European Journal of Pharmacology, 178 (1990) 313–320
Elsevier

EJP 51232

3,4-Methylenedioxymethamphetamine-induced release of serotonin and inhibition of dorsal raphe cell firing: potentiation by L-tryptophan

Jeffrey S. Sprouse [1], Charles W. Bradberry [1,2], Robert H. Roth [1,2] and George K. Aghajanian [1,2]

Departments of [1] Psychiatry and [2] Pharmacology, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508, U.S.A.

Received 28 December 1989, accepted 9 January 1990

The effects of the serotonin (5-HT) precursor L-tryptophan on MDMA (3,4-methylenedioxymethamphetamine)-induced inhibition of dorsal raphe neuronal firing were characterized using extracellular single-unit recording and microdialysis techniques in the in vitro midbrain slice preparation. Pretreatment with L-tryptophan (100 μM) lowered the doses of MDMA required to inhibit unit activity. Based upon IC_{50} values, L-tryptophan increased the potency of MDMA by approximately 3-fold. In a parallel series of experiments, microdialysis probes resting on the brain slice surface provided a means to estimate 5-HT release from the dorsal raphe nucleus. Pretreatment with L-tryptophan increased MDMA-induced 5-HT release in a manner consistent with the suppression of dorsal raphe cell firing: compared to untreated preparations, peak 5-HT release, total release and the duration of release were all increased. Taken together, these data suggest that the enhancement by L-tryptophan of MDMA-induced 5-HT release and inhibition of dorsal raphe neuronal firing is due to an increase in the amount of 5-HT available for release. The question is raised as to what effect L-tryptophan may have on the psychotropic and neurotoxic actions of MDMA.

MDMA (3,4-methylenedioxymethamphetamine); L-Tryptophan; Dorsal raphe nucleus; Single unit recording; Microdialysis

1. Introduction

MDMA (3,4-methylenedioxymethamphetamine), a synthetic amphetamine derivative with unique psychotropic effects, has become increasingly popular among recreational drug users (Peroutka, 1987) and may represent a novel class of psychoactive agents (Nichols et al., 1986). From a neurochemical standpoint, MDMA closely resembles the serotonergic neurotoxin, p-chloroamphetamine (PCA). Like PCA, administration of MDMA to laboratory animals produces an immediate and a long-term decrease in brain serotonin (5-HT). The immediate decrease in brain 5-HT appears to be the result of inhibition of 5-HT biosynthesis and stimulation of carrier-mediated 5-HT release (Stone et al., 1986; Schmidt et al., 1987). The long-term decrease in brain 5-HT may represent selective nerve terminal damage (Schmidt et al., 1986; Schmidt, 1987). Release of 5-HT is stereoselective, with (+)-MDMA being the more potent releasing agent in vitro, and susceptible to blockade by 5-HT uptake inhibitors (Schmidt et al., 1987). Neurotoxicity is a property of the (+) enantiomer (Schmidt, 1987; Schmidt et al., 1987).

In an earlier report (Sprouse et al., 1989), we examined the relationship between the neurochemical effects of PCA, (+)-MDMA and (−)-MDMA and the electrophysiological responses which they may elicit. Serotonergic dorsal raphe neurons were recorded in the midbrain slice pre-

Correspondence to: J.S. Sprouse, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215, U.S.A.

paration and the effects of these compounds on single unit activity were assessed. As predicted from neurochemical studies, (+)-MDMA was more potent than (−)-MDMA in suppressing neuronal firing rate, and fluoxetine, a selective 5-HT uptake inhibitor, blocked the suppressant effects of these compounds without altering baseline firing rate. Parallel experiments using microdialysis probes resting on the brain slice surface to estimate 5-HT efflux supported the notion that MDMA indirectly inhibits dorsal raphe neurons via release of 5-HT. The purpose of the present study was to examine the effect of L-tryptophan, the initial precursor in 5-HT biosynthesis, on MDMA-induced inhibition of dorsal raphe cell firing. As in our earlier report, combined electrophysiological and neurochemical approaches were utilized.

2. Materials and methods

2.1. Brain slice preparation

Brain slices were obtained as previously described (Sprouse et al., 1989). Briefly, male rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and perfused with ice-cold sucrose-enriched artificial cerebrospinal fluid (ACSF) before decapitation. 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 petrie dish containing ice-cold sucrose-enriched ACSF and trimmed to expose the posterior midbrain. Coronal sections (500 μm) then were 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 recording. Both the sucrose-enriched ACSF and the standard ACSF contained no added L-tryptophan or other amino acids.

