Neurochem. Int. Vol. 17, No. 4, pp. 509–513, 1990
Printed in Great Britain.

0197-0186/90 $3.00 + 0.00

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED RELEASE OF ENDOGENOUS SEROTONIN FROM THE RAT DORSAL RAPHE NUCLEUS *IN VITRO*: EFFECTS OF FLUOXETINE AND TRYPTOPHAN

CHARLES W. BRADBERRY*, JEFFREY S. SPROUSE†, GEORGE K. AGHAJANIAN and ROBERT H. ROTH

Departments of Pharmacology and Psychiatry, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, U.S.A.

(*Received* 26 *January* 1990; *accepted* 12 *March* 1990)

Abstract—The serotonin releasing action of 3,4-methylenedioxymethamphetamine on slices of dorsal raphe nucleus from rat was investigated. The slices were maintained in a gas–liquid interface perfusion chamber used for electrophysiological recording. Microdialysis probes designed for use on the slice surface were employed to measure the release of endogenous serotonin which was determined using liquid chromatography with electrochemical detection. Three minute duration exposure of the slices to 100 micromolar 3,4-methylenedioxymethamphetamine caused a long lasting release of endogenous serotonin. Fluoxetine, a serotonin transport inhibitor, reduced the amount of serotonin release. Tryptophan added to the perfusion solution increased both the duration and amount of serotonin released. These results further support earlier work on the mechanism of 3,4-methylenedioxymethamphetamine induced inhibition of serotonin neuronal firing.

The drug of abuse, 3,4-methylenedioxymethamphetamine (MDMA), has recently come under close scrutiny because of reports of possible use as a psychotherapeutic aid (Greer, 1983), widespread recreational use (Peroutka, 1987) and neurotoxic effects on serotonin neurons (Schmidt *et al.*, 1986; O'Hearn *et al.*, 1988; Insel *et al.*, 1989). In laboratory animals, MDMA possesses a serotonin (5-HT) releasing action on brain tissue *in vitro* (Nichols *et al.*, 1982; Schmidt *et al.*, 1987) which can be blocked by 5-HT transport inhibitors (Schmidt *et al.*, 1987) and a less potent dopamine releasing action *in vitro* Johnson *et al.*, 1986). The reader is referred to McKenna and Peroutka (1990) for a recent review.

We have been studying the electrophysiological effects of this compound on serotonin neurons in the dorsal raphe brain slice preparation. In this preparation, MDMA causes a stereoselective, concentration-dependent inhibition of 5-HT neuronal firing which can be blocked by the 5-HT transport inhibitor fluoxetine (Sprouse *et al.*, 1989a). Because of the stereoselectivity of the inhibition and its blockade by fluoxetine, it was proposed that MDMA was acting to inhibit the firing of 5-HT neurons by releasing 5-HT which could subsequently interact with impulse-regulating somatodendritic autoreceptors, previously shown to be of the 5-HT_{1a} subtype (Sprouse and Aghajanian, 1987). In an attempt to correlate the effects of MDMA on neuronal firing with endogenous 5-HT release, we developed a method of positioning microdialysis probes on the surface of the dorsal raphe slice preparation in the recording chamber (Sprouse *et al.*, 1989a).

Our previously reported data using this technique supported the hypothesis that MDMA-induced 5-HT release was responsible for the observed inhibition of neuronal firing in that the duration of MDMA-induced endogenous 5-HT release correlated with the period of inhibition of firing (Sprouse *et al.*, 1989a). In this communication, we report the results of further experiments on MDMA-induced endogenous 5-HT release from the dorsal raphe brain slice preparation. In these experiments, we have examined the effects

* Author to whom all correspondence should be addressed.
† Present address: Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215, U.S.A.

of tryptophan and fluoxetine on basal and MDMA-induced serotonin release. The results of these studies further support a mechanism of action in which MDMA releases serotonin which can subsequently interact with somatodendritic impulse-regulating autoreceptors to inhibit 5-HT neuronal firing.

