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ALTERED FOREBRAIN NEUROTRANSMITTER RESPONSES TO IMMOBILIZATION STRESS FOLLOWING 3,4-METHYLENEDIOXYMETHAMPHETAMINE

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Abstract—(±)3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is an increasingly popular drug of abuse that acts as a neurotoxin to forebrain serotonin neurons. The neurochemical effects of the serotonin depletion following high doses of MDMA were investigated in response to acute immobilization stress. Male rats were treated with a neurotoxic dosing regimen of MDMA (10 mg/kg, i.p. every 2 h for four injections) or equivalent doses of saline. Seven days after treatment, *in vivo* microdialysis was used to assess extracellular dopamine and serotonin in the dorsal hippocampus and prefrontal cortex during 1 h of immobilization stress. In saline treated control rats, serotonin in the hippocampus and serotonin and dopamine in the prefrontal cortex were increased during immobilization stress. Rats pretreated with MDMA, however, showed blunted neurotransmitter responses in the hippocampus and the prefrontal cortex. In the drug pretreated rats, basal serotonin levels in the hippocampus, but not the prefrontal cortex, were lower compared to saline pretreated controls. Stress-induced increases in plasma corticosterone and body temperature were not affected by the pretreatment condition.

From these studies we suggest that depletion of serotonin stores in terminal regions with the neurotoxin MDMA compromises the ability of the serotonergic neurons to activate central systems that respond to stressful stimuli. This altered responsiveness may have implications for long-term functional consequences of MDMA abuse as well as the interactions between the serotonergic system and stress.

Key words: MDMA, immobilization stress, prefrontal cortex, hippocampus, serotonin, dopamine.

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is a commonly abused 'club drug' with long-term neurochemical effects on the CNS. Administration of high doses of MDMA results in depletions of serotonin (5-hydroxytryptamine or 5-HT) in the frontal cortex, hippocampus, and striatum of rodents and non-human primates (Callahan et al., 2001; Gibb et al., 1990; Schmidt, 1987; Stone et al., 1986). Neuronal damage to the serotonergic system is evidenced by decreased tryptophan hydroxylase activity, decreased number of 5-HT reuptake sites and immunocytochemical damage to 5-HT axons following high doses of MDMA (Battaglia et al., 1987; Commins et al., 1987; O'Hearn et al., 1988; Schmidt and Taylor, 1987; Stone et al., 1987). The degeneration of 5-HT axons has been reported to be limited to fine-diameter axons from the dorsal raphe nucleus that project to forebrain regions, such as the hippocampus and frontal cortex (Mamounas et al., 1991; O'Hearn et al., 1988; Wilson et al., 1989).

Although the neurochemical alterations following neurotoxic treatment with MDMA have been well characterized, the functional consequences of MDMA at neurotoxic doses are relatively unknown. Several studies have reported that 7 days after treatment with a neurotoxic regimen of MDMA, extracellular 5-HT responses to an acute injection of MDMA or D-fenfluramine are suppressed, as are behavioral and hyperthermic responses (Series et al., 1994; Shankaran and Gudelsky, 1999). Prior exposure to a neurotoxic regimen of MDMA also decreased the sensitivity of rhesus monkeys to the subsequent effects of an acute injection of dexfenfluramine on a behavioral task, or of rats to MDMA in a

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Abbreviations: 5-HT, 5-hydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; ANOVA, analysis of variance; EDTA, ethylenediaminetetra-acetate; HPLC-EC, high-performance liquid chromatography with electrochemical detection; MDMA, (±)3,4-methylenedioxymethamphetamine; VTA, ventral tegmental area.

drug discrimination task (Frederick et al., 1995; Schechter, 1991). However, in a behavioral paradigm without the use of a drug challenge, MDMA pretreatment in rats had no effect on a place navigational learning-set task, or maze performance (Ricaurte et al., 1993; Robinson et al., 1993; Seiden et al., 1993; Slikker et al., 1989). Thus, the effects of MDMA on the functioning of the CNS may only be apparent during an activated or stimulated state, such as following a drug injection or an environmental stressor.