The otherwise silent 5-HT neurons in the brain slice were induced to fire with 2.5 μM phenylephrine in the ACSF (Vandermaelen and Aghajanian, 1983). Solutions of MDMA (NIDA) in standard ACSF containing phenylephrine were introduced into the slice chamber by means of a stopcock arrangement. The (+) enantiomer of MDMA was used in all experiments. Perfusion fluid routinely flowed at 1-1.5 ml/min. The period of drug applications was fixed at 3 min; the period of pretreatment with L-tryptophan (Sigma) was minimally 10 min for the electrophysiological experiments and 40 min for the neurochemical experiments.

2.2. Recording

Extracellular single unit recordings were performed using single barrel glass micropippetes filled with 2 M NaCl. Tip diameters ranged from 1-2 μm; in vitro impedences ranged from 2 to 4 MΩ when measured at 60 Hz. Integrated firing rate was computed in 10 s samples from an electronic counter triggered by individual neuronal spikes. Putative 5-HT neurons were identified on the basis of their wide-duration action potentials (1-2 ms, positive-negative spikes), regular rhythm and slow firing rate (Vandermaelen and Aghajanian, 1983). Cells with these characteristics in the dorsal raphe nucleus have been confirmed to be 5-HT neurons by intracellular double labeling (Aghajanian and Vandermaelen, 1982).

As previously shown in an in vivo model (Gallager and Aghajanian, 1976), L-tryptophan by itself reduced dorsal raphe unit activity in the brain slices. Firing rates decreased by 54 ± 9% (N = 7) following the addition of L-tryptophan to the ACSF (final concentration of 100 μM). To avoid the possible bias introduced by recording from neurons firing at such a reduced rate, cells were sought in subsequent electrode passes which exhibited firing rates similar to those observed in the control (untreated) group.

2.3. Data analysis

The percenta
calculated by co
for a 3 min peri
maximal drug re
min following di
corded, a concen
determined for
quired to produc
firing (IC_{50}) was
gression analysis.
Student's t-test.

2.4. Estimation of

In a parallel s
sis probes were
slice surface, pro
release from the d
of a concentric d
previously reporte
dez et al., 198
(Carnegie Medici
structed using Cu
hollow fibers (300
in a section of 23
mic tubing. The fi
2.5 mm beyond th
active surface of 1
mM: KCl 2.4, N
NaH_2PO_4 0.9, N
pH 7.4) was pump
silica (170 μm O.D
which extended to
hypodermic tubing
bly was bent 90°
brain slice surface
and perpendicular
slice. Collection pe
the flow set at 2
centage recovery'
probes was 9 ± 1.

5-HT content in
termined by HPL
tion. Columns, 10
μm C-18 particles
junction with a thi

2.3. Data analysis

The percentage suppression of firing was calculated by comparing the baseline firing rate for a 3 min period prior to drug exposure to the maximal drug response, which occurred within 5 min following drug application. In each cell recorded, a concentration-response relationship was determined for MDMA. The concentration required to produce a 50% suppression of a cell firing (IC_{50}) was interpolated following linear regression analysis. Differences were determined by Student's t-test.

2.4. Estimation of 5-HT release

In a parallel series of experiments, microdialysis probes were positioned directly on the brain slice surface, providing a means to estimate 5-HT release from the dorsal raphe nucleus. Probes were of a concentric design, similar to several others previously reported (Justice et al., 1983; Hernandez et al., 1986) or commercially available (Carnegie Medicin, Stockholm). They were constructed using Cuprophan (Enka, West Germany) hollow fibers (300 μm I.D., 330 μm O.D.) housed in a section of 23 gauge (0.64 mm O.D.) hypodermic tubing. The fiber extended approximately 2.0-2.5 mm beyond the tip of the tubing exposing an active surface of 1.5-2.0 mm. Perfusion buffer (in mM: KCl 2.4, NaCl 125, $CaCl_2$ 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 a section of vitreous silica (170 μm O.D., Scientific Glass Engineering) which extended to the tip of the hollow fiber. The hypodermic tubing containing the dialysis assembly was bent 90° to allow the probe to rest on the brain slice surface over the dorsal raphe nucleus and perpendicular to the flow of ACSF over the slice. Collection periods were fixed at 10 min with the flow set at 2.0 μl/min. The average 'percentage recovery' (Ungerstedt, 1984) for these probes was 9 ± 1.

