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EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT

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Summary—The effects of (±)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") (5, 10, 15 or 30 mg/kg. i.p.) on local cerebral utilization of glucose were studied by the quantitative autoradiographic 2-deoxy-D-[1-^{14}C]glucose method in awake adult male Fischer-344 rats. Statistically significant effects on local utilization of glucose, 5 min after the administration of MDMA were observed in 20 of 60 areas of brain sampled. Marked stimulation was seen in components of the extrapyramidal motor system (substantia nigra, globus pallidus, entopenduncular nucleus, subthalamic nucleus, cerebellar vermis). The limbic system showed decrements in the medial cortex and hippocampal dentate gyrus (outer blade) and the lateral habenula, while there was stimulation in the mammillary body and the basolateral amygdaloid nucleus. Glucose utilization in MDMA-treated rats was reduced in the superior colliculus and medial terminal nucleus of the accessory optic system, but was unchanged in the visual cortex and components of the auditory system. Some of the effects of MDMA on cerebral utilization of glucose resembled those previously reported with *l*-cocaine, *d*-amphetamine, and phencyclidine, implicating some common mechanisms in the actions of these drugs.

Key words—2-deoxy-D-[1-^{14}C]glucose, glucose utilization, methoxyamphetamine derivatives, methylenedioxymethamphetamine, psychomotor stimulants, drug abuse.

(±)3,4-Methylenedioxymethamphetamine(MDMA), first synthesized and patented in 1914 to be used as an appetite suppressant (Merck, 1914), is structurally similar to its parent compounds, methylenedioxyamphetamine (MDA), and mescaline. Aside from a study on the toxicity and behavioural effects of mescaline and several analogues (including MDMA), MDMA received little attention until 1968, when its nonmedical use was seen in the western United States (Seymour, 1986). Recreational use of MDMA became widespread by the late 1970's (Siegel, 1986) and continued until 1985, when it was placed under Schedule I by the Drug Enforcement Agency (Shulgin, 1986).

Various effects of MDMA on the central nervous system have been noted in dogs and monkeys (Hardman, Haavik and Seevers, 1973). These include influences on motor activity, emesis, apprehension or fright and evidence of hallucinations. Behavioural studies have demonstrated that subhuman primates self-administer MDMA (Beardsley, Balstar and Harris, 1986; Lamb and Griffiths, 1987) and that rats, trained to discriminate serotonergic and/or dopaminergic drugs, generalize to MDMA (Glennon and Young, 1984; Schechter, 1986). Acute reactions reported in humans include increased acuity to cold and color, vomiting, visual hallucinations, ataxia and crying (Hayner and McKinney, 1986). However, many positive subjective effects of MDMA have been reported by human subjects. The effects, including feelings of euphoria or love, enhanced self-confidence, lowering of defenses and heightening of awareness, have led some psychiatrists to suggest that MDMA may be a beneficial adjunct to psychotherapy (Greer and Tolbert, 1986).

The purpose of the present study was to identify areas in the brain that might be activated or inhibited by acute treatment with MDMA and which might be important to the psychotropic actions of the drug. The autoradiographic 2-deoxy-D-1-[^{14}C]glucose ([^{14}C]DG) method was used to measure rates of local cerebral utilization of glucose in rats treated with one of several doses of MDMA (Sokoloff, Reivich, Kennedy, Des Rosiers, Patlak, Pettigrew, Sakurada and Shinohara, 1977). The [^{14}C]DG method has been used to help map the cerebral actions of several psychomotor stimulants, including *d*-amphetamine (Wechsler, Savaki and Sokoloff, 1979), *l*-cocaine (London, Wilkerson, Goldberg and Risner, 1986) and phencyclidine (Weissman, Dam and London, 1987).

METHODS

Preparation of animals

Male Fischer-344 rats were used at 16–21 weeks of age, after a 3–5 week period of habituation in a vivarium. Food and water were freely available until

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the night before the experiment, when the food was removed in order to stabilize levels of glucose in the plasma. On the day of the experiment, catheters were inserted into a right femoral artery and vein of each rat, under 2–2.5% halothane anesthesia. The wound was irrigated with a local anesthetic (bupivacaine HCl, 0.1 ml of 0.05%) at the beginning and end of the operation. Immediately after surgery, each rat was partially restrained in a loose-fitting plaster cast and was placed in a sound-insulated box (London, Nespor, Ohata and Rapoport, 1981). The animals were allowed to recover for approximately 3.5 hr before experimentation. In order to regulate body temperature during the recovery and experimental period, a rectal thermoprobe was inserted and attached to a feedback device which heated the chamber when the temperature of the rat fell below 37°C (YSI indicating Controller Model 73ATA; Yellow Springs Instruments, Yellow Springs, Ohio).