The integrity of the central serotonergic system appears to be important for some types of neurochemical responses to the presentation of an acute stressor. In rats, lesions of hypothalamic 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) attenuate the increase in plasma corticosterone concentrations following photic stimuli (Feldman, 1985; Feldman et al., 1984). In contrast, dopamine metabolism in prefrontal cortex and nucleus accumbens following 30 min of restraint stress was enhanced in rats that received injections of 5,7-DHT into the dorsal raphe (Morrow and Roth, 1996). Therefore, a sufficient serotonergic tone may be important in maintaining the appropriate neurochemical responses to an acute stressor.

Exposure to an environmental stressor increases 5-HT and dopamine levels in several brain regions, including areas innervated by 5-HT and depleted by MDMA (for review see Chaouloff, 1993). The application of acute stressors has been correlated with increased 5-HT release in the prefrontal cortex, hippocampus, striatum, hypothalamus, amygdala and periaqueductal gray of the rat as measured by *in vivo* microdialysis or voltammetry (Adell et al., 1997; Amat et al., 1998; Boutelle et al., 1990; Joseph and Kennett, 1983, 1986; Kawahara et al., 1993; Kirby et al., 1997; Shimizu et al., 1992; Takahashi et al., 1998; Yoshioka et al., 1995). In a similar manner, extracellular dopamine and dopamine turnover increase in the prefrontal cortex, striatum and nucleus accumbens in response to tail shock/pressure, restraint, foot shock or handling stress (Abercrombie et al., 1989; Cenci et al., 1992; Finlay et al., 1995; Finlay and Zigmond, 1997; Gresch et al., 1994; Imperato et al., 1991; Kawahara et al., 1999; Keefe et al., 1993; Nakahara and Nakamura, 1999; Sorg and Kalivas, 1993; Taber and Fibiger, 1997; Thierry et al., 1976). The hippocampus and prefrontal cortex may play important roles in the interaction between neurotransmitter and neuroendocrine responses to stress due to the large number of corticosterone receptor sites in each, their projections to hypothalamic structures, and their dense monoamine innervation (Chaouloff, 1993; Diorio et al., 1993; Herman and Cullinan, 1997; McEwen et al., 1986).

It remains to be determined whether 5-HT terminal damage produced by exposure to high doses of MDMA alters the stress-induced release of monoamines in brain regions targeted by MDMA. The objective of the present study was to measure extracellular concentrations of 5-HT in the hippocampus, as well as 5-HT and dopamine in the prefrontal cortex, during immobilization stress of rats that were administered MDMA 7 days earlier.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (175–250 g) were purchased from Zivic Miller Labs (Allison Park, PA, USA). Rats were housed individually, with food and water available *ad libitum*, on a 12-h light–dark cycle in a temperature controlled room. All procedures were in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the local institutional animal care committee.

Drugs

All rats were given i.p. injections with 10 mg/kg MDMA hydrochloride salt (National Institutes of Drug Abuse, Bethesda MD, USA) or an equivalent volume of saline (0.9% NaCl), every 2 h for a total of four injections. Drug injections were given in a volume of 1 ml/kg. The above injection procedure has been shown previously to decrease 5-HT neurotransmitter content in the striatum (Shankaran and Gudelsky, 1999).

Surgical procedures

Three days after the i.p. injections, all rats were anesthetized with a combination of xylazine (6 mg/kg) and ketamine (70 mg/kg) and placed into a Kopf stereotaxic frame. The skull was exposed and two 21-gauge stainless steel guide cannulae (Small Parts, Miami Lakes, FL, USA) were positioned above the cortex (prefrontal cortex: 3.2 mm anterior and 0.5 mm medial to bregma) and dorsal hippocampus (3.2 mm posterior and 2.0 mm medial to bregma). The cannulae and a metal female connector were secured to the skull with three stainless steel screws and cranioplastic cement. Obturators fashioned from 31-gauge stainless steel wire, ending flush with the guide cannulae, were inserted into the cannulae after surgery.

