Psychopharmacology (1997) 131 : 411–419

ORIGINAL INVESTIGATION

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Abnormal ACTH and prolactin responses to fenfluramine in rats exposed to single and multiple doses of MDMA

Received: 12 November 1996/Final version: 14 January 1997

Abstract The present study examined the persistent functional consequences associated with exposure to single and multiple doses of (±) 3,4-methylenedioxymethamphetamine (MDMA) as reflected by the neuroendocrine responses to *d,l*-fenfluramine (FEN). Adult male rats were administered a single dose of MDMA (20 mg/kg, SC) and challenged 2 weeks later with saline or FEN (2, 4, 6 and 8 mg/kg, SC). The corticotropin (ACTH) response to FEN (6 and 8 mg/kg) was blunted and the prolactin response to FEN (4 and 6 mg/kg) was enhanced in MDMA pre-treated rats. The ACTH and prolactin responses to FEN (6 mg/kg, SC) were then evaluated 4, 8 and 12 months after exposure to single and multiple doses MDMA (20 mg/kg, SC and 20 mg/kg, SC, bid, × 4 days, respectively). The ACTH response to FEN was significantly reduced at 4 and 8 months in both MDMA treatment groups, and at 12 months in the multiple dose group only. In contrast, the prolactin response to FEN was enhanced in both groups of MDMA treated rats at 4 months, but only in the multiple dose group at 8 months. By 12 months, the prolactin response to FEN had normalized. Following multiple doses of MDMA, 5-HT concentrations were reduced significantly in the frontal cortex at 4 and 12 months. The results indicate that exposure to single or multiple doses of MDMA can produce functional alterations which can persist for months, whereas the biochemical sequelae were less robust and shorter lived.

Key words 3,4-Methylenedioxymethamphetamine (MDMA) · ACTH · Prolactin · Fenfluramine · Neurotoxicity · Serotonin (5-HT)

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Introduction

It is well established that the substituted amphetamine (±) 3,4-methylenedioxymethamphetamine (MDMA), also known as Ecstasy, can be neurotoxic to serotonin (5-HT) systems in the brains of rats and non-human primates (Schmidt 1987a; Battaglia et al. 1990; McKenna and Peroutka 1990; Ricaurte et al. 1992; Green et al. 1995). As manifested by decreased 5-HT concentrations and 5-HT reuptake sites, administration of a single dose of MDMA can disrupt 5-HT systems for weeks (Commins et al. 1987), whereas multiple doses are affective for months (De Souza and Battaglia 1989). Despite considerable data on the neurochemical effects of MDMA, it remains unclear as to whether functional alterations are linked to the neurochemical changes (Nencini et al. 1988; Poland 1990; Winslow and Insel 1990; Sharkey et al. 1991; Rezvani et al. 1992; LeSage et al. 1993; Ricaurte et al 1993; Robinson et al. 1993; Romano and Harvey 1994; McNamara et al. 1995). Because the central nervous system (CNS) is the predominate regulator of neuroendocrine function (Tuomisto and Männistö 1985), with pituitary ACTH and prolactin secretion strongly influenced by 5-HT pathways in the brain (Van de Kar 1991; Fuller 1992), an abnormal response of these hormones to 5-HT agonist administration might serve as a potential functional marker of MDMA neurotoxicity.

Previous studies have demonstrated that animals with 5-HT lesions induced by electrolytic procedures or treatment with 5,7-dihydroxytryptamine (5,7-DHT) show blunted corticosterone and prolactin responses to acute challenge with indirectly acting 5-HT agonists such as fenfluramine (FEN) (Quattrone et al. 1979; Schettini et al. 1979). In order to determine if MDMA produces comparable neuroendocrine sequelae, the present study was undertaken to ascertain the long-term neurochemical and functional consequences of single and multiple doses of MDMA, as measured by brain 5-HT concentrations and by neuroendocrine responses to FEN, respectively.