Treatment with drugs

The MDMA (NIDA, Research Technology Branch, Rockville, Maryland) was dissolved in 0.9% NaCl and given at 5, 10, 15 or 30 mg/kg (i.p.). Control rats received an equal volume of the vehicle (1 ml/kg 0.9% NaCl). The rats were given injections of MDMA or 0.9% NaCl 5 min before [^{14}C]DG. The dose range employed included doses greater than that which was used effectively in drug discrimination studies in rats (Schechter, 1986) and spanned those which have produced alterations in dopaminergic and serotonergic neurochemical markers *in vivo* (Schmidt, Wu and Lovenberg, 1986; Schmidt, 1987).

Physiological measurements

Prior to and during the [^{14}C]DG experiment, several physiological parameters were measured. Body temperature was recorded. Blood pressure and heart rate were measured by connecting a strain gauge transducer (Gould Statham Instruments, Hatorey, Puerto Rico) to the femoral artery catheter and were recorded on a chart recorder (Model 2200, Gould, Cleveland, Ohio). Arterial blood was withdrawn for the assay of blood gases (Pa_{O_2}, Pa_{CO_2}) and pH (pH-blood gas analyzer, System 1302, Instrumentation Laboratory, Lexington, Massachusetts). Some samples were centrifuged for the assay of the concentration of glucose in plasma.

Determination of local cerebral utilization of glucose

The [^{14}C]DG procedure was performed as described previously (Sokoloff *et al.*, 1977). Rats were killed 45 min after the injection of [^{14}C]DG by an intravenous injection of 50 mg pentobarbital. The brains were then quickly removed and frozen in 2-methylbutane at approximately −60°C. They were stored at −70°C until they were cut into 20 μm sections on a cryostat (Hacker Instruments Inc., Fairfield, New Jersey) maintained at −18 to −20°C. The sections were thaw-mounted onto coverslips, dried on a hot plate and then apposed to X-ray film (Kodak SB5) in cassettes for 1 week, along with [^{14}C]methylmethacrylate standards.

Regions of the brain were identified with the aid of an atlas of the brain of the rat (Paxinos and Watson, 1982). The radioactivity of specific areas of the brain was determined by measuring and comparing point measurements (60×72 μm diaphragm) of optical densities in corresponding autoradiograms to those in the standards on the same X-ray film, using a DADS 560 image analysis system (E. Leitz, Inc., Rockleigh, New Jersey). The average rate of utilization of glucose for each area of the brain was taken from readings on autoradiograms of 6–8 sections of the brain. Rates of local cerebral utilization of glucose were calculated from the concentrations of radioactivity in the brain and the plasma and concentrations of glucose, using the operational equation for the [^{14}C]DG method and published values of the rate constants for the transport and phosphorylation of [^{14}C]DG and the lumped constant (Sokoloff *et al.*, 1977).

Statistical analysis

Statistical significance of the effects of MDMA on the utilization of glucose was determined by a separate one-way analysis of variance for each region of the brain. If the F statistic indicated an effect of treatment which was statistically significant at $P < 0.05$, the individual means from groups given various doses of MDMA were compared with the control mean, using Dunnett's test. Data on physiological parameters were analysed by 2-way analysis of variance, with time as a repeated measure. In the one case, in which a statistically-significant interaction was obtained, the individual means were compared with Tukey's ω procedure.

RESULTS

Behavioral effects

Methylenedioxymethamphetamine produced stereotyped behavior (head weaving, sniffing and grooming) which began 2–3 min after the administration of the drug and lasted for approximately 40 min. In addition, MDMA-treated rats exhibited struggling within the confines of their restraints. This behavior was not seen in the control rats. Rats given 10–30 mg/kg MDMA were hyperreactive to touch, exhibiting marked irritability and vocalization in response to any tactile stimulus. Also present in the MDMA-treated animals was the Straub tail response, which was mainly observed at the larger doses (15 and 30 mg/kg). Exophthalmos was seen at all doses of MDMA tested.

Physiological parameters (Table 1)

Although there was some tachycardia, which was noted during the experimental procedure compared to pre-drug baseline conditions, neither the effect of