Experiment 1: Measurement of monoamines with in vivo microdialysis during restraint stress

Three days after surgery, the obturators were removed from the guide cannulae and replaced with microdialysis probes. The probes were constructed as previously described (Lowy et al., 1993) from a 27-gauge thin wall stainless steel tube, fitted with a dialysis membrane (13 000 Da cut off, 210 μm outer diameter; Spectrum Laboratories, Rancho Domingues, CA, USA) at one end, and a 3 cm piece of polyethylene 20 tubing (Fisher Scientific, Pittsburg, PA, USA) at the other end, to serve as the inlet for the perfusion medium. The dialysis membrane was 4 mm or 2 mm$\times$210 μm diameter for the prefrontal cortex or dorsal hippocampus, respectively. A 4 cm length of capillary tubing (125 μm outer diameter, 50 μm inner diameter; Polymicro Technologies, Phoenix, AZ, USA) served as the outlet from the dialysis membrane. The vertical placement of the microdialysis probe was determined during construction of the probe by gluing a ring of polyethylene 20 tubing, which acts as a mechanical 'stop', at a measured distance along the length of the probe. The positioning permitted the exposed portion of the dialysis membrane to extend beyond the guide cannulae and into the prefrontal cortex (ventral from dura -1.0 to -5.0) or dorsal hippocampus (ventral from dura -2.0 to -4.0). The rats were placed in microdialysis cages and attached to a swivel (Instech Laboratories, Plymouth Meeting, PA, USA), with food and water available *ad libitum*.

Eighteen hours after probe insertion, Dulbecco's phosphate-buffered saline medium (138 mM NaCl, 2.1 mM KCl, 0.5 mM

$MgCl_2$, 1.5 mM KH_2PO_4, 8.1 mM $NaHPO_4$, 1.2 mM $CaCl_2$, and 5 mM D-glucose, pH 7.4) was pumped through the microdialysis probes with a Harvard Model 22 syringe infusion pump (Hollison, MA, USA). The Dulbecco's medium was perfused at a rate of 1.5 μl/min. A 3-h perfusion period was allowed prior to sample collection. Twenty-minute samples were then collected during the following conditions: three baseline samples, three samples during immobilization stress, and three samples following the immobilization stress. The immobilization stress procedure was initiated between 11:30 h and 13:00 h. For the immobilization stress, rats were placed with their ventral surface on a Plexiglas board and secured with a 2-inch Velcro strap across their midregion and a 1-inch Velcro strap behind their head. Tape was used to secure their paws. In five saline and five MDMA pretreated rats, rectal temperatures were monitored every 10 min during the 1-h immobilization stress with a Thermalert TH-8 monitor (Physitemp Instruments, Clinton, NJ, USA). After the 1-h immobilization, the tape and Velcro strips were removed and the rats returned to their microdialysis cages for the remaining three samples.

Experiment 2: Plasma corticosterone concentrations prior to and during restraint stress

Separate groups of rats given injections of either MDMA or saline were killed and trunk blood assayed for corticosterone concentrations. These rats did not have microdialysis cannulae implanted. One week after injections, saline ($n = 12$) or MDMA treated ($n = 12$) rats were killed by rapid decapitation either without immobilization stress ($n = 12$) or 10 min into the immobilization stress procedure ($n = 12$). Trunk blood was collected into a 4-ml vial with 0.1 ml heparin sodium sulfate (1000 U/ml, Pharmacia&Upjohn, Kalamazoo, MI, USA) and centrifuged for 15 min ($4000 \times g$).

Histology

After the microdialysis study or the blood collection, brains were quickly removed from the skull and frozen on dry ice. For the microdialysis experiment, probe placements were verified from frozen coronal sections and only rats with probes located in the prefrontal cortex or dorsal hippocampus were used for statistical analysis.