Materials and methods

Animals

Viral antibody free adult male Sprague Dawley rats (190–200 g) were purchased from Harlan Sprague-Dawley, San Diego, Calif., USA. Animals were housed three or four/cage and maintained under a 12–12 h light-dark cycle with food and water available ad libitum. Rats were acclimated to the housing conditions for 1 week prior to beginning the experiment. All injections were administered subcutaneously (SC) at 2 ml/kg between 0530 and 0730 hours and between 1630 and 1830 hours. Animals were handled daily for 1 week prior to injection and death. Rats were killed by decapitation 15 min after FEN administration. All FEN studies were performed between 0500 and 0730 hours. Trunk blood was collected into chilled plastic tubes containing EDTA (10 mg), aprotinin (500 kallikrein units) and sodium azide (0.081 mg). Blood was spun at 500 *g* for 20 min at 4°C. Plasma aliquots were placed into plastic vials using plastic pipettes and stored at −70°C.

Experimental procedures

Experiment I

Animals received saline or a single dose of MDMA (20 mg/kg). Fourteen days later, the rats were challenged with saline or FEN (2, 4, 6 or 8 mg/kg) and killed 15 min later. Regional brain 5-HT concentrations were measured in a sub-group of saline and MDMA treated animals.

Experiment II

Animals received saline, a single dose of MDMA (20 mg/kg) or multiple doses of MDMA (20 mg/kg, bid, × 4 days). All animals received a total of eight injections approximately 12 h apart; 1) eight injections of saline, 2) a single injection of MDMA (20 mg/kg) followed by seven injections of saline over the next 4 days, and 3) eight injections of MDMA (20 mg/kg, bid, × 4 days). Four, 8 and 12 months later, rats received saline or FEN (6 mg/kg) and were killed 15 min post-challenge. Regional brain 5-HT concentrations were measured in a sub-group of saline and MDMA treated animals.

Hormone assays

Plasma ACTH and prolactin were analyzed by specific radioimmunoassays as described previously (Poland et al. 1980; 1989, Pechnick et al. 1990). ACTH antiserum was obtained from INC-STAR Corp (Stillwater, Minn., USA). Minimum sensitivity was 35 pg/ml. Materials for the prolactin radioimmunoassay were obtained from the National Pituitary Agency with results expressed as RP-1 units. Minimum sensitivity was 0.5 ng/ml. ACTH and prolactin were analyzed in duplicate. As determined by multiple pool replicates, maximum inter- and intra-assay variability of all hormone assays was 12.2%.

Biochemical measures

Serotonin concentrations

Brain tissue was dissected on ice, weighed, and homogenized in 5 vol perchloric acid (0.04 M). The homogenate was centrifuged at 13000 *g* for 5 min at 4°C, and the supernatant was transferred to clean tubes and frozen at −70°C until chromatography. Analytes were separated on a reversed phase column (Axiom ODS, 4.6 × 150 mm, 5 μm particle size) using a mobile phase which consisted of an aqueous phase (33 mM citrate, 33 mM phosphate, 0.27 mM EDTA) adjusted to pH 4.1 with sodium phosphate and mixed with acetonitrile to 10%. The mobile phase was pumped at 1 ml/min at ambient temperature. Detection was by fluorescence (Shimadzu RF-551), and detector output was captured using a Rainin Dynamax data acquisition system. Quantitation was achieved with reference to regression lines from detector response to external standards. As determined by brain tissue pools, inter- and intra-run variations were 8% and 5%, respectively. Minimum sensitivity was equivalent to 25 ng/g tissue.

Materials

(±) MDMA HCL was provided by the National Institute of Drug Abuse, Rockville, Md., USA. *d,l*-FEN HCL was obtained from Sigma Chemical Corporation, St Louis, Mo., USA. All doses were calculated as free base equivalents.

Statistical analysis

Hormone and biochemical data initially were analyzed by two-way analysis of variance (ANOVA). If significant, one-way ANOVAs and *t*-tests were performed to determine the location of significant differences. In order to reduce the likelihood of a type I statistical error, significance levels were corrected for the number of *t*-tests performed (Jacobs 1976). $P \leq 0.05$ was considered statistically significant.