EXPERIMENTAL PROCEDURES

Brain slice preparation

Slices of dorsal raphe were obtained from male Sprague–Dawley rats (125–175 g) which had been anesthetized and perfused with an ice-cold sucrose-enriched artificial cerebrospinal fluid (ACSF) as described previously (Sprouse *et al.*, 1989a). The sucrose-enriched ACSF was of a standard composition (in mM: KCl 5.0, $CaCl_2$ 2.0, $MgSO_4$ 2.0, $NaHCO_3$ 28, NaH_2PO_4 1.25, D-glucose 10) except that NaCl, normally present at 126 mM, was replaced by a calculated equiosmolar concentration of sucrose (252 mM). The substitution of sucrose for NaCl in the ACSF for the preparatory and recovery phases of the experiment improved viability (Aghajanian and Rasmussen, 1989). Immediately following decapitation, the brain was removed rapidly, placed in a Petri dish containing ice-cold sucrose-enriched ACSF and trimmed to expose the posterior midbrain. Coronal sections (500 μm) were then cut through the dorsal raphe using a vibrating-knife microtome (WPI, Vibroslice) and transferred to the stage of a gas–fluid interface brain slice chamber (Haas *et al.*, 1979). One hour from the time of decapitation, sucrose-enriched ACSF was replaced by standard ACSF containing NaCl. An additional period of 1 h was allowed prior to the experiment. Before initiating the release studies, 2.5 μM phenylephrine was added to the ACSF to induce the otherwise silent 5-HT neurons to fire (Vandermaelen and Aghajanian, 1983). Viability of the slice was determined by probing for active 5-HT neurons identified by their wide-duration action potentials (1–2 ms, positive–negative spikes), regular rhythm and slow firing rate (Vandermaelen and Aghajanian, 1983). Tryptophan, MDMA and Fluoxetine (Eli Lilly) were added to the ACSF flowing over the slice at the indicated concentrations.

In vitro microdialysis

A microdialysis assembly containing an exposed section of perfused microdialysis tubing 330 μm in diameter, 1.5–2.0 mm long was lowered onto the surface of the brain slice so that the length of the dialysis tubing rested along the medial–lateral axis of the exposed dorsal raphe, perpendicular to the flow of ACSF. Perfusion buffer (in mM: NaCl 120, KCl 2.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, NaH_2PO_4 0.9, Na_2HPO_4 1.4, ascorbic acid 0.3, pH 7.4) was pumped through the dialysis assembly at a flow rate of 2.0 μl/min. Samples were collected at 10 min intervals. Because of the consistent percentage recoveries (Ungerstedt, 1984) obtained for the microdialysis probes, no correction was made for them (i.e. data were compared directly on the basis of fmol of 5-HT per sample). Probe recoveries measured in a static solution at room temperature averaged 9.7$\pm$0.7 (SEM) %. Statistical significance of differences in cumulative release and duration of release between experimental groups was determined with a Mann–Whitney *U*-test.

5-HT determination

5-HT content of the microdialysis perfusates was determined by reverse-phase liquid chromatography with electrochemical detection. Ten cm $\times$ 2.1 mm i.d. columns were packed with 3 μm C-18 spherical particles (Shandon). A thin-layer amperometric detection electrode assembly (Bioanalytical Systems) was used in conjunction with a laboratory-constructed low-noise potentiostat for detection of 5-HT. A laboratory-constructed pulseless pneumatic displacement fluid pump was used to pump mobile phase through the column. The chromatographic system had a routine detection limit of 2 fmol (at a signal to noise ratio of 2).

RESULTS

Initial experiments using a dialysis probe on the surface of the dorsal raphe brain slice examined the effect of a 3 min exposure of the slice to 100 μM MDMA. The effect on 5-HT neuronal firing rate of this concentration and duration of exposure had previously been characterized (Sprouse *et al.*, 1989a). Figure 1 illustrates the duration and amount of endogenous 5-HT release induced by MDMA. Basal levels of 5-HT prior to MDMA are below the detection limit of 2 fmol. Samples of the slice superfusate (not the dialysate), collected during the period when 5-HT release was maximal, were injected into the chromatograph but did not contain measureable amounts of 5-HT, thus illustrating the ability of a microdialysis probe placed directly on the slice surface to measure neurotransmitter release not detectable in the superfusate. Prior treatment of the slice with 100 μM fluoxetine significantly reduced the ability of the MDMA to release 5-HT (Table 1). The addition of fluoxetine did not raise basal 5-HT to detectable levels.

Preliminary experiments (Sprouse *et al.*, 1989b) indicated that prior exposure of the slice to Trp

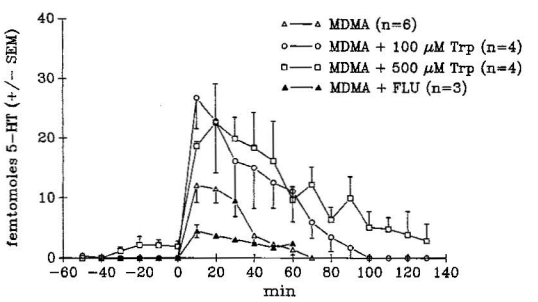

Fig. 1. MDMA-induced release of 5-HT. Dorsal raphe slices were all exposed to 100 micromolar MDMA for 3 min starting at $t = 0$. Trp pretreatment began at $t = -40$ and was maintained throughout the remainder of the experiment. Fluoxetine (100 μM) pretreatment began at $t = -20$ and was maintained throughout.