Table 1. Physiological variables in saline- or MDMA-treated rats

	Time (min)*	Saline Control ($n = 6$)	5 mg/kg MDMA ($n = 6$)	10 mg/kg MDMA ($n = 6$)	15 mg/kg MDMA ($n = 5$)	30 mg/kg MDMA ($n = 5$)
Temperature** (°C)	0	37.1 ± 0.13	37.1 ± 0.42	36.9 ± 0.19	36.8 ± 0.21	36.9 ± 0.12
	30	36.6 ± 0.16	37.1 ± 0.29	37.9 ± 0.51†	38.2 ± 0.37†	37.9 ± 0.56†
Arterial plasma glucose* (mg/100 ml)	0	143 ± 4	144 ± 4	143 ± 7	143 ± 17	154 ± 6
	35	156 ± 9	198 ± 17	174 ± 13	197 ± 13	171 ± 9
Arterial blood pH*	0	7.4 ± 0.01	7.4 ± 0.01	7.4 ± 0.01	7.4 ± 0.01	7.4 ± 0.01
	36	7.4 ± 0.01	7.3 ± 0.04	7.3 ± 0.04	7.3 ± 0.05	7.4 ± 0.02
Arterial blood P_aCO_2* (mm Hg)	0	36.1 ± 1.2	37.2 ± 1.4	36.9 ± 0.50	37.3 ± 0.94	37.1 ± 1.1
	36	35.0 ± 0.82	34.4 ± 1.8	31.4 ± 2.0	29.4 ± 3.2	30.6 ± 2.9
Arterial blood P_a0_2* (mm Hg)	0	91.3 ± 2.7	86.2 ± 2.8	88.8 ± 2.5	94.0 ± 2.6	94.8 ± 2.4
	36	97.7 ± 1.5	93.4 ± 5.8	91.4 ± 6.4	103 ± 9.4	98.3 ± 5.8
Heart rate* (beats/min)	0	405 ± 25	396 ± 20	432 ± 22	420 ± 17	408 ± 16
	36	415 ± 26	429 ± 21	447 ± 37	461 ± 24	455 ± 39
Mean arterial blood pressure*	0	121 ± 2	102 ± 6	118 ± 2	112 ± 4	120 ± 5
(mm Hg)	36	108 ± 8	113 ± 10	135 ± 9	126 ± 9	132 ± 10

*Time of measurement was relative to the intravenous injection of [^{14}C]DG, which was 5 min after the intraperitoneal injection of MDMA or 0.9% NaCl.
*Significant effect of treatment with drug and time of measurement by two-way analysis of variance, $P < 0.05$.
**Significant interaction (dose × time) by two-way analysis of variance, $P < 0.05$.
†Significant from mean temperature at time 0 and mean of saline control by Tukey's ω procedure, $P < 0.05$.

drug nor time, nor the interaction between the drug and time, reached statistical significance ($P > 0.05$). There were no statistically significant effects of MDMA or interactions between MDMA and time on arterial blood pressure or blood gases and pH; however, each of these parameters showed statistically significant effects of time as follows: mean arterial blood pressure [$F(1,24) = 5.8$, $P < 0.05$], pH [$F(1,24) = 8.2$, $P < 0.01$] Pa_{CO_2} [$F(1,24) = 16.4$, $P < 0.01$] and Pa_{O_2} [$F(1,24) = 4.6$, $P < 0.01$] all showed significant effects of time. Changes in temperature showed statistical significance in the effect of time [$F(1,24) = 9.8$, $P < 0.01$] and the interaction between time and treatment with MDMA [$F(4,24) = 4.05$, $P < 0.01$], reflecting hyperthermia in rats treated with 10, 15 or 30 mg/kg of MDMA.

Local cerebral utilization of glucose

Methylenedioxymethamphetamine produced statistically significant effects on the utilization of glucose in 20 of the 60 regions assayed (Table 2). In most cases, the effects did not vary as a function of dose. The greatest effect, in terms of the magnitude and number of regions affected, occurred in the extrapyramidal motor system. Mean increases of 42–73% and 48–98% over control were observed in the globus pallidus and substantia nigra pars reticulata, respectively, in response to all doses of MDMA. The entopeduncular nucleus also showed stimulation in response to all doses tested; the magnitude of the increase was 34–53% over control. A uniform effect was not obtained in the substantia nigra reticulata. While the ventromedial portion showed a significant increase, utilization of glucose in the dorsolateral portion remained unchanged (Fig. 1). The 15 and 30 mg/kg doses stimulated utilization of glucose in the subthalamic nucleus by 30–35%, but the zona incerta was unaffected. All doses of MDMA increased utilization of glucose in the cerebellar vermis by 56–75%.

Effects varied within the cerebral cortex. Utilization of glucose in the anterior cingulate cortex was reduced by 25% in response to the largest dose of MDMA tested. The posterior cingulate and retrosplenial cortices showed reductions of 21–30% and 32–38%, respectively, in response to 10, 15 and 30 mg/kg MDMA. There were no effects in the frontoparietal motor, striate and entorhinal areas of cortex.

Although the striate cortex was unaffected, as was the dorsal lateral geniculate nucleus, other components of the visual pathways showed responses to MDMA. The medial terminal nucleus of the accessory optic tract and the superficial layer of the superior colliculus both showed a 30% decrease in utilization of glucose in response to 30 mg/kg MDMA. In addition, a reduction of 27% in response to 5 mg/kg MDMA reached statistical significance in the medial terminal nucleus. In addition, the principal oculomotor nucleus showed mean increases of 42–67% over control at all doses of MDMA.

Various subcortical limbic areas showed effects of MDMA. There was a 23% decrease in the outer blade of the dentate gyrus, in response only to the largest dose of MDMA. The basolateral amygdaloid nucleus showed an increase in response to MDMA, which reached statistical significance only at the 5 mg/kg dose (24%). Other limbic areas, the lateral habenula (mean decreases of 26–45%) and the mammillary body (mean increases of 28–34%), showed significant effects of 5 mg/kg, as well as larger doses of MDMA. The accumbens nucleus and ventral tegmental area showed no significant effects.