Neurochemical analysis of monoamines (high-performance liquid chromatography)

Microdialysis samples were assayed for dopamine and/or 5-HT by high-performance liquid chromatography with electrochemical detection (HPLC-EC). Samples from the prefrontal cortex were assayed for dopamine and 5-HT, and samples from the hippocampus were assayed for 5-HT only. Samples (20 μl) were loaded via a Rheodyne injector (Cotati, CA, USA) onto a 3-μm C18 column (100×2.0 mm, Phenomenex, Torrance, CA, USA). The mobile phase (pH 4.2) consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM EDTA, 0.215 mM octyl sodium sulfate and 3% methanol. Compounds were detected with an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA), with a 6 mm glassy carbon working electrode maintained at a potential of +0.7 V relative to an Ag/AgCl reference electrode. Data were collected using a Hewlett Packard Integrator.

Analysis of 5-HT and dopamine tissue concentrations of the frontal cortex and 5-HT tissue concentrations of the dorsal hippocampus were performed on an HPLC-EC, as described above for the monoamines. The tissues were sonicated in 0.1 M perchloric acid, centrifuged at $13\,000 \times g$ for 6 min. The supernatant was assayed for 5-HT and dopamine. The pellet was resuspended in 1 N NaOH and protein content determined by a Bradford Protein Assay.

Radioimmunoassay of corticosterone

Corticosterone was measured in plasma (5 μl) samples that were diluted with 100 μl sterile water and stored at 4°C until assayed. The radioimmunoassay was adapted from a previously reported procedure (Keith et al., 1978) and employed [^{125}I]corticosterone from ICN Pharmaceuticals (Costa Mesa, CA, USA) and antisera from Ventrex (Portland, ME, USA). Counts per minute were normalized and fit to a least-squares regression equation produced by log–logit transformation of the standards. Mass of samples was calculated by interpolation of the standards. The detectable range of the assay was from 0.1 to 400 μg corticosterone per 100 ml plasma. Intra- and inter-assay coefficients of variation were less than 10%. The specificity of the assay is very high, with only 4% cross-reactivity to deoxycorticosterone, 1% cross-reactivity to 5β-pregnanedione, and less than 0.6% cross-reactivity to other endogenous steroids.

Statistical analysis

Thirty rats were used for the microdialysis experiment. Of the 15 rats treated with MDMA and the 15 rats treated with saline prior to surgery, six rats were excluded from data analysis due to misplaced cannulae or microdialysis difficulties. Further data were excluded from analysis if the average basal monoamine concentrations were below a 3:1 signal-to-noise limit of detection. Twenty-four rats were used to determine the concentration of corticosterone in plasma.

Two-way, repeated-measures analyses of variance (ANOVAs) were computed to compare rats pretreated with MDMA to those pretreated with saline, across all samples (baseline samples, stress samples and post-stress samples) for each neurotransmitter. Individual one-way repeated-measures ANOVAs were also computed separately when appropriate for the saline and MDMA pretreated groups to assess possible differences due to immobilization stress. Post-hoc Newman–Keuls pairwise tests were used to analyze any significant treatments. Independent *t*-tests were used to compare the pretreatment groups (MDMA vs. saline) on the following measures: 5-HT and dopamine tissue content in the frontal cortex or the dorsal hippocampus; the average of the baseline samples of each neurotransmitter; and peak rectal temperatures. A two-way ANOVA was used to compare the corticosterone plasma concentrations prior to and during immobilization stress, in saline and MDMA pretreated rats. Statistical significance was fixed at $P < 0.05$ for all tests.

RESULTS

Neurochemical content of monoamines

Serotonin tissue content of rats treated with MDMA

Table 1. Serotonin and dopamine tissue levels (μg/g protein) in the dorsal hippocampus and frontal cortex 7 days following treatment with MDMA (10 mg/kg i.p. × 4 injections, every 2 h) or an equivalent volume of saline

Brain region and neurotransmitter measured	Saline treated	MDMA treated
Hippocampus 5-HT	2.38 ± 0.15	1.08 ± 0.13*
Frontal cortex 5-HT	2.44 ± 0.30	1.27 ± 0.22*
Frontal cortex dopamine	0.82 ± 0.18	0.72 ± 0.07

All data are presented as the mean ± S.E.M. *$P < 0.05$ vs. saline injected control by an independent Student's *t*-test.