Table 1. Summary of release data. Amount and duration of cumulative 5-HT release from dorsal raphe slices induced by 3 min MDMA exposure. See Experimental Procedures for details of fluoxetine and Trp pretreatments. * $P < 0.05$ vs control, ** $P < 0.01$ vs control, † $P < 0.05$ vs Trp (100 μM)

Group	Release (fmol)	Duration (min)	N
Control	39.5±7.1	47.5±5.7	6
Fluoxetine	15.6±2.9*	50.0±10.0	3
Trp (100 μM)	111.3±36.1*	75±6*	4
Trp (500 μM)	148.1±30.2**	112.5±2.5**,†	4

increased the inhibitory effect of MDMA on 5-HT neuronal firing and increased the amount of 5-HT released by 100 μM MDMA. Figure 1 shows the results of pretreatment of the slice with two concentrations of Trp. Pretreatment with 100 μM Trp significantly increased both the amplitude and duration of the 5-HT release curve ($P < 0.05$). At this concentration of Trp, basal levels of 5-HT were still undetectable. Pretreatment with 500 μM Trp increased basal levels of 5-HT to the point of just being detectable. The amount of 5-HT released from slices pretreated with 500 μM Trp exposure was not significantly greater than seen with 100 μM Trp; however, the duration of measureable release was significantly greater ($P < 0.05$) with the 500 μM pretreatment than with the 100 μM pretreatment. Table 1 summarizes amount and duration of release for the four experimental groups.

DISCUSSION

All previous reports of release of 5-HT from the dorsal raphe either *in vivo* (see Hery *et al.*, 1986 for review), or *in vitro* (Ennis and Cox, 1981; Collinge and Pycock, 1982; Daszuta, *et al.*, 1984; Frankhuijzen *et al.*, 1988) have used labeled 5-HT recently taken up or newly synthesized. By using liquid chromatography with highly sensitive electrochemical detection methods, we avoided the necessity of using such labeled 5-HT methods which have the accompanying problems of interpretation due to the selective labeling of a recently taken up or synthesized pool.

Inhibitors of 5-HT transport have been shown to block the ability of MDMA to release [^{3}H]5-HT *in vitro* (Schmidt *et al.*, 1987), to block the long-term neurotoxic effects of MDMA (Gehlert *et al.*, 1985; Schmidt *et al.*, 1987), and also to block the inhibition of 5-HT neuronal firing caused by MDMA while not altering firing itself (Sprouse *et al.*, 1989a). The effects of the reuptake blocker *in vitro* appear to be due to an inhibition of carrier-mediated release, analogous to the effects of reuptake blockers on amphetamine-induced release of catecholamines (Liang and Rutledge, 1982; Hurd and Ungerstedt, 1989). As can be seen in Fig. 1, and Table 1, the 5-HT reuptake inhibitor fluoxetine significantly decreases the ability of MDMA to release endogenous 5-HT in the dorsal raphe brain slice preparation. Local application of fluoxetine has been shown to cause a large enhancement of basal release of labeled 5-HT from the dorsal raphe *in vivo* (Hery *et al.*, 1982), suggesting that reuptake processes are operating in the cell body region in a similar fashion to that in the nerve terminal regions. Thus, it appears that MDMA-induced release of 5-HT from the dorsal raphe cell body region exhibits essentially similar characteristics to that from telencephalic nerve terminal regions (Nichols *et al.*, 1982; Johnson *et al.*, 1986; Schmidt *et al.*, 1987).