Thalamic nuclei were not universally affected by

MDMA. A statistically significant decrease of 17% was seen in the gelatinosus nucleus in response to 30 mg/kg MDMA, whereas an increase of 39% was seen in the reticular nucleus with only the 15 mg/kg dose. All doses of MDMA produced significant increases of 26–39% in the ventromedial nucleus.

Table 2. Effects of MDMA on local cerebral utilization of glucose

Region of brain	Atlas Figure[a]	Saline Control ($n = 6$)	5 mg/kg MDMA ($n = 6$)	10 mg/kg MDMA ($n = 6$)	15 mg/kg MDMA ($n = 5$)	30 mg/kg MDMA ($n = 5$)
Cerebral cortex						
Medial prefrontal		82 ± 10	84 ± 5	87 ± 9	74 ± 3	73 ± 15
Anterior cingulate	15	107 ± 9	103 ± 6	86 ± 5	91 ± 4	80 ± 4*
Somatosensory, layer 4	18	110 ± 9	110 ± 7	102 ± 7	105 ± 6	98 ± 7
Frontoparietal Motor, layer 4	18	111 ± 9	118 ± 6	110 ± 9	116 ± 10	101 ± 09
Posterior cingulate	20	118 ± 6	111 ± 8	87 ± 6*	93 ± 8*	83 ± 5*
Auditory, layer 4	26	143 ± 6	144 ± 9	136 ± 8	146 ± 11	129 ± 13
Retrosplenial cortex	26	107 ± 6	89 ± 8	73 ± 6*	73 ± 7*	66 ± 4*
Striate, layers 1–3	27	96 ± 6	88 ± 6	80 ± 8	87 ± 8	80 ± 6
layer 4	27	110 ± 4	109 ± 6	99 ± 9	114 ± 11	95 ± 7
Entorhinal	31	74 ± 3	74 ± 4	69 ± 8	69 ± 5	62 ± 4
Accumbens nucleus (n.)	10	92 ± 7	105 ± 5	109 ± 7	118 ± 9	104 ± 6
Lateral septum, intermediate	14	75 ± 7	88 ± 5	84 ± 6	92 ± 7	85 ± 7
Globus pallidus	16	52 ± 3	74 ± 5*	80 ± 6*	90 ± 7*	81 ± 5*
Caudate putamen	17	79 ± 6	92 ± 5	91 ± 6	92 ± 4	88 ± 9
Basolateral amygdaloid n.	20	89 ± 5	110 ± 5*	100 ± 5	108 ± 8	91 ± 6
Dentate gyrus:						
Molecular layer	22	88 ± 4	92 ± 5	76 ± 4	87 ± 5	76 ± 4
Outer blade	23	61 ± 4	59 ± 3	50 ± 3	53 ± 5	47 ± 3*
Dorsal hippocampus, CA1	22	58 ± 4	59 ± 4	51 ± 4	55 ± 4	50 ± 3
CA3	22	67 ± 5	76 ± 6	67 ± 6	71 ± 6	65 ± 4
Subiculum	29	84 ± 5	79 ± 4	74 ± 8	78 ± 8	67 ± 7
Parasubiculum	30	82 ± 6	77 ± 5	68 ± 7	74 ± 7	65 ± 9
Anterior hypothalamus	18	57 ± 4	64 ± 4	63 ± 4	66 ± 5	57 ± 3
Thalamic nuclei:						
Reticular	17	84 ± 5	104 ± 7	103 ± 4	117 ± 9*	98 ± 9
Anteroventral	17	97 ± 4	115 ± 8	108 ± 4	113 ± 4	101 ± 7
Lateral dorsal	19	89 ± 5	95 ± 5	85 ± 5	86 ± 5	77 ± 3
Rhomboid	19	77 ± 4	100 ± 7	94 ± 5	96 ± 6	87 ± 6