5-HT content in the dialysate aliquots was determined by HPLC with electrochemical detection. Columns, 10 × 2.1 mm I.D., packed with 3 μm C-18 particles (Shandon) were used in conjunction with a thin-layer amperometric detection electrode assembly (Bioanalytical Systems). A lab-designed potentiostat (Bradberry et al., in preparation) was used for the application of a potential to the detector electrode (+0.6 V versus Ag/AgCl reference) and conversion of the resulting current to voltage for output to a strip chart recorder. A pneumatic fluid pump (Bradberry and Roth, in preparation) was used for pumping mobile phase through the column, providing extremely smooth flow and isolation from electrical noise. Mobile phase consisted of 0.05 M Na_2HPO_4, 350 mg/l sodium octanesulfonate, 0.1 mM disodium EDTA, 300 μl/l triethylamine and 150 ml/l methanol. pH was unadjusted and was 5.6. Flow rate was 0.2 ml/min. The limit of detection attainable was routinely 0.1 nM injected in a volume of 20 μl or 2 fmol.

3. Results

3.1. Effects of L-tryptophan on MDMA-induced inhibition of dorsal raphe cell firing

In slices superfused with standard ACSF, MDMA inhibited dorsal raphe cell firing as a function of concentration (fig. 1A). The onset of the response generally occurred after 2-3 min of MDMA exposure; peak effects occurred during the first few min following drug application. The time required for recovery from the peak effect to the baseline firing rate was related directly to the MDMA concentration. For the 10 μM applications, the time to recovery averaged 8 ± 3 min (range 2-18 min). In preparations continuously superfused with L-tryptophan in the ACSF (100 μM for minimally 10 min), lower concentrations of MDMA were required to inhibit unit activity with no change in time to onset and peak effect (fig. 1B). The time required for recovery from the peak drug effect in L-tryptophan-treated slices generally appeared to be longer. For the 10 μM applications, five of eight cells tested showed incomplete recovery 12-45 min following the peak effect; in the remaining three cells which showed complete recovery, the time required averaged 14 ± 4 min (range 8-21 min).

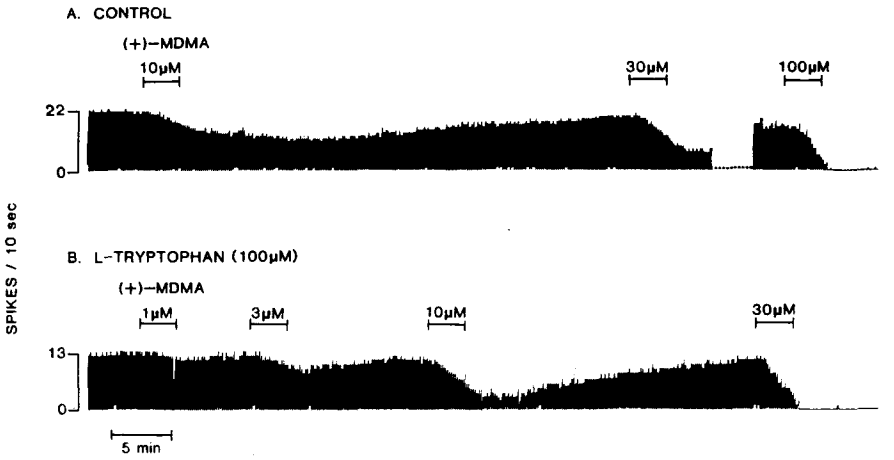

Fig. 1. Effects of L-tryptophan on (+)-MDMA-induced suppression of dorsal raphe cell firing recorded in midbrain slices. (+)-MDMA was added to the superfusate to produce the concentrations indicated; the duration of drug exposure, indicated by the length of the bar, was fixed at 3 min. (A) In (untreated) control slices, (+)-MDMA reversibly inhibited dorsal raphe cell firing in a concentration-dependent manner. IC_{50} for (+)-MDMA with this cell was 13 μM. The dotted line indicates 42 min of elapsed time between tracings. (B) In a slice pretreated for 60 min with L-tryptophan (100 μM), lower concentrations of (+)-MDMA were required to inhibit unit activity. IC_{50} for (+)-MDMA with this cell was 5 μM.

Concentration-response curves constructed from these data for MDMA in the absence or presence of L-tryptophan appeared to be parallel, i.e. the hypothesis of parallelism could not be rejected (Tallarida and Murray, 1981) (fig. 2). Based upon IC_{50} values, the presence of L-tryptophan increased the potency of MDMA approximately 3-fold, and effect which could not be attributed to differences in the baseline firing rates of the cells sampled (table 1). The latter is an important consideration given that L-tryptophan

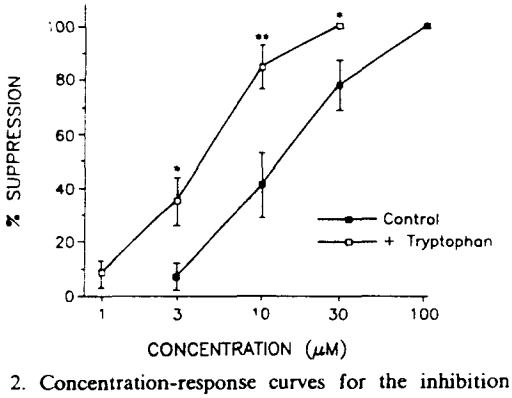

Fig. 2. Concentration-response curves for the inhibition of dorsal raphe cell firing by (+)-MDMA in the absence (control ■) or presence (+ tryptophan □) of L-tryptophan. Pretreatment with L-tryptophan (100 μM) was minimally 10 min. Points represent the mean ± S.E.M. for N = 8. * P < 0.05, ** P < 0.01.