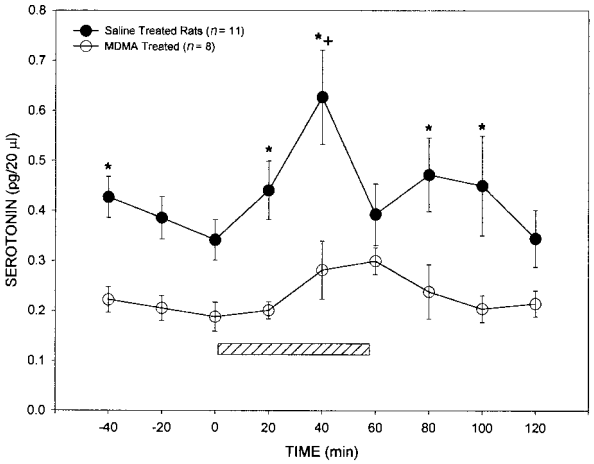

Fig. 1. Effects of 1-h immobilization stress on extracellular levels of 5-HT in the dorsal hippocampus of rats pretreated 7 days earlier with MDMA (10 mg/kg i.p. for four injections, every 2 h) or an equivalent volume of saline. Baseline microdialysis samples were collected for 1 h, after which rats were immobilized (hatched bar) for 1 h followed by a 1-h post-stress interval. Basal concentrations of 5-HT were higher in saline compared to MDMA pretreated rats ($P < 0.05$). Concentrations of 5-HT were elevated in saline pretreated rats during the second period of immobilization stress (time = 40 min) compared to the final baseline (time = 0 min) (*$P < 0.05$ versus respective MDMA pretreated group; $^{+}P < 0.05$ versus time = 0 of saline treated group).

was significantly decreased by 55% in the dorsal hippocampus ($t(28) = 6.28$, $P < 0.01$) and 48% in the frontal cortex ($t(28) = 3.09$, $P < 0.01$) compared to saline treated rats (Table 1). There was no effect of MDMA pretreatment on the concentration of dopamine in the frontal cortex ($t(26) = 0.674$).

Extracellular serotonin response to stress

In the dorsal hippocampus, there was a significant difference between the treatment groups in 5-HT concentrations throughout the experiment as indicated by the main effect of treatment ($F(1,109) = 14.63$, $P < 0.001$; Fig. 1) and through post-hoc comparisons at −40, 20, 40 80 and 100 min ($P < 0.05$). This difference was highlighted by a significant increase in extracellular 5-HT after 40 min of the immobilization stress compared to baseline (time = 0 min) in saline treated rats ($F(8,109) = 2.46$, $P < 0.05$), but not of MDMA treated rats.

In the prefrontal cortex, there was a significant interaction between drug pretreatment (saline or MDMA) across time for extracellular 5-HT concentrations ($F(8,147) = 2.52$, $P < 0.05$, Fig. 2). Post-hoc comparisons indicate that there was a significant increase in 5-HT in the saline, but not the MDMA pretreated group, during the first 20 min of immobilization stress compared to baseline.

Extracellular dopamine response to stress

There was an overall increase in extracellular dopamine concentrations in the prefrontal cortex ($F(8,105) = 2.33$, $P < 0.05$). The saline pretreated group showed a significant increase in dopamine during the first 20 min of immobilization compared to baseline (one-way ANOVA, $F(8,59) = 3.06$, $P < 0.01$; Fig. 3). In contrast, extracellular dopamine concentrations in the MDMA treated group were unaffected by stress and did not change across time ($F(8,46) = 0.66$).