Results

Figure 1 shows the mean (± SEM) ACTH (top) and prolactin (bottom) responses to FEN (2, 4, 6 and 8 mg/kg) in rats treated 2 weeks previously with saline or a single dose of MDMA (20 mg/kg × 1). FEN significantly affected ACTH concentrations ($F_{4,99} = 18.4$, $P \leq 0.0001$). As compared to acute saline challenge (open and hatched bars on extreme left of figure), the ACTH responses to FEN (4, 6 and 8 mg/kg) were significantly increased in both the saline and MDMA pretreated rats. A significant MDMA effect was detected ($F_{1,99} = 4.1$, $P \leq 0.05$), but no significant interaction was observed ($F_{4,99} = 0.31$). As compared

to the corresponding saline pretreated counterparts (open bars), the ACTH responses to FEN (6 and 8 mg/kg) were significantly reduced in the MDMA rats ($P \leq 0.05$) (hatched bars). FEN significantly altered prolactin concentrations ($F_{4,99} = 12.4$, $P \leq 0.0001$). As compared to acute saline challenge (open and hatched bars on extreme left of figure), the prolactin responses to all doses of FEN were significantly increased in both the saline and MDMA pretreated rats. A significant MDMA effect was detected ($F_{1,99} = 14.1$, $P \leq 0.001$), but no interaction was observed ($F_{4,99} = 0.92$, NS). As compared to the corresponding saline pretreated counterparts (open bars), the prolactin responses to FEN (4 and 6 mg/kg) were significantly enhanced in the MDMA animals ($P \leq 0.05$) (hatched bars). As shown in Table 1, 5-HT concentrations were reduced significantly in the frontal cortex, hippocampus and hypothalamus ($P \leq 0.05$).

Figure 2 shows ACTH (top) and prolactin (bottom) responses to a single dose of FEN (6 mg/kg) in rats treated 4, 8 and 12 months previously with saline, MDMA (20 mg/kg) and MDMA (20 mg/kg, bid × 4 days). The cross-hatched bars represent the mean neuroendocrine responses to acute saline challenge in the saline and MDMA pretreated rats. There were no differences in the response among the groups to saline challenge, so the results were combined and presented for comparison purposes. As compared to this group, FEN (6.0 mg/kg) significantly increased ACTH and prolactin concentrations in all groups irrespective of pretreatment ($P \leq 0.05$).

For ACTH, a significant MDMA effect was observed ($F_{2,109} = 7.0$, $P \leq 0.002$). There was no effect of time ($F_{2,109} = 2.3$, NS) and there was no interaction ($F_{4,109} = 0.41$, NS). As compared to their saline treated counterparts (open bars), the ACTH response to FEN was reduced significantly in both MDMA treatment groups at 4 and 8 months, but reduced only in the multiple dose group at 12 months ($P \leq 0.05$) (closed bars). For prolactin, significant effects of MDMA