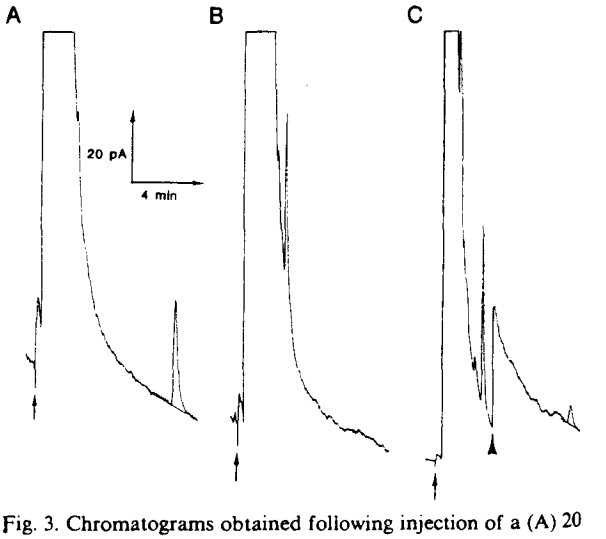

Fig. 3. Chromatograms obtained following injection of a (A) 20 fmol 5-HT standard, (B) brain slice dialysate prior to MDMA exposure, showing no detectable level of 5-HT, and (C) brain slice dialysate collected during the period 40-50 min following MDMA exposure, showing a 5-HT peak near the limit of detection. The arrowhead in (C) marks a gain change to that of panels (A) and (B).

TABLE 1

Effect of L-tryptopl
dorsal raphe cell firi
means ± S.E.M. IC_{5}
produce a 50% suppr
μM) pretreatment w

Control
L-Tryptophan

* P < 0.02.

by itself partial
Materials and m

3.2. Effects of L-
lease of 5-HT

Sample chron
standard and di

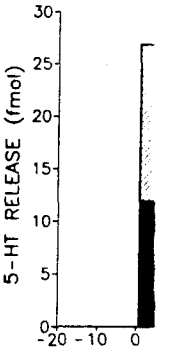

Fig. 4. (+)-MDMA-i
raphe nucleus as mea
the brain slice surfa
release was below th
collection periods pri
the collection period
fused (100 μM for 3
increased to 12 ± 3 f
gradually (filled bars;
with L-tryptophan (10
with (+)-MDMA was
min collection periods
fusion, 5-HT release
N = 4). Total fmol of
in control and L-tryp
inset. * P < 0.02

TABLE 1

Effect of L-tryptophan on (+)-MDMA-induced inhibition of dorsal raphe cell firing recorded in midbrain slices. Values are means ± S.E.M. IC_{50} is the drug concentration required to produce a 50% suppression in unit activity. L-Tryptophan (100 μM) pretreatment was minimally 10 min.

	N	Firing rate (Hz)	IC_{50} (μM)
Control	8	1.6 ± 0.2	16 ± 4
L-Tryptophan	8	1.3 ± 0.3	5 ± 1 [a]

[a] $P < 0.02$.

by itself partially suppressed unit activity (see Materials and methods).

3.2. *Effects of L-tryptophan on MDMA-induced release of 5-HT*

Sample chromatograms obtained with a 5-HT standard and dialysis perfusates before and after MDMA are shown in fig. 3. In untreated preparations, peak levels of 5-HT in the dialysis perfusate occurred during the first or second 10 min collection periods following MDMA application and averaged 13 ± 3 fmol (N = 6; fig. 4), uncorrected for probe efficiency. Thereafter, 5-HT release gradually declined but remained detectable for 48 ± 6 min (range 30-60 min). In slices continuously exposed to L-tryptophan (100 μM), peak levels of 5-HT also occurred during the first or second collection periods following MDMA, but were significantly higher at 28 ± 6 fmol (N = 4, $P < 0.03$). Detectable levels of 5-HT persisted for 75 ± 6 min (range 60-90 min), significantly longer than in untreated slices ($P < 0.01$). The total number of fmol measured in the dialysis perfusate from all collection samples was increased approximately 3-fold by L-typtophan pretreatment (fig. 4, inset). Baseline levels of 5-HT, prior to the addition of MDMA, were below the detection limit in the absence or presence of L-tryptophan.