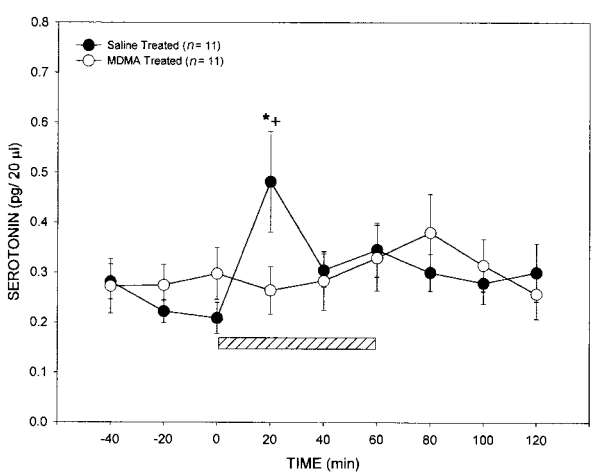

Fig. 2. Effects of 1-h immobilization stress on extracellular levels of serotonin in the prefrontal cortex of rats pretreated 7 days earlier with MDMA (10 mg/kg i.p. for four injections, every 2 h) or an equivalent volume of saline. Baseline microdialysis samples were collected for 1 h, after which rats were immobilized (hatched bar) for 1 h followed by a 1-h post-stress interval. Saline pretreated rats showed an increase in 5-HT concentrations during the first period of immobilization stress (time = 20 min). No change in 5-HT was observed in MDMA pretreated rats (*$P < 0.05$ versus respective MDMA pretreated group; $^{+}P < 0.05$ versus time = 0 of saline treated group).

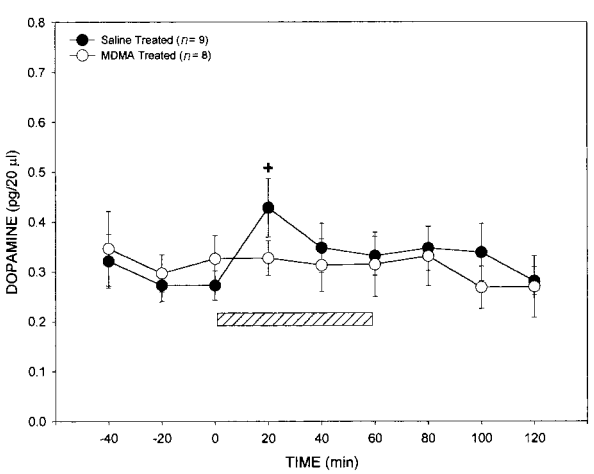

Fig. 3. Effects of 1-h immobilization stress on extracellular levels of dopamine in the prefrontal cortex of rats pretreated 7 days earlier with MDMA (10 mg/kg i.p. for four injections, every 2 h) or an equivalent volume of saline. Baseline microdialysis samples were collected for 1 h, after which rats were immobilized (hatched bar) for 1 h followed by a 1-h post-stress interval. Saline pretreated rats showed an increase in dopamine concentrations during the first period of immobilization stress (time = 20 min). No change in dopamine was observed in MDMA pretreated rats ($^{+}P < 0.05$ versus time = 0 of saline pretreated group).

Basal serotonin and dopamine levels following MDMA or saline pretreatment

Extracellular concentrations of 5-HT in the dorsal hippocampus were significantly lower in rats treated with MDMA compared to saline treated rats during baseline (0.21 ± 0.02 pg/20 µl vs. 0.38 ± 0.02 pg/20 µl; $t(47) = 4.91$, $P < 0.001$). There were no differences in basal concentrations of dopamine or 5-HT in the prefrontal cortex between MDMA and saline pretreated rats.

Corticosterone and hyperthermic responses to stress

No significant differences in plasma corticosterone concentrations were observed between the saline and MDMA treated rats, either prior to or 10 min into the immobilization stress procedure ($F(1,20) = 0.80$). Immobilization stress increased plasma corticosterone levels in both groups ($F(1,20) = 23.41$, $P < 0.001$, Table 2). There was no difference in peak rectal temperatures during the immobilization procedure between the two groups: saline treated 38.50 ± 0.16, MDMA treated 38.3 ± 0.22°C ($t(9) = 0.04$).