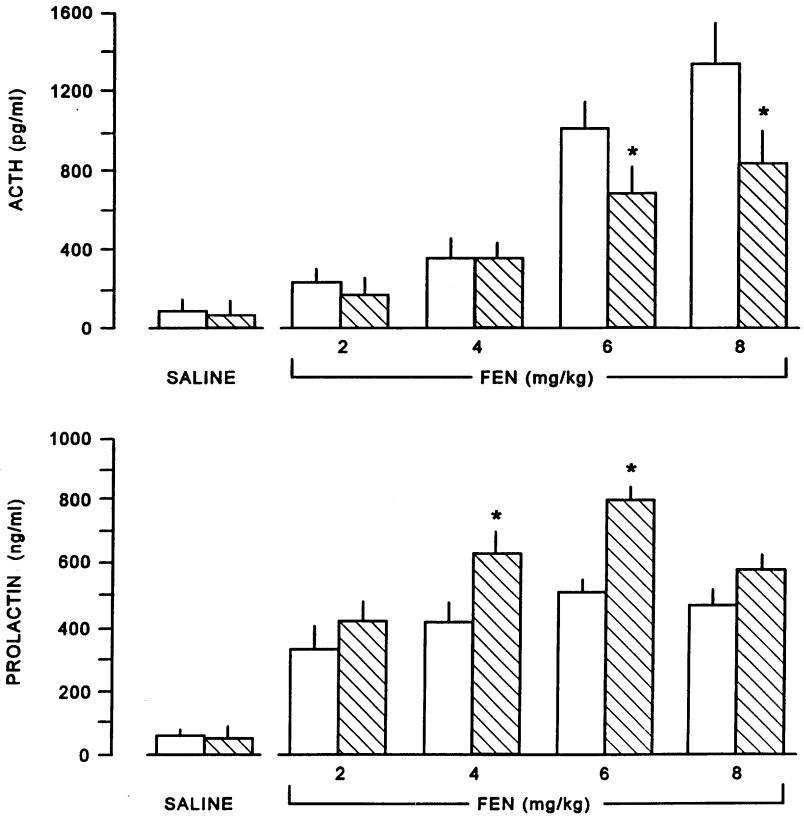

Fig. 1 Effect of saline and increasing doses of fenfluramine (2–8 mg/kg) on mean (±SEM) plasma ACTH (*top*) and prolactin (*bottom*) concentrations in rats exposed to a single dose of saline (□) or MDMA (▨) (20 mg/kg × 1) 2 weeks previously. *$P \leq 0.05$ as compared to corresponding saline control. (See text for results of additional comparisons.) ($n = 12$–24/group)

Table 1 Mean (±SEM) 5-HT concentrations 2 weeks after a single dose of MDMA (20 mg/kg × 1) ($n = 6$/group)

	2 Weeks (5-HT ng/g)		
	Frontal cortex	Hippocampus	Hypothalamus
Saline	339 ± 17	362 ± 23	812 ± 38
MDMA (20 mg/kg × 1)	228 ± 15*	247 ± 9*	560 ± 25*

*$P \leq 0.05$

($F_{2,109} = 9.8$, $P \leq 0.001$) and time ($F_{2,109} = 12.3$, $P \leq 0.001$) were found, but there was no interaction ($F_{4,109} = 1.81$, $P = 0.14$, NS). As compared to their saline treated counterparts (open bars), the prolactin response to FEN was significantly enhanced in both groups of MDMA treated rats at 4 months, but only in the multiple dose group at 8 months ($P \leq 0.05$) (closed bars). By 12 months, the prolactin response to FEN had normalized in both MDMA groups. The prolactin response to FEN was significantly reduced in both MDMA groups at 8 and 12 months as compared to their pretreatment counterparts at 4 months.

The effects of MDMA on 5-HT concentrations in the frontal cortex, hippocampus and hypothalamus at 4, 8 and 12 months are shown in Table 2. In frontal cortex, there was a significant effect of MDMA ($F_{2,39} = 5.2$ $P \leq 0.01$) and time ($F_{2,39} = 3.2$, $P \leq 0.05$), but there was no interaction ($F_{4,39} = 0.45$, NS). As compared to respective controls at each time point, 5-HT concentrations were significantly reduced in the multiple dose MDMA groups at 4 and 12 months ($P \leq 0.05$).