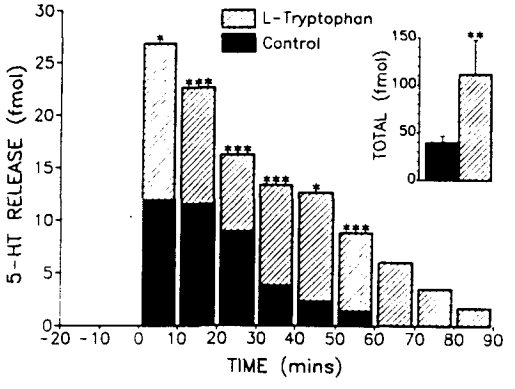

Fig. 4. (+)-MDMA-induced release of 5-HT from the dorsal raphe nucleus as measured by microdialysis probes resting on the brain slice surface. In (untreated) control slices, 5-HT release was below the limit of detection in the two 10 min collection periods prior to superfusion with (+)-MDMA. In the collection period during which (+)-MDMA was superfused (100 μM for 3 min starting at time 0), 5-HT release increased to 12 ± 3 fmol; thereafter, 5-HT release declined gradually (filled bars; N = 6). In slices pretreated for 40 min with L-tryptophan (100 μM), 5-HT release prior to superfusion with (+)-MDMA was also below the limit of detection (two 10 min collection periods shown). Following (+)-MDMA superfusion, 5-HT release increased to 27 ± 5 fmol (hatched bars; N = 4). Total fmol of 5-HT released for the entire time course in control and L-tryptophan-treated slices is shown in the inset. * $P < 0.02$, ** $P < 0.05$, *** $0.05 < P < 0.1$.

4. Discussion

In our earlier report (Sprouse et al., 1989), we presented evidence that (+)-MDMA indirectly inhibits the firing of dorsal raphe neurons via release of endogenous 5-HT. The released 5-HT in turn suppresses cell firing by binding to the inhibitory somatodendritic autoreceptor of dorsal raphe neurons. In the present study, pretreatment with L-tryptophan, the initial precursor in 5-HT biosynthesis, increased the potency of MDMA to inhibit dorsal raphe unit activity. There are several lines of evidence to suggest that it is through conversion to 5-HT that L-tryptophan exerts its electrophysiological effects. (1) Large systemic doses of L-tryptophan are reported to raise tissue levels of 5-HT and rates of 5-HT synthesis in rat brain, suggesting precursor control of 5-HT content and metabolism (Fernstrom and Wurtman, 1971; Knott and Curzon, 1974; Carlsson and Lindqvist, 1978). In hippocampal slices, low concentrations of L-tryptophan (10 μM) have been shown to increase the rate of 5-HT synthesis and release during sustained K^+-induced depolarizations (Auerbach and Lipton, 1985). (2) Tryptophan

hydroxylase, the enzyme which converts L-tryptophan to 5-hydroxytryptophan (5-HTP), has a reported K_m of 50-79 μM for the partially purified form (Friedman et al., 1972; Boadle-Biber, 1978; Nakata and Fujisawa, 1982) or 25-120 μM as determined in in vivo and in vitro tissue studies (Carlsson and Lindqvist, 1978; Elks et al., 1979). Addition of L-tryptophan to the ACSF in the present study (final concentration of 100 μM) therefore should increase the synthesis of 5-HT. (3) The systemic administration of L-tryptophan to rats depresses the firing of dorsal raphe neurons recorded in vivo (Gallager and Aghajanian, 1976). This L-tryptophan-induced suppression is prevented by Ro-4-4602, an L-aromatic amino acid decarboxylase inhibitor which blocks the conversion of 5-HTP to 5-HT, the final step in 5-HT biosynthesis (Bedard et al., 1971; Carlsson and Lindqvist, 1970). Similarly, Ro-4-4602 prevents L-tryptophan-induced suppression of dorsal raphe cell firing in brain slices (Aghajanian, unpublished observations). (4) Finally, in the present study, dialysis measurements showed that pretreatment with L-tryptophan (100 μM) increased MDMA-induced 5-HT release in a manner consistent with MDMA-induced inhibition of neuronal firing.