DISCUSSION

MDMA pretreatment attenuates stress-induced neurotransmitter release

The primary, novel finding of the current study is that MDMA pretreatment can inhibit acute 5-HT and dopamine responses to a behavioral challenge, i.e. immobilization stress. The attenuated 5-HT responsiveness is consistent with previous studies using less subtle pharmacological challenges that activate striatal and cortical 5-HT systems (Series et al., 1994; Shankaran and Gudelsky, 1999). A similar attenuation of 5-HT release following a pharmacological challenge has also been reported following other neurochemical lesions of 5-HT neurons, e.g. 5,7-DHT, *p*-chloramphetamine, or fenfluramine (Baumann et al., 1998; Kirby et al., 1995; Romero et al., 1998; Sabol et al., 1992; Series et al., 1994).

To our knowledge, this is the first report that depleting 5-HT with a serotonergic neurotoxin leads to an attenuation of extracellular dopamine concentrations in response to an environmental challenge. Other reports have suggested 5-HT depletions may augment the responsiveness of dopamine neurons to dopaminergic agents. In MDMA- or 5,7-DHT-induced 5-HT lesioned rats, a challenge injection of cocaine enhanced extracellular levels of dopamine in the nucleus accumbens, increased dopamine metabolism in the prefrontal cortex, and increased conditioned place preference (Horan et al., 1997, 2000; Morrow and Roth, 1996). Restraint stress also increased dopamine metabolism to a greater extent in rats with 5,7-DHT lesions compared to sham controls (Morrow and Roth, 1996). The assessment of dopaminergic activity with ex vivo tissue concentrations rather than *in vivo* microdialysis may explain the discrepancies between Morrow and Roth (1996) and the present findings. Of more interest is the possibility that the toxicity of MDMA to the serotonergic system produces a unique response profile with regard to its longer-term impact on dopaminergic activity.

The blunted stress-induced dopamine response observed in our study may be due to the attenuated stress-induced release of 5-HT in the prefrontal cortex. Increasing 5-HT levels through blockade of 5-HT uptake with fluoxetine or the perfusion of 5-HT itself through a microdialysis probe in the prefrontal cortex increased dopamine levels (Iyer and Bradberry, 1996; Matsumoto et al., 1999). Alternatively, the removal of the normal 5-HT mediated inhibition of GABAergic tone on dopamine cell bodies of the ventral tegmental area (VTA) (Johnson et al., 1992; Kalivas, 1993) may explain the inhibition of stress-induced cortical dopamine release after neurotoxic doses of MDMA. The VTA contains 5-HT fibers, which in part, originate from the dorsal raphe nucleus, a region targeted by MDMA (Herve et al., 1987; Imai et al., 1986; Vertes, 1991). If 5-HT release in the VTA is attenuated following MDMA, GABAergic tone would be enhanced and terminal dopamine release inhibited.

MDMA pretreatment affects basal serotonin levels in the dorsal hippocampus

In addition to the effects on stimulated 5-HT release, basal 5-HT concentrations were lower in rats pretreated with MDMA in the dorsal hippocampus, but not in the prefrontal cortex. The magnitude of 5-HT depletion produced by MDMA may contribute to these regional differences in basal 5-HT concentrations 1 week after MDMA. Decreased basal 5-hydroxyindoleacetic acid levels but not 5-HT concentrations were reported in rats treated with high doses of MDMA in the ventral hippocampus, prefrontal cortex or striatum (Gartside et al., 1996; Series et al., 1994; Shankaran and Gudelsky, 1999). It is possible that a 40–50% decrease in tissue 5-HT as observed previously in the striatum (Shankaran and Gudelsky, 1999) or frontal cortex (Table 2) is not

Table 2. Corticosterone concentrations (µg/dl) in trunk blood plasma collected 7 days following treatment with MDMA (10 mg/kg ip. × 4 injections, every 2 h) or an equivalent volume of saline

Time of corticosterone measurement	Saline treated	MDMA treated
Prior to restraint stress	3.0 ± 2.2	4.5 ± 4.4
During exposure to restraint stress	20.3 ± 4.1*	20.6 ± 3.2*

All data are presented as the mean ± S.E.M. *$P < 0.05$ vs. prior to restraint stress by a two-way ANOVA.

sufficient to produce changes in basal extracellular concentrations of 5-HT.