The serotonin precursor Trp has been shown to increase synthesis, storage and metabolism of 5-HT (Lookingland *et al.*, 1986). There have also been reports that Trp administration can enhance the release of 5-HT *in vivo* (Schwartz *et al.*, 1988; Carboni *et al.*, 1989) and *in vitro* (Auerbach and Lipton, 1985; Acworth *et al.*, 1988). The effect of Trp at enhancing MDMA-induced release of 5-HT is of interest for two reasons. First, the parallel between increased 5-HT release and increased inhibitory effect of MDMA on 5-HT neuronal cell firing (Sprouse *et al.*, 1989b) further supports the proposed mechanism of the electrophysiological effects of MDMA. The observation that Trp can enhance the action of MDMA is also important because of the wide availability and use of Trp as a nutritional supplement, and the possibility that MDMA users might attempt to augment the actions of MDMA which they find pleasureable. This could be very dangerous because of the possibility that the well documented neurotoxic effects of MDMA and parachloroamphetamine may be dependent on release of 5-HT (Berger *et al.*, 1989). Thus, the damaging action of MDMA on 5-HT neurons might be increased by increasing brain Trp levels. It should be noted that the concentrations of Trp used to enhance the effects of MDMA in this study are not within the normally encountered physiological range (Young *et al.*, 1980), but with large dietary supplementation, CSF levels of Trp are increased approx. 8-fold over basal levels (Young and Gauthier, 1981), and thus may approach a range at which MDMA effects are enhanced. Also, because the background ACSF used in the present experiments did not contain any Trp, the slice might be considered to be Trp starved, thus enhancing the effect of the addition of Trp to the bath. Experiments aimed at elucidating any

neurotoxicity enhancing effects of Trp are currently in progress.

In an earlier communication (Sprouse *et al.*, 1989a), we reported that the time course of the release induced by MDMA closely paralleled the time course of inhibition of dorsal raphe 5-HT neuronal firing suggesting that it is elevated extracellular 5-HT acting upon somatodendritic autoreceptors which is responsible for the inhibition. The additional experiments described herein offer further support for this hypothesis. The electrophysiological effects of MDMA on the dorsal raphe 5-HT neuronal firing were shown to be blocked by fluoxetine (Sprouse *et al.*, 1989a). Here we have shown that 5-HT release from the dorsal raphe is blocked by fluoxetine. It has been shown that Trp is able to enhance the inhibitory effects of MDMA on unit firing (Sprouse *et al.*, 1989b), and as presented herein, Trp also enhances the MDMA-induced release of 5-HT. Taken together, these observations strongly support the previously proposed mechanism of action of MDMA on 5-HT neuronal firing (Sprouse *et al.*, 1989a).

Acknowledgements—This work was supported by MH 14092, 14276, 17871, 25642, DA 05119 and the State of Connecticut.

REFERENCES

Acworth I. N., During M. J. and Wurtman R. J. (1988) Processes that couple amino acid availability to neurotransmitter synthesis and release. In: *Amino Acid Availability and Brain Function in Health and Disease* (Heuther G., ed.), Nato ASI Series H, Vol. 20, pp. 117–136. Springer, Berlin.

Aghajanian G. K. and Rasmussen K. (1989) Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. *Synapse* **3,** 331–338.

Auerbach S. and Lipton P. (1985) Regulation of serotonin release from the *in vitro* rat hippocampus: effects of alterations in levels of depolarization and in rates of serotonin metabolism. *J. Neurochem.* **44,** 1116–1130.

Berger U. V., Grzanna R. and Molliver M. E. (1989) Depletion of serotonin using *p*-chlorophenylalanine (PCPA) and reserpine protects against the neurotoxic effects of *p*-chloroamphetamine (PCA) in the brain. *Exp. Neurol.* **103,** 111–115.

Carboni E., Cadoni C., Tanda G. L. and Di Chiara G. (1989) Calcium-dependent, tetrodotoxin-sensitive stimulation of cortical serotonin release after a tryptophan load. *J. Neurochem.* **53,** 976–978.

Collinge J. and Pycock C. (1982) Differential actions of diazepam on the release of [^{3}H]5-hydroxytryptamine from cortical and midbrain raphe slices in the rat. *Eur. J. Pharmac.* **85,** 9–14.

Daszuta A., Faudon M. and Hery F. (1984) *In vitro* ^{3}H-serotonin (5-HT) synthesis and release in BALBc and C57BL mice. II. Cell body areas. *Brain Res. Bull.* **12,** 565–570.

Ennis C. and Cox B. (1982) Pharmacological evidence for the existence of two distinct serotonin receptors in rat brain. *Neuropharmacology* **21,** 41–44.

Frankhuijzen A. L., Wardeh G., Hogenboom F. and Mulder A. (1988) Alpha-2 adrenoceptor mediated inhibition of the release of radiolabelled 5-hydroxytryptamine and noradrenaline from slices of the dorsal region of the rat brain. *Naunyn-Schmiedeberg's Arch. Pharamac.* **337,** 255–260.

Gehlert D. R., Schmidt C. J., Wu L. and Lovenberg W. (1985) Evidence for specific methylenedioxymethamphetamine (ecstasy) binding sites in the rat brain. *Eur. J. Pharmac.* **119,** 135–136.