While the addition of L-tryptophan may induce an increase in 5-HT synthesis (see Elks et al., 1979), it is not clear whether 5-HT release is altered by the presence of precursor. Differential pulse voltammetry studies in rat striatum have shown that L-tryptophan given orally at 150 mg/kg does not alter 5-HIAA levels in the extracellular space (De Simoni et al., 1987). L-tryptophan given i.p. at 100 mg/kg did not increase 5-HT release in perfused rat lateral ventricles until a transient peak occurred in the second hour of perfusion (Ternaux et al., 1976). As described above, however, in a functional test of 5-HT release, L-tryptophan given i.p. at 100 mg/kg inhibited dorsal raphe cell firing, an effect which could be blocked by a 5-HT synthesis inhibitor (Gallager and Aghajanian, 1976). Furthermore, transcerebral dialysis measurements in freely moving rats recently demonstrated a calcium-dependent, tetrodotoxin-sensitive stimulation of cortical 5-HT release after L-tryptophan loads (Carboni et al., 1989). In the present study, it was not possible to determine whether the presence of L-tryptophan by itself increased 5-HT release because baseline levels of 5-HT (i.e. prior to the addition of MDMA) were below the detection limit of the assay system. In experiments to be reported elsewhere, measurable baseline levels of 5-HT release were observed using 500 μM L-tryptophan (Bradberry et al., in preparation). Far lower concentrations of L-tryptophan (10 μM) have been reported to increase the rate of 5-HT release in brain (hippocampal) slices, but in these studies release was induced by continuous application of high K^+ concentrations (60 mM) in the presence of an uptake inhibitor and a nerve terminal autoreceptor antagonist (Auerbach and Lipton, 1985).

MDMA-induced 5-HT release as revealed by microdialysis measurements closely correlates with MDMA-induced inhibition of dorsal raphe neuronal firing (Sprouse et al., 1989). Following the addition of MDMA (100 μM), firing rate drops to zero at the time 5-HT release peaks. Thereafter, firing rate and 5-HT release begin to return to baseline. In the present study, this correlation between 5-HT release and cell firing is preserved in the presence of L-tryptophan. Compared to untreated preparations, pretreatment with L-tryptophan produced a prolonged release of 5-HT and a prolonged inhibition of neuronal firing. Furthermore, in the presence of L-tryptophan there was a 3.2-fold shift in the IC_{50} for neuronal firing (16 vs. 5 μM; table 1) with similar shifts in peak 5-HT release (13 vs. 28 fmol; ratio of 2.2) and total fmol of 5-HT released (40 vs. 111 fmol; ratio of 2.8; fig. 4).

In our earlier study, MDMA-induced 5-HT release was shown to be independent of impulse flow as release proceeded during prolonged periods of neuronal silence (Sprouse et al., 1989). While independent of cell firing, the present results indicate that MDMA-induced 5-HT release is dependent upon the quantity of 5-HT available for release in that increasing extracellular L-tryptophan (and presumably intracellular 5-HT) increases the quantity of 5-HT released (fig. 4). This additional 5-HT release is functionally significant as the shift in the IC_{50} in the presence of L-tryptophan attests (table 1). Despite this apparent dependence on available 5-HT, in our brain slice

preparation M
5-HT depletio
neuronal inhib
liminary exper
MDMA for 6
release and sus
ing the 60 min
firing returned
time course as
tions.

The release
mine has been
synthesized, ex
model comes f
deplete the ma
to inhibit vesic
and Moore, 1
exact nature o
releases 5-HT
L-tryptophan
lease comes fro
synthesized neu
pool of neurotr
The release of s
the absence of
5-HT is synthesi
newly synthesiz
storage pool o
pool is also not
the pool(s) is cy
ment against M
comes from our
was shown to
MDMA on dor
blocking carrier-
et al., 1989).

Finally, the p
to what effect
psychotropic an
The effect of L
neurotoxicity hir
nerve endings is
projection areas,
to neither situati
worsen MDMA-i
5-HT accelerates
metabolite (Com
L-tryptophan ma
toxicity if the rel

preparation MDMA does not appear to produce 5-HT depletion as reflected in tachyphylaxis for neuronal inhibition (Sprouse et al., 1989). In preliminary experiments, sustained applications of MDMA for 60 min produced sustained 5-HT release and sustained neuronal inhibition. Following the 60 min application, 5-HT release and cell firing returned to baseline levels with the same time course as that following the 3 min applications.