Gelatinosus	19	123 ± 5	127 ± 4	111 ± 6	115 ± 5	102 ± 4*
Ventromedial	20	104 ± 7	136 ± 5*	135 ± 5*	145 ± 8*	131 ± 9*
Lateral posterior	22	96 ± 5	105 ± 7	107 ± 8	111 ± 6	101 ± 3
Medial geniculate body	25	120 ± 8	127 ± 5	123 ± 8	133 ± 8	118 ± 8
Dorsal lateral geniculate n.	23	77 ± 3	81 ± 3	71 ± 4	80 ± 4	74 ± 4
Subthalamic n.	22	86 ± 3	103 ± 6	98 ± 6	116 ± 9*	112 ± 7*
Mammillary body	24	115 + 6	154 ± 8*	139 ± 8	140 ± 11	147 ± 11*
Habenula, medial	22	69 ± 5	74 ± 5	65 ± 4	72 ± 3	63 ± 4
lateral	22	130 ± 3	96 ± 4*	80 ± 4*	85 ± 4*	71 ± 4*
Zona incerta	22	81 ± 4	94 ± 4	91 ± 4	96 ± 7	85 ± 5
Entopeduncular n.	19	53 ± 3	71 ± 4*	72 ± 5*	81 ± 5*	73 ± 5*
Inferior colliculus	30	176 ± 12	176 ± 8	163 ± 7	175 ± 13	155 ± 14
Superior colliculus, superficial grey layer	26	76 ± 5	74 ± 6	60 ± 5	65 ± 6	53 ± 5*
deep grey layer	26	81 ± 6	100 ± 8	97 ± 6	97 ± 13	90 ± 7
Anterior pretectal area	25	96 ± 3	122 ± 7	123 ± 9	126 ± 11	117 ± 6
Substantia nigra, compacta	25	74 ± 5	84 ± 9	80 ± 5	88 ± 6	84 ± 5
Substantia nigra, reticulata:						
ventromedial portion	25	58 ± 7	86 ± 8*	96 ± 7*	115 ± 8*	110 ± 7*
dorsolateral portion	25	52 ± 6	61 ± 5	60 ± 5	64 ± 5	61 ± 6
Medial terminal n.	25	74 ± 4	54 ± 4*	56 ± 5	58 ± 6	52 ± 6*
Red n.	26	80 ± 4	95 ± 7	97 ± 8	106 ± 11	96 ± 7
Ventral tegmental area	26	63 ± 4	78 ± 6	76 ± 5	87 ± 8	78 ± 5
Interpeduncular n.	26	112 ± 5	121 ± 8	107 ± 7	120 ± 9	105 ± 10
Principal oculomotor n.	28	86 ± 7	131 ± 9*	128 ± 9*	144 ± 12*	122 ± 8*
Central grey	30	67 ± 5	75 ± 4	73 ± 5	85 ± 9	77 ± 4
N. of Darkschewitsch	25	88 ± 5	113 ± 8	107 ± 6	110 ± 8	105 ± 8
Pontine reticular formation	32	61 ± 4	72 ± 4	73 ± 4	84 ± 6*	73 ± 5
Dorsal raphe n.	30	90 ± 6	97 ± 5	93 ± 6	102 ± 6	92 ± 9
Median raphe n.	30	105 ± 5	100 ± 5	94 ± 6	104 ± 6	94 ± 11
Raphe magnus n.	35	54 ± 4	61 ± 4	61 + 4	75 + 6*	68 ± 3
Raphe obscurus n.	39	57 ± 3	73 ± 5	70 ± 6	80 ± 7	74 ± 5
Locus Coeruleus	33	82 ± 3	92 ± 4	86 ± 7	95 ± 9	92 ± 9
Cerebellar vermis	35	93 ± 6	147 ± 7*	148 ± 12*	163 ± 13*	145 ± 13*
Fiber pathways:						
Corpus collosum	15	32 ± 5	32 ± 3	32 ± 2	33 ± 5	27 ± 2
Internal capsule	19	38 ± 3	42 ± 3	36 ± 4	39 ± 4	37 ± 3