Greer G. (1983) *MDMA: A New Psychotropic Compound and its Effects in Humans.* Greer, Santa Fe, N.M.

Haas H. L., Schaerer B. and Vosmansky M. (1979) A simple perfusion chamber for the study of nervous tissue slices *in vitro*. *J. Neurosci. Meth.* **1,** 323–325.

Hery F., Faudon M. and Fueri C. (1986) Release of serotonin in structures containing serotoninergic nerve cell bodies: dorsalis raphe nucleus and nodose ganglia of the cat. *Ann. N.Y. Acad. Sci.* **473,** 239–255.

Hery F. Faudon M. and Ternaux J.-P. (1982) *In vivo* release of serotonin in two raphe nuclei (Raphe Dorsalis and Magnus) of the cat. *Brain Res. Bull.* **8,** 123–129.

Hurd Y. L. and Ungerstedt U. (1989) Ca^{2+} dependence of the amphetamine, nomifensine, and Lu 19-1905 effect on *in vivo* dopamine transmission. *Eur. J. Pharmac.* **166,** 261–269.

Insel R. T., Battaglia G., Johannessen J. N., Marra S. and De Souza E. (1989) 3,4-Methylenedioxymethamphetamine ("ecstasy") selectively destroys brain serotonin terminals in rhesus monkeys. *J. Pharmac. exp. Ther.* **249,** 713–720.

Johnson M. P., Hoffman A. J. and Nichols D. E. (1986) Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused ratbrain slices. *Eur. J. Pharmac.* **132,** 269–276.

Liang N. Y. and Rutledge C. O. (1982) Evidence for carrier-mediated efflux of dopamine from corpus striatum. *Biochem. Pharmac.* **31,** 2479–2484.

Lookingland K. J., Shannon N. J., Chapin D. S. and Moore K. E. (1986) Exogenous tryptophan increases synthesis, storage, and intraneuronal metabolism of 5-hydroxytryptamine in the rat hypothalamus. *J. Neurochem.* **47,** 205–212.

McKenna D. J. and Peroutka S. J. (1990) Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *J. Neurochem.* **54,** 14–22.

Nichols D. E., Lloyd D. H., Hoffman A. J., Nichols M. B. and Yim G. K. (1982) Effects of certain hallucinogenic amphetamine analogues on the release of [^{3}H]serotonin from rat brain synaptosomes. *J. med. Chem.* **25,** 530–535.

O'Hearn E., Battaglia G., De Souza E. B., Kuhar M. J. and Molliver M. E. (1988) Methylenedioxamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J. Neurosci.* **8,** 2788–2803.

Peroutka S. J. (1987) Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") on an undergraduate campus. *New Engl. J. Med.* **317,** 1542–1543.

Schmidt C. J., Levin J. A. and Lovenberg W. (1987) *In vitro*

and *in vivo* neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem. Pharmac.* **36,** 747–755.

Schmidt C. J., Wu L. and Lovenberg W. A. (1986) Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. *Eur. J. Pharmac.* **124,** 175–178.

Schwartz D. H., McClane S., Hernandez L. and Hoebel B. (1988) Tryptophan and food increase extracellular serotonin in the lateral hypothalamus as measured by microdialysis in the rat. *Soc. Neurosci. Abstr.* **14,** 25.

Sprouse J. S. and Aghajanian G. K. (1987) Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5HT1B agonists. *Synapse* **1,** 3–9.

Sprouse J. S., Bradberry C. W., Roth R. H. and Aghajanian G. K. (1989a) MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin. *Eur. J. Pharmac.* **167,** 375–383.

Sprouse J. S., Bradberry C. W., Roth R. H. and Aghajanian G. K. (1989b) MDMA-induced release of serotonin (5-HT) and inhibition of neuronal firing in dorsal raphe (DR) brain slices: potentiation by L-tryptophan (TRP). *Soc. Neurosci. Abstr.* **15,** 417.

Ungerstedt U. (1984) *Measurement of Neurotransmitter Release In Vivo* (Marsden C. A., ed.), pp. 81–105. Wiley, Chichester, England.

Vandermaelen C. P. and Aghajanian G. K. (1983) Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Res.* **289,** 109–119.

Young S. N. and Gauthier S. (1981) Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. *J. Neurol. Neurosurg. Psychiat.* **44,** 323–327.

Young S. N., Anderson G. M. and Purdy W. C. (1980) Indoleamine metabolism in rat brain studied through measurements of tryptophan, 5-hydroxyindoleacetic acid, and indoleacetic acid in cerebrospinal fluid. *J. Neurochem.* **34,** 309–315.