The release of catecholamines by d-amphetamine has been hypothesized to come from a newly synthesized, extravesicular pool. Support for this model comes from studies utilizing reserpine to deplete the main catecholamine storage pool and to inhibit vesicular catecholamine uptake (Chiueh and Moore, 1975; Masuoka et al., 1982). The exact nature of the pool(s) from which MDMA releases 5-HT is not known. Our results with L-tryptophan suggest that MDMA-induced release comes from a pool(s) which contains newly synthesized neurotransmitter and from a sizable pool of neurotransmitter which has been 'stored'. The release of stored 5-HT seems likely because in the absence of L-tryptophan presumably little 5-HT is synthesized in the brain slice. Whether the newly synthesized 5-HT is added to an existing storage pool or forms a new MDMA-sensitive pool is also not known nor is it known whether the pool(s) is cytoplasmic or vesicular. One argument against MDMA-induced vesicular release comes from our earlier report in which fluoxetine was shown to block the inhibitory effects of MDMA on dorsal raphe neurons presumably by blocking carrier-mediated efflux of 5-HT (Sprouse et al., 1989).

Finally, the present results raise the question as to what effect L-tryptophan may have on the psychotropic and neurotoxic actions of MDMA. The effect of L-tryptophan on MDMA-induced neurotoxicity hinges on whether the loss of 5-HT nerve endings is related to the release of 5-HT into projection areas, to its depletion in the terminals, to neither situation or to both. L-tryptophan may worsen MDMA-induced neurotoxicity if release of 5-HT accelerates the production of a neurotoxic metabolite (Commins et al., 1987). Alternatively, L-tryptophan may lessen MDMA-induced neurotoxicity if the release of 5-HT delays the release of damaging quantities of dopamine (Yamamoto and Spanos, 1988; Stone et al., 1988) or if the increased amount of 5-HT in the nerve terminals in some way serves a protective function. L-tryptophan may facilitate the psychotropic actions of MDMA if release of 5-HT is causative (Schechter, 1989) or L-tryptophan may diminish the psychotropic actions if its presence produces greater neurotoxicity. With regard to this latter possibility, preinjection of PCA, which should cause the degeneration of 5-HT neurons, strongly attenuates the PCA-induced 5-HT syndrome (Kutscher and Yamamoto, 1979) and PCA-induced impairment of avoidance acquisition, nociception and hyperactivity (Ögren and Johansson, 1985). Additional studies examining these possibilities are warranted. Furthermore, the effect of L-tryptophan in a human population of MDMA users with differing dietary habits and with access to amino acid supplements deserves attention.

Acknowledgements

This work was supported by MH 14092, 14276, 17871, 25642, DA 05119 and the State of Connecticut. The authors wish to thank Nancy Margiotta and Mary Dooley (Merrell Dow Research Institute) for their assistance in the completion of this manuscript.

References

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

Aghajanian, G.K. and C.P. Vandermaelen, 1982, Intracellular identification of central noradrenergic and serotonergic neurons by a new double labeling procedure, J. Neurosci. 2, 1786.

Auerbach, S. and P. Lipton, 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

Bedard, P., A. Carlsson, K. Fuxe and M. Lindqvist, 1971, Origin of 5-hydroxytryptophan and L-dopa accumulating in brain following decarboxylase inhibition, Naunyn-Schmiedeb. Arch. Pharmacol. 269, 1.

Boadle-Biber, M.C., 1978, Activation of tryptophan hydroxylase from central serotonergic neurons by calcium and depolarization, Biochem. Pharmacol. 27, 1069.

Carboni, E., C. Cadoni, G.L. Tanda and G. Di Chiara, 1989,

Calcium-dependent, tetrodotoxin-sensitive stimulation of cortical serotonin release after a tryptophan load, J. Neurochem. 53, 976.

Carlsson, A. and M. Lindqvist, 1970, Accumulation of 5-hydroxytryptophan in mouse brain after decarboxylase inhibition, J. Pharm. Pharmacol. 22, 276.

Carlsson, A. and M. Lindqvist, 1978, Dependence of 5-HT and catecholamine synthesis on concentrations of precursor amino acids in rat brain, Naunyn-Schmiedeb. Arch. Pharmacol. 303, 157.

Chiueh, C.C. and K.E. Moore, 1975, d-Amphetamine-induced release of 'newly synthesized' and 'stored' dopamine from the caudate nucleus in vivo, J. Pharmacol. Exp. Ther. 192, 642.

Commins, D.L., G. Vosmer, R.M. Virus, W.L. Woolverton, C.R. Schuster and L.S. Seiden, 1987, Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain, J. Pharmacol. Exp. Ther. 241, 338.

De Simoni, M.G., A. Sokola, F. Fodritto, G. Dal Toso and S. Algeri, 1987, Functional meaning of tryptophan-induced increase of 5-HT metabolism as clarified by in vivo voltammetry, Brain Res. 411, 89.

Elks, M.L., W.W. Youngblood and J.S. Kizer, 1979, Serotonin synthesis and release in brain slices: independence of tryptophan, Brain Res. 172, 471.