Values are the means ± SEM (μmol/100 g/min).

[a]The numbers indicate figures in a stereotaxic atlas (Paxinos and Watson, 1982) which was used to determine the anatomical location of the various regions of the brain sampled.

*$P < 0.05$ by one-way analysis of variance and Dunnett's test.

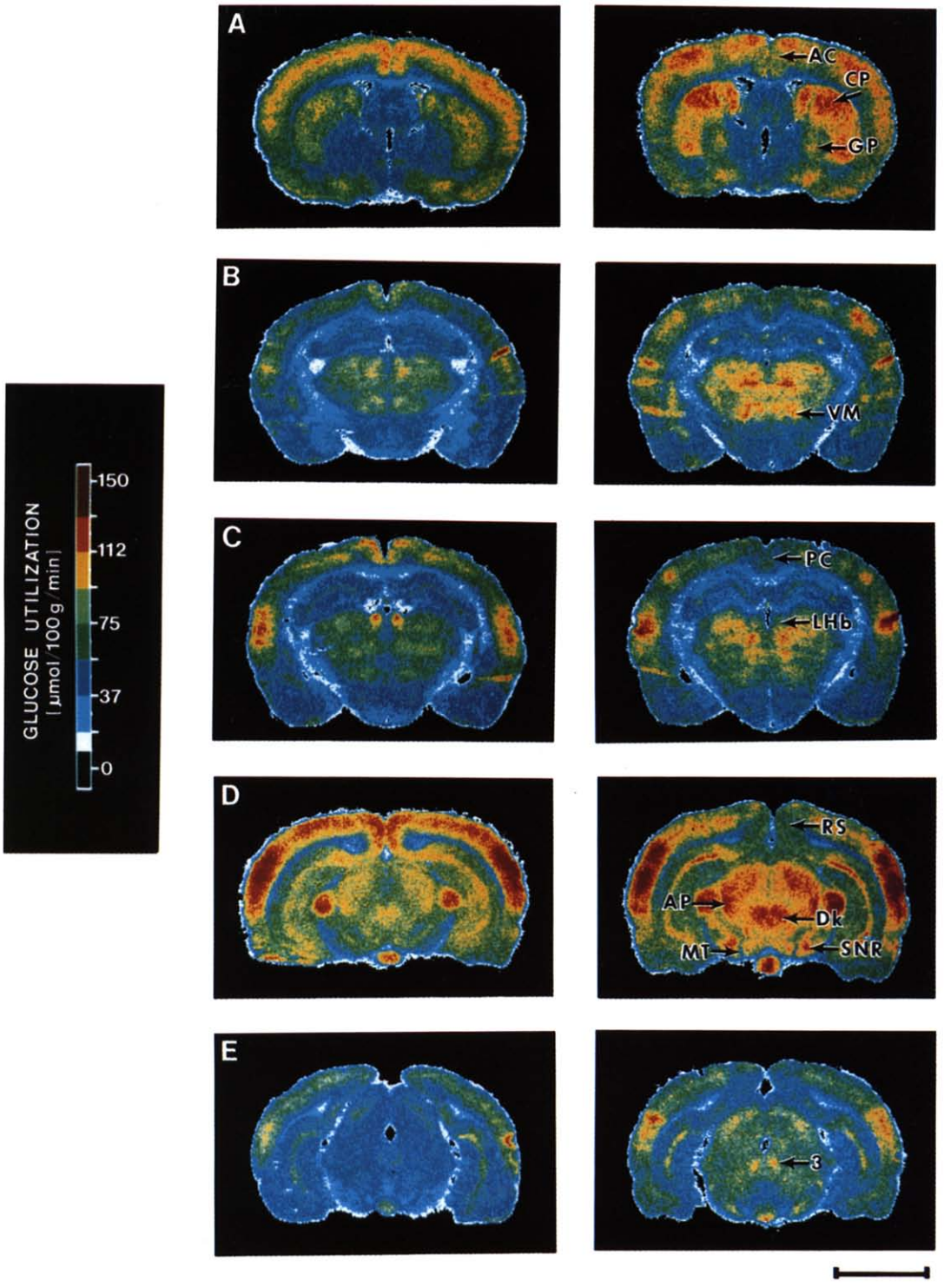

Fig. 1. Color-coded transforms of autoradiograms, showing rates of local cerebral utilization of glucose in a rat given an injection of 0.9% NaCl (left) and another rat given 30 mg/kg MDMA (right) intraperitoneally 5 min before [^{14}C]DG. (A) Sections at the level of the corpus striatum showed a statistically-significant decrease of utilization of glucose in the anterior cingulate cortex (AC) and an increase in the globus pallidus (GP). Stimulation in the caudate-putamen (CP) did not reach statistical significance. (B) At the level of the thalamus, increases were noted in several nuclei, including the ventromedial n. (VM). (C) At the level of the epithalamus, MDMA decreased utilization of glucose in the lateral habenula (LHb) and posterior cingulate cortex (PC). (D) Sections at the level of the substantia nigra showed marked stimulation in the ventromedial portion of the substantia nigra pars reticulata (SNR) and decreases in utilization of glucose in the retrosplenial cortex (RS) and medial terminal n. (MT). Increases in the anterior pretectal area (AP) and n. of Darkschewicz (Dk) did not reach statistical significance. (E) At the level of the superior colliculus, MDMA markedly stimulated utilization of the glucose in the principal oculomotor n. (3). Magnification bar = 5 mm.

Several brainstem nuclei were assayed. Most of these regions, including the dorsal and median raphe nuclei, the raphe obscurus and the locus coeruleus, showed no statistically significant effects. However, the raphe magnus nucleus and the pontine reticular formation showed mean increases of 39% and 38%, respectively, in response to 15 mg/kg MDMA.

DISCUSSION

Most previous work, aimed at identifying the neurotransmitter systems which mediate the effects of MDMA in the brain, have pointed to a major role of serotonin (5-HT). The drug MDMA causes release of [^{3}H]5-HT from slices of hippocampus *in vitro* (Johnson, Hoffman and Nichols, 1986; Schmidt, 1987) and binds to 5-HT binding sites with affinities in the micromolar range (Lyon, Glennon and Titeler, 1986; Battaglia, Brooks, Kulsakdinun and De Souza, 1988). Acute treatment with MDMA produces a dose-dependent loss of 5-HT and 5-hydroxyindoleacetic in the neostriatum (Schmidt *et al.*, 1986) and a long-term reduction in concentrations and uptake of 5-HT in the cortex (Schmidt, 1987). Subacute treatments with MDMA depress the activity of tryptophan hydroxylase and levels of 5-HT and 5-hydroxyindoleacetic acid in the neostriatum, hippocampus and cortex (Stone, Stahl, Hanson and Gibb, 1986; Battaglia, Yeh, O'Hearn, Molliver, Kuhar and De Souza, 1987), although one study showed the loss of 5-HT in the hippocampus to be nonsignificant (Battaglia *et al.*, 1987). Evidence that MDMA destroys serotonergic nerve terminals in the brain of the rat derives from immunocytochemical data (O'Hearn, Battaglia, De Souza, Kuhar and Molliver, 1988) and the loss of binding of [^{3}H]paroxetine, a probe for uptake sites for 5-HT (Battaglia *et al.*, 1987). Furthermore, in rats trained to discriminate fenfluramine and tetrahydro-β-carboline, serotonergic agents, MDMA generalized to the cues of the serotonergic drugs (Schechter, 1986).