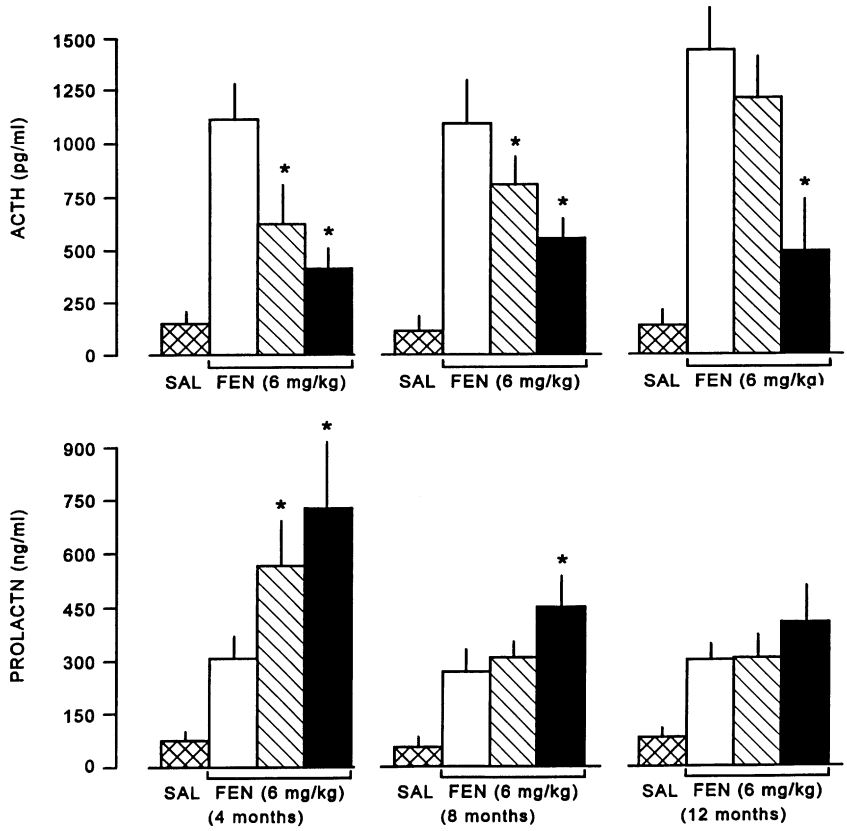

Fig. 2 Mean (±SEM) plasma ACTH (*top*) and prolactin (*bottom*) concentrations in response to fenfluramine (6 mg/kg) 4, 8 and 12 months after exposure to single and multiple doses of saline (□) or MDMA (20 mg/kg × 1 (▨) and 20 mg/kg × 8 (■)). *Hatched bar* represents the hormone responses of the combined saline and MDMA pretreated rats to saline challenge. *$P \leq 0.05$ as compared to corresponding saline control. (See text for results of additional comparisons.) (n = 12–15/group)

Table 2 Mean (± SEM) 5-HT concentrations in frontal cortex, hippocampus and hypothalamus at 4, 8, and 12 months following single (20 mg/kg × 1) and multiple (20 mg/kg × 8) doses of MDMA (n = 4–8/group)

	Frontal cortex	Hippocampus	Hypothalamus
4 Months (5-HT ng/g)			
Saline	336 ± 29	517 ± 25	731 ± 64
MDMA (20 mg/kg × 1)	299 ± 18	543 ± 46	747 ± 55
MDMA (20 mg/kg × 8)	237 ± 23*	408 ± 69	701 ± 118
8 Months (5-HT ng/g)			
Saline	398 ± 64	567 ± 34	929 ± 92^{+}
MDMA (20 mg/kg × 1)	332 ± 47	520 ± 33	792 ± 58
MDMA (20 mg/kg × 8)	297 ± 21	386 ± 21	743 ± 72
12 Months (5-HT ng/g)			
Saline	457 ± 21^{+}	449 ± 54	995 ± 46^{+}
MDMA (20 mg/kg × 1)	436 ± 17^{++}	548 ± 154	970 ± 15^{++}
MDMA (20 mg/kg × 8)	294 ± 24*	569 ± 212	1070 ± 56^{++}

* $P \leq 0.05$ as compared to saline at same time
$^{+}P \leq 0.05$ as compared to corresponding group at 4 months
$^{++}P \leq 0.05$ as compared to corresponding groups at 4 and 8 months

The 5-HT concentration in the saline group at 12 months was significantly higher than at 4 months ($P \leq 0.05$). The 5-HT concentration in the single dose MDMA group was significantly greater at 12 months than at 4 or 8 months ($P \leq 0.05$). In hippocampus, there were no significant effect of MDMA ($F_{2,39} = 0.85$, NS) or time ($F_{2,39} = 0.18$, NS) and there was no interaction ($F_{4,39} = 1.2$, NS). In the hypothalamus, no MDMA effect was found ($F_{2,39} = 0.70$, NS). There was a significant effect of time ($F_{2,39} = 7.6$, $P \leq 0.002$), but there was no interaction ($F_{4,39} = 1.4$, NS). In the saline controls, 5-HT concentrations were significantly higher at 8 and 12 months as compared to 4 months ($P \leq 0.05$). In both MDMA groups, 5-HT concentrations were significantly greater at 12 months as compared to their respective controls at 4 and 8 months ($P \leq 0.05$).