Fernstrom, J.D. and R.J. Wurtman, 1971, Brain serotonin content: physiological dependence on plasma tryptophan levels, Science 173, 149.

Friedman, P.A., A.H. Kappelman and S. Kaufman, 1972, Partial purification and characterization of tryptophan hydroxylase from rabbit hindbrain, J. Biol. Chem. 247, 4165.

Gallager, D.W. and G.K. Aghajanian, 1976, Inhibition of firing of raphe neurons by tryptophan and 5-hydroxytryptophan: blockade by inhibiting serotonin synthesis with Ro-4-4602, Neuropharmacology 15, 149.

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

Hernandez, L.B., B.G. Stanley and B.G. Hoebel, 1986, A small removable dialysis probe, Life Sci. 39, 2629.

Justice, J.B., S.A. Wages and A.C. Michael, 1983, Interpretations of voltammetry in the striatum based on chromatography of striatal dialysate, J. Liq. Chromatogr. 6, 1873.

Knott, P.J. and G. Curzon, 1974, Effect of increased rat brain tryptophan on 5-hydroxytryptamine and 5-hydroxyindolyl acetic acid in the hypothalamus and other brain regions, J. Neurochem. 22, 1065.

Kutscher, C.L. and B.K. Yamamoto, 1979, A frequency analysis of behavior components of the serotonin syndrome produced by p-chloroamphetamine, Pharmacol. Biochem. Behav. 11, 611.

Masuoka, D.T., A.F. Alcaraz and H.F. Schott, 1982, [^{3}H] Dopamine release by d-amphetamine from striatal synaptosomes of reserpinized rats, Biochem. Pharmacol. 31, 1969.

Nakata, H. and H. Fujisawa, 1982, Purification and properties of tryptophan 5-monooxygenase from rat brain-stem, European J. Biochem. 122, 41.

Nichols, D.E., A.J. Hoffman, R.A. Oberlander, P. Jacob and A.T. Shulgin, 1986, Derivatives of 1-(1,3-benzodioxyl-5-yl)-2-butanamide: representatives of a novel therapeutic class, J. Med. Chem. 29, 2009.

Ögren, S.-O. and C. Johansson, 1985, Separation of the associative and non-associative effects of brain serotonin released by p-chloroamphetamine: dissociable serotonergic involvement in avoidance learning, pain and motor function, Psychopharmacology 86, 12.

Peroutka, S.J., 1987, Incidence of recreational use of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') on an undergraduate campus, N. Engl. J. Med. 317, 1542.

Schechter, M.D., 1989, Serotonergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'), Pharmacol. Biochem. Behav. 31, 817.

Schmidt, C.J., 1987, Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, J. Pharmacol. Exp. Ther. 240, 1.

Schmidt, C.J., J.A. Levin and W. Lovenberg, 1987, In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain, Biochem. Pharmacol. 36, 747.

Schmidt, C.J., L. Wu and W. Lovenberg, 1986, Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analog, European J. Pharmacol. 124, 178.

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

Stone, D.M., M. Johnson, G.R. Hanson and J.W. Gibb, 1988, Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine, J. Pharmacol. Exp. Ther. 247, 79.

Stone, D.M., D.C. Stahl, G.R. Hanson and J.W. Gibb, 1986, The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain, European J. Pharmacol. 128, 41.

Tallarida, R.J. and R.B. Murray, 1981, Manual of Pharmacologic Calculations with Computer Programs (Springer-Verlag, New York) p. 9.

Ternaux, J.P., A. Boireau, S. Bourgoin, M. Hamon, F. Hery and J. Glowinski, 1976, In vivo release of 5-HT in the lateral ventricle of the rat: effects of 5-hydroxytryptophan and tryptophan, Brain Res. 101, 533.

Ungerstedt, U., 1984, Measurement of Neurotransmitter Release in Vivo, ed. C.A. Marsden (John Wiley & Sons, England) p. 81.

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

Yamamoto, B.K. and L.J. Spanos, 1988, The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat, European J. Pharmacol. 148, 195.

European Journal o
Elsevier

EJP 51235

Cha

Department of Veter

Ring segments
mediated contract
norepinephrine >
about 42% of tha
Phentolamine (10
whereas yohimbir
10^{-7} M) but were
prazosin was dete
The pA_2 value of
between receptor
the irreversible an
and $2.5 \pm 0.8 \times 10$
response to norep
contraction to α-a
ceptors, and there
α_2-adrenoceptors

1. Introduction

α-Adrenocept
lar smooth mus
controlling the
the possible ana
(Langer, 1974)
sponses to selec
α_1- and α_2-recep
differentiate thes

Correspondence to:
Physiology and Phar
Iowa State Universit

0014-2999/90/$03.5