A similar relationship between the severity of tissue depletion and the extracellular basal concentrations has been observed following other 5-HT neurotoxic regimens and other monoamine neurotoxins, such as 6-hydroxydopamine. As reported for hippocampal 5-HT in the present study, depletions of tissue norepinephrine content greater than 50% seem to be required for alterations in basal hippocampal norepinephrine (Abercrombie and Zigmond, 1989). In the striatum, Hall et al. (1999) found decreased basal extracellular 5-HT levels following 65% or greater depletions of striatal 5-HT tissue content following 5,7-DHT lesions. However, another study reported no changes in extracellular levels of 5-HT after depletions of 76–93% in striatal 5-HT (Kirby et al., 1995). This later study measured 5-HT levels in anesthetized rats 3 h following probe implantation, rather than in freely moving, awake animals 18 h after probe insertion. Therefore, a longer period for stabilization of extracellular 5-HT concentrations, such as used in this study, may be necessary to measure subtle differences in basal extracellular concentrations of neurotransmitters (Hall et al., 1999).

MDMA pretreatment does not affect stress-induced endocrine or autonomic responses

Central serotonergic systems can modulate stress-stimulated activity of the hypothalamic–pituitary–adrenal axis (for review see Chaouloff, 1993). MDMA, possibly through its ability to increase extracellular 5-HT levels, acutely increases plasma corticosterone (Aguirre et al., 1997; McNamara et al., 1995; Nash et al., 1988). However, these effects of MDMA on corticosterone are not persistent. There were no differences in basal or stress-stimulated corticosterone responses 7 days following MDMA pretreatment (Table 2; Aguirre et al., 1997). This lack of a long-term effect of MDMA on corticosterone responsiveness may be due to the regional selectivity of MDMA-induced 5-HT depletions. MDMA more severely damages 5-HT axons in cortical and limbic terminal regions than in hypothalamic regions (for review see Sprague et al., 1998; Battaglia et al., 1987; O'Hearn et al., 1988; Stone et al., 1986; however see Callahan et al., 2001). Previous studies have reported variable effects of 5-HT lesions on corticosterone levels depending on the neurotoxin used to produce the lesion and the type of stimulus (Baumann et al., 1998; Chung et al., 1999; Poland, 1990). These differences in corticosterone responses may also be due to the varying degrees of 5-HT depletions in hypothalamic nuclei observed in the studies (Baumann et al., 1998; Chung et al., 1999).

Acute stressors have been shown to produce hyperthermic responses (Kluger et al., 1987; Long et al., 1990; Morimoto et al., 1991; Terlouw et al., 1996). In this experiment, the stress-induced rise in core body temperature occurred in both saline and MDMA pretreated groups. These findings corroborate previous reports that basal, acute stress- or drug-stimulated thermic responses remained intact following 5-HT depletions (McNamara et al., 1995; Chung et al., 1999). Thus, while 5-HT depletions with MDMA may alter neurotransmitter responses to an acute stressor, the autonomic responses appear to remain intact.

OVERALL CONCLUSIONS

Pretreatment with MDMA resulted in 5-HT tissue depletions in the hippocampus and the frontal cortex. MDMA pretreatment blunted the dopamine and 5-HT increases during acute immobilization stress observed in non-depleted or saline pretreated rats. While previous studies have shown that the release of 5-HT in response to a pharmacological agent may be impaired by MDMA pretreatment, the present study indicates that the release of neurotransmitters induced by an environmental change (i.e. stress) also is disrupted. The importance of the various neurotransmitter responses that have been correlated with the onset of an acute stressor is unknown. The increases in neurotransmitter release may be specific to a particular stressor, or may be a more general response to any relevant arousing stimuli. Regardless of the specific behavioral significance, MDMA treated rats exhibited abnormal physiological responses that may have significant implications for long-term functional consequences of MDMA abuse as well as the interactions between the serotonergic system and stress.

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