The present findings suggest a presynaptic serotonergic action of MDMA, as the distribution of the effects of MDMA on utilization of glucose was similar to that of 5-HT$_1$ binding sites in the brain of the rat (Pazos and Palacios, 1985). A good correlation has been found between the affinities of several drugs for 5-HT, sites and their effects in models of presynaptic 5-HT receptors (for review see Pazos and Palacios, 1985). In this regard, the globus pallidus, the dentate gyrus, substantia nigra and the entopeduncular nucleus, all show high densities of 5-HT$_1$ receptors. They also showed effects of MDMA on utilization of glucose. In contrast, the entorhinal cortex and dorsal raphe nucleus, which also have high densities of 5-HT$_1$ receptors showed no response of local cerebral utilization of glucose to MDMA. Intermediate levels of 5-HT$_1$ binding have been obtained in the following areas in which MDMA altered utilization of glucose: anterior cingulate cortex, basolateral amygdaloid nucleus, medial mammillary nucleus, superficial grey layer of the superior colliculus and principal oculomotor nucleus. However, intermediate levels of binding to 5-HT$_1$ receptors are seen in the following regions of the brain, where MDMA did not significantly alter the utilization of glucose: frontoparietal motor and somatosensory areas of cortex, caudate-putamen and accumbens nucleus. Many of the areas of the brain which showed no cerebral metabolic response to MDMA also show only low densities of 5-HT$_1$ receptors. Exceptions are the lateral habenula and the reticular thalamic nucleus, which did show MDMA-induced alterations in utilization of glucose.

The distribution of the effects of MDMA were not well-matched to the distribution of 5-HT$_2$ receptors (Nakada, Wieczorek and Rainbow, 1984; Pazos, Cortēs and Palacios, 1985), despite a greater affinity of MDMA at 5-HT$_2$ than 5-HT$_1$ recognition sites (Battaglia *et al.*, 1988). Most areas which were reported to have moderate to high densities of 5-HT$_2$ receptors, including neocortical areas, the accumbens nucleus, caudate-putamen and inferior colliculus, showed no effects of MDMA. However, the superficial layer of the superior colliculus and the globus pallidus, which showed intermediate levels of binding in one study (Nakada *et al.*, 1984), did show metabolic responses to MDMA. This discrepancy may reflect a selective agonistic action of MDMA at 5-HT$_2$ receptors in the high affinity state (Battaglia, Zaczek and De Souza, in press).

The effects of MDMA to reduce the utilization of glucose in the cingulate cortex and dentate gyrus were similar to those obtained after treatment with 15 μg (i.v.) lysergic acid diethylamine (LSD), a serotonergic agonist, although a larger dose of LSD generally reduced cerebral utilization of glucose (Grome and Harper, 1986). This similarity further supports the view that the effects of MDMA in the cingulate cortex and dentate gyrus are mediated by an action on serotonergic neurons.

The present findings support a dopaminergic involvement in MDMA-induced changes in local cerebral utilization of glucose. Evidence that MDMA increases levels of dopamine in the striatum (Stone *et al.*, 1986; Schmidt *et al.*, 1986), alters levels of homovanillic acid and dihydroxyphenylacetic acid (Stone *et al.*, 1986; Schmidt *et al.*, 1986) and causes release of [^{3}H]dopamine from slices of striatum (Johnson *et al.*, 1986; Schmidt, 1987) has suggested a dopaminergic component in the action of this drug. Rats trained to discriminate dopaminergic drugs showed generalization to MDMA (Glennon and Young, 1984; Schechter, 1986) and MDMA produced amphetamine- and apomorphine-like effects on rotational behaviour (Kulmala, Boja and Schechter, 1987). The effects of MDMA on cerebral utilization of glucose were markedly similar to those obtained with other dopaminergic drugs. Specifically, stimulation of the utilization of glucose in the extra-

pyramidal motor system and a reduction in the lateral habenula, have been observed in response to treatment with *d*-amphetamine (Wechsler *et al.*, 1979; Orzi, Dow-Edwards, Jehle, Kennedy and Sokoloff, 1983), *l*-cocaine (London *et al.*, 1986) and apomorphine (McCulloch, Savaki and Sokoloff, 1980; McCulloch, Savaki, McCulloch, Jehle and Sokoloff, 1982). The effects of apomorphine on the utilization of glucose in the habenular and on the uptake of [^{14}C]-DG in extrapyramidal areas were blocked by prior treatment with haloperidol (Brown and Wolfson, 1978; McCulloch *et al.*, 1980), suggesting that the actions of these drugs, as well as MDMA, resulted from dopaminergic interactions. The anatomy of central dopaminergic systems has been reviewed (Björklund and Lindvall, 1984). The caudate-putamen, subthalamic nucleus and globus pallidus all receive a dopaminergic input from the substantia nigra, while the dopaminergic projection to the lateral habenula is from the ventral tegmental area. These dopaminergic projection areas all showed effects of MDMA on the utilization of glucose, although the increase in the caudate-putamen did not reach statistical significance. As the serotonergic agonist LSD did not stimulate utilization of glucose in components of the extrapyramidal motor system, the stimulatory effects of MDMA in these regions were apparently not mediated serotonergically (Grome and Harper, 1986).