Discussion

The administration of MDMA can affect various physiologic processes acutely (e.g. nocioception, brain glucose utilization, temperature, learning, alcohol consumption and neuroendocrine regulation) (Nash et al. 1988; Crisp et al. 1989; Wilkerson and London 1989; Rezvani et al. 1992; Romano and Harvey 1994). Delayed effects from MDMA administration also have been reported. For example, responsiveness to morphine analgesia was increased (Nencini et al. 1988), ultrasonic vocalization decreased (Winslow and Insel 1990) and glucose utilization in the hippocampus increased (Sharkey et al. 1991) in rats exposed to multiple doses of MDMA 2 weeks previously. The neuroendocrine response to 8-OH-DPAT, a 5-HT_{1A} agonist, also was found to be abnormal 2 weeks after a single dose of MDMA (Poland 1990). In contrast, persistent effects of MDMA on memory have not been found (LeSage et al. 1993; Ricaurte et al. 1993; Robinson et al. 1993). To our knowledge, no functional abnormalities have been reported beyond 2 weeks.

For the present study, FEN was selected as the 5-HT probe because we believed that a drug which recruits both pre- and post-synaptic elements of the 5-HT neuron into the response would be more likely to detect an abnormality. FEN also can be administered to humans (Yatham and Steiner 1993), so that comparable studies might be undertaken in human subjects with MDMA experience. FEN robustly stimulated both ACTH and prolactin secretion. FEN (6 mg/kg) was chosen for the long-term studies because this dose elicited abnormal responses of both ACTH and prolactin at 2 weeks. However, a full dose-response study with FEN needs to be performed to elucidate further the mechanisms underlying the abnormal responses. In addition, other doses of FEN might detect abnormalities which the 6 mg/kg dose could not.

The neuroendocrine response to FEN was altered for up to 1 year in rats exposed to multiple doses of MDMA. The exact mechanisms underlying the differential effect of MDMA on the ACTH and prolactin responses to FEN are unknown. Previous studies have found that the ACTH (or corticosterone) and prolactin responses to FEN (or other indirectly acting 5-HT agonists) were reduced or unaffected in animals with (non-MDMA induced) 5-HT lesions (Quattrone et al. 1979; Schettini et al. 1979; Van de Kar and Bethea 1982; Willoughby et al. 1982; McElroy et al. 1984; Fuller 1992), although an augmented response to fenfluramine in 5,7-DHT pretreated animals also has been reported (Kuhn et al. 1981). In one published study with MDMA, no changes in the ACTH or prolactin response to *d*-FEN (7.5 mg/kg) were found in animals treated 2 weeks previously with MDMA (20 mg/kg, bid × 4 days), although there were trends for both hormones to show blunted responses in the MDMA treated rats (Series et al. 1995). Explanations for the divergent findings are not readily apparent, although differences in route of administration (IM versus SC) for both FEN and MDMA, duration of "handling" prior to the FEN challenge, FEN dose, as well as use of the *d* versus *dl* isomer might be involved. In regard to dose, the dose of *d*-FEN (7.5 mg/kg) used by Series et al. (1995) is equivalent to 15 mg/kg of the racemic mixture, which is more than twice the dose utilized herein. The issue surrounding the specificity of *d,l*-fenfluramine for 5-HT systems remains unresolved. However, it appears that both the *d* and *l* isomers are capable of stimulating prolactin secretion at least in humans (Feeney et al. 1993), so that the augmented prolactin response following *d,l*-fenfluramine might be mediated, at least in part, by *l*-fenfluramine's neuroleptic-like properties (Bettini et al. 1987; Invernizzi et al. 1989).

In contrast to studies utilizing indirectly acting 5-HT agonists, the prolactin and ACTH responses to directly acting 5-HT agonists such as *m*-chlorophenylpiperazine (MCPP) and RU 24969 were found to be augmented in parachloroamphetamine and 5,7-DHT lesioned animals (Quattrone et al. 1981; Meltzer et al. 1982; Van de Kar et al. 1989a). Consistent with results of these studies, Bluet-Pajot et al. (1995) reported that prolactin response to 8-OH DPAT was augmented in animals with bilateral electrolytic lesions of the paraventricular nucleus (PVN) in the hypothalamus, a major site of neuroendocrine regulation which receives 5-HT projections from the brain stem (Van de Kar and Bethea 1982; Korte et al. 1991). However, in the same study, the ACTH response to 8-OH DPAT was blunted. The latter hormone response profile was identical to that observed previously in animals pretreated with MDMA and challenged with 8-OH DPAT (Poland 1990), and to the response profile measured herein following FEN.

