcortical areas (hippocampus and striatum), were weighed, digested in tissue solubilizer (Soluene, Packard), and processed for liquid scintillation analysis to measure tissue tracer concentrations. Neocortical areas were chosen to provide samples from different cerebrovascular territories (anterior, middle, and posterior cerebral arteries). Both LCMRglu and LCBF were calculated from ^{14}C concentrations measured in brain tissue samples and in blood samples taken during the experiments, using the appropriate operational equations for the techniques [13,16].

All data are presented throughout as mean ± standard error of the mean. Statistical analysis of blood flow and glucose use measurements were performed using Student's t-test with acceptable levels of significance set at $P < 0.05$.

There were no significant differences in physiological status between groups of rats before treatment, or after injection with saline alone. The injection of MDMA produced transient hypertension (MABP in the range 160–190 mmHg) which was rapid in onset, but lasted no more than 2–3 min after the end of the injection, and was totally resolved (MABP = 130 ± 8 mmHg) before the initiation of the measurement procedures. This hypertensive episode coincided with a period of intense motor activity which had many of the components of the 'serotonergic syndrome' [8]. By 15 min post-injection, when the blood flow measurements were initiated, plasma glucose in MDMA-treated rats (2.46 ± 0.4 g/l) was significantly increased above both pre-injection levels (1.65 ± 0.18) and the appropriate saline-injected controls (1.65 ± 0.18).

Despite the intense motor activity in MDMA-treated rats, there were no significant changes in LCMRglu in any of the areas of the brain analysed in this study (Table 1), although a 19% increase in striatum (= 2.023) just failed to reach statistical significance (critical $t = 2.145$). In marked contrast to the apparent lack of effect upon LCMRglu, increases in LCBF were measured in frontal cortex (+107%) and parietal cortex (+67%) of MDMA-treated rats (Table 1). However, no changes were evident either in more caudal neocortical areas or in subcortical regions.

In control animals, LCBF was closely coupled to LCMRglu in all brain areas analysed (correlation coefficient, $r = 0.718$), and the overall flow/metabolism ratio, as illustrated by the slope (m) of the best fitting straight line through the data points, was 1.90 (Fig. 1A). This relationship between LCBF and LCMRglu was also evident in occipital cortex, striatum and hippocampus of MDMA-treated rats (Fig. 1A). However, in frontal and parietal cortices, LCBF was greatly in excess of metabolic demand and the ratio of LCBF to LCMRglu in these two areas (4.56 and 3.92, respectively), could be clearly identified as 'outliers' from the overall mean ratio of 1.9 using box-and-whisker analysis (Fig. 1B).

Under normal circumstances, a close physiological relationship is maintained between synaptic activity, energy generation and blood flow in the mammalian brain [15]. This dynamic relationship remains largely intact, even in the face of a variety of physiological and pharmacological challenges, including treatment with amphetamines [3]. In the present study, however, acute

Table 1
Local cerebral blood flow and glucose utilization in saline- and MDMA-treated rats

Brain area	Saline treatment		MDMA treatment	
	LCMRglu (μmol/100 g/min)	LCBF (ml/100 g/min)	LCMRglu (μmol/100 g/min)	LCBF (ml/100 g/min)
Frontal cortex	80 ± 13	174 ± 20	79 ± 12	360 ± 121 *
Parietal cortex	75 ± 17	199 ± 30	85 ± 15	333 ± 145 *
Occipital cortex	63 ± 7	157 ± 22	59 ± 6	160 ± 55
Striatum	79 ± 11	154 ± 15	94 ± 19	174 ± 31
Hippocampus	55 ± 11	130 ± 11	53 ± 14	120 ± 10

Data are presented as mean ± S.E.M.
* Significantly different from appropriate saline control group; $P < 0.05$.