There is, however, no clear-cut relationship between dopaminergic innervation and an effect of MDMA on utilization of glucose in mesolimbic and mesocortical dopaminergic projections. For example, the nucleus accumbens, a major dopaminergic terminal field (Björklund and Lindvall, 1984), showed no effect of MDMA on the utilization of glucose. Another example is presented by the heterogeneity of the effect in the substantia nigra pars reticulata. Previous studies have characterized the histology and content of dopamine of the ventromedial and dorsolateral zones of the substantia nigra pars reticulata (Fallon and Loughlin, 1985; Björklund and Lindvall, 1984). The ventromedial zone of the substantia nigra pars reticulata, which showed an MDMA-induced increase in utilization of glucose, contains small nondopaminergic neurons. Conversely, the larger neurons in the dorsolateral zone of this area, where MDMA produced no effect, are predominately dopaminergic. As apomorphine and psychomotor stimulants with indirect dopaminergic activity (e.g. *d*-amphetamine, *l*-cocaine) stimulate the utilization of glucose of the pars reticulata in its entirety (Brown and Wolfson, 1978; Wechsler *et al.*, 1979; London *et al.*, 1986), the heterogeneous effect of MDMA in this area may reflect an effect on a nondopaminergic system. In light of the putative role of serotonin in the action of MDMA, it is noteworthy that the substantia nigra shows a large concentration of serotonin (Brownstein and Palkovitz, 1984).

Stimulation of the utilization of glucose in components of the extrapyramidal motor system is consistent with a role of these areas of the brain in stereotyped behaviour produced by psychomotor stimulants (Iversen and Iversen, 1975). In addition to MDMA, *d*-amphetamine, *l*-cocaine and phencyclidine also produce stereotyped behaviour and stimulated utilization of glucose in the extrapyramidal motor system (Wechsler *et al.*, 1979; London *et al.*, 1986; Weissman *et al.*, 1987). These effects were not secondary to limb movements, as they were observed in paralyzed, artificially ventilated rats treated with MDMA, *l*-cocaine, *d*-amphetamine or phencyclidine (Kimes, Wilkerson, Ori and London, personal communication).

The effects of MDMA on the utilization of glucose in components of the visual system may have bearing on reports that MDMA produces blurred vision and visual hallucinations in humans (for reviews, see Hayner and McKinney, 1986; Siegel, 1986). Utilization of glucose was reduced in the medial terminal nucleus of the accessory optic tract and the superficial layer of the superior colliculus. The 3rd principal oculomotor nucleus showed an increase in the utilization of glucose which may have resulted from exophthalmos. Effects of MDMA in visual pathways are consistent with actions on dopaminergic and serotonergic neuronal systems. One of the first demonstrations of dopamine-containing nerve cells was performed in the mammalian retina (Häggendal and Malmfors, 1963), where the DA-containing cell group consists of amacrine cells in the inner nuclear layer (review Björklund and Lindvall, 1984). The high density of 5-HT$_1$ receptors in the superficial grey layer of the superior colliculus has already been mentioned.

It was of particular interest to determine if MDMA altered the utilization of glucose in the accumbens nucleus and medial prefrontal cortex, which have been implicated in the reinforcing effects of psychomotor stimulants (Wise, 1983; Goeders and Smith, 1986). However, these regions of the brain showed no significant effect on the utilization of glucose. The negative finding does not mean that these areas of the brain do not mediate reinforcing effects of MDMA, as negative findings with [^{14}C]DG could reflect dilution of effects on discrete neuronal populations by overall metabolic activity in a region of the brain. Furthermore, it is noteworthy that self-administration of MDMA has not been demonstrated in rats and there is no indication that the acute treatments with MDMA administered were reinforcing. The present results, as well as previous findings with *d*-amphetamine (Wechsler *et al.*, 1979), *l*-cocaine (London *et al.*, 1986) and phencyclidine (Weissman *et al.*, 1987), all demonstrate a reduction of utilization of glucose in the lateral habenula, which has been implicated in self-stimulation, that is mediated by a serotonergic input (Nakajima, 1984). It seems reasonable that the habenula may be involved in the reinforcing properties of these drugs. Nonetheless, further research

is needed to clarify the involvement of various regions of the brain in the potential reinforcing effects of MDMA.

The present findings support the view that the effects of MDMA on behaviour reflect actions on serotonergic and dopaminergic neuronal systems. The major systems affected include the extrapyramidal motor system, and limbic and visual areas. Metabolic changes in these regions of the brain may underlie the stereotyped behavior, reinforcing and visual effects of MDMA.

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