These results suggest that the abnormal responses to FEN might be mediated by changes in 5-HT_{1A}

receptor-coupled events. However, the 5-HT receptor subtypes involved in the neuroendocrine responses to FEN have not been fully delineated. For example, in rats the 5-HT_2 antagonist, LY53857, did not antagonize the prolactin or the corticosterone response to FEN (Lorens and Van de Kar 1989; Van de Kar et al. 1989b), whereas the prolactin response to FEN was significantly reduced by pretreatment with the 5-HT_2 antagonist ritanserin (Di Renzo et al. 1989). In humans, the prolactin response to FEN was blocked by metergoline (Quattrone et al. 1983), a non-selective 5-HT antagonist, as well as with ritanserin and amesergide, relatively selective 5-$HT_{2A/2C}$ antagonists (Goodall et al. 1995; Coccaro et al. 1996). Cyproheptadine, a non-selective and somewhat non-specific 5-HT antagonist, antagonized the ACTH and cortisol responses as well (Lewis and Sherman 1984). Data for involvement of 5-HT_{1A} receptors as mediators of the ACTH and prolactin responses to FEN are limited. One study in male volunteers found that pretreatment with pindolol, a beta and 5-HT_{1A} antagonist, attenuated the prolactin (but not the cortisol) response to *d*-FEN (Palazidou et al. 1995). In contrast, Park and Cowen (1995) reported no effect of pindolol on the prolactin response to *d*-FEN in normal subjects. In rats, the prolactin and ACTH responses to *d,l*-FEN (2 and 6 mg/kg, SC) were significantly attenuated by pretreatment with (−) propanolol (3 mg/kg) (Poland et al., unpublished). Thus, available data suggest that both 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors might be involved in the neuroendocrine response to FEN.

Although FEN might be acting via a 5-HT_{1A} mechanism, this still does not resolve the issue as to why the animals showed an augmented prolactin and a blunted ACTH response. However, a number of factors could account for this. First, the contribution of pre- and post-synaptic 5-HT_{1A} receptors involved in the regulation of the two axes, and their adaptive responses, might not be the same (Aguirre et al. 1995). For example, animals and humans treated with antidepressants frequently show divergent ACTH and prolactin responses to 5-HT agonists, including fenfluramine (Aulakh et al. 1991; Poland and Frazer 1991; Poland et al. 1995). Second, the origin of the 5-HT pathways involved in the regulation of these two hormone axes differs (Van de Kar and Bethea 1982; Fessler et al. 1984; Liposits et al. 1987; Van de Kar 1991; Fuller 1992), as does their sensitivity to compounds such as MDMA (Mamounas and Molliver 1988; Jacobs and Azmitia 1992). There appears to be (at least) two classes of 5-HT axon terminals which differ in regional distribution, axon morphology, cells of origin and response to psychotropic compounds. 5-HT fibers from the dorsal raphe are extremely fine and innervate the hypothalamus to control prolactin secretion, whereas the regulation of ACTH secretion by 5-HT is less well defined (Van de Kar et al. 1982, 1985). Some investigators suggest that neither the dorsal nor the medial raphe contribute 5-HT projections to the PVN, whereas others believe that projections from both nuclei contribute to the regulation of the ACTH secretion (Liposits et al. 1987; Fuller 1996). Fibers (terminals) emanating from the dorsal raphe are sensitive to destruction by MDMA, while those from the medial raphe are coarser and more resistant to MDMA induced damage. It is possible that the augmented prolactin response was due to incomplete destruction of 5-HT terminals involved in prolactin regulation coupled with post-synaptic supersensitivity. In contrast, the 5-HT system involved in ACTH secretion might have been more severely affected by MDMA resulting in a blunted ACTH response to *d,l*-fenfluramine. Finally, as opposed to corticotropin regulation, prolactin secretion is under strong inhibitory control by the tuberoinfundibular dopamine system (Tuomisto and Männistö 1985). Destruction of 5-HT pathways by MDMA which are "upstream" to this system might sensitize 5-HT_{1A} receptors on dopaminergic neurons.

In regard to the biochemical effects of MDMA, 2 weeks after administration of a single dose of MDMA (20 mg/kg), significant reductions