A)

Local Cerebral Blood Flow (ml/100g/min)

B)

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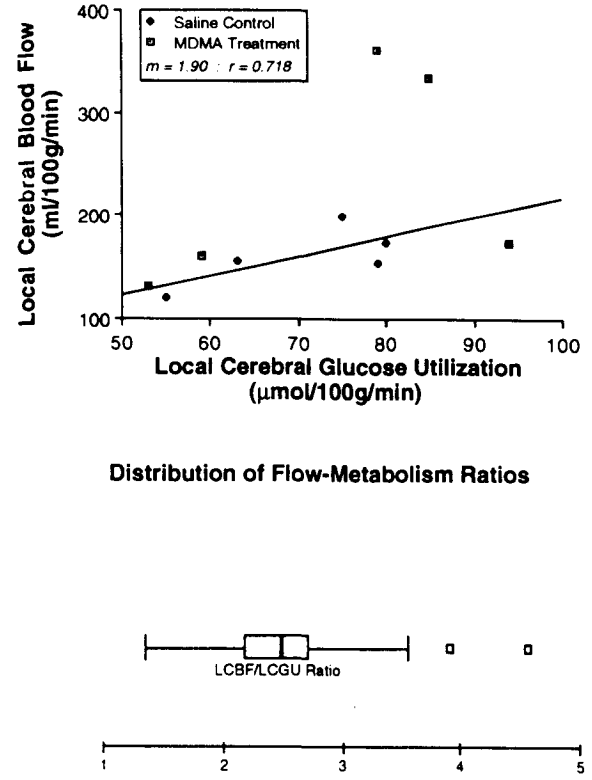

Fig. 1. A: the relationship between mean LCMRglu and mean LCBF in the five brain areas measured in saline- and MDMA-treated rats. The best fitting straight line through the control data points is illustrated. B: box-and-whisker plot of LCBF/LCMRglu ratios in areas of the brain from both control and MDMA-treated rats, indicating the median, upper and lower quartiles (box) ±1.5 times the interquartile range (whiskers). The ratio of flow to metabolism in both frontal and parietal cortices of MDMA-treated rats are identified as 'outliers', being greater than 1.5 times the interquartile range.

exposure to the amphetamine derivative MDMA on a single occasion resulted in focal uncoupling of blood flow from metabolic demand in frontal and parietal cortex of the conscious rat. In these two areas, the marked increase in flow–metabolism ratios indicate that blood flow was greatly in excess of the metabolic demand of brain tissues for blood-borne glucose which, in keeping with low doses of MDMA used in previous studies of LCMRglu [18], remained relatively unchanged. This focal hyperaemia could represent a direct action of the drug upon cerebrovascular receptors to induce dilatation, but all available pharmacological evidence indicates that MDMA should induce constriction in cerebral smooth muscle via direct interaction with serotonergic and adrenergic receptors [1]. Moreover, within the neuropil of the brain as a whole, the principal actions of MDMA are thought to be mediated via its capacity to inhibit monoamine, but in particular serotonergic re-uptake mechanisms [1], and if a similar effect were to be induced in perivascular monoaminergic nerve terminals, again a constrictory decrease in flow would be expected [6].

The resistance vessels of the cerebrovascular bed are endowed with the dynamic capacity to alter their calibre in response to fluctuations in perfusion pressure. The resulting changes in vascular resistance ensure the maintenance of a constant cerebral blood flow over a wide range of arterial blood pressures, a phenomenon known as autoregulation [11]. However, autoregulatory capacity is not infinite, and if blood pressure exceeds the upper limit of autoregulation, forced dilatation of cerebral blood vessels ensues and cerebral blood flow increases in direct proportion to the lowered resistance [10]. If blood pressure continues to rise, cerebral blood vessels may suffer lasting physical disruption, with loss of constrictor capacity, and blood flow remains elevated even after arterial blood pressure returns to levels normally within the autoregulatory range [17]. In this study the hypertension induced by MDMA (range 160–190 mmHg) was significantly above the normal upper limit of autoregulation [11].

A possible mechanism for MDMA-induced cortical hyperaemia may therefore lie in the pronounced, but transient hypertension induced in the first few minutes after injection, the effects of which could outlast the actual cardiovascular phenomenon [17]. Acute hypertension is also a feature of the response to MDMA in man [5], and it is intriguing to note that there are close parallels between the pattern of hyperaemia produced in this study and the distribution of intracerebral haemorrhage reported clinically [9]. In man, however, where the route of ingestion is not normally parenteral, the hypertension is slower in onset but longer lasting. Whatever the cause of the cerebrovascular dysfunction identified in this study, it would appear that if this phenomenon were also to be manifest in the human cerebrovascular bed, then intracerebral haemorrhage could result from normally sustainable physiological challenge to blood vessels in which focal loss of cerebrovascular tone has been established.

These studies, together with a growing number of clinical observations, lend added weight to the assertion that 'ecstasy' is not a safe recreational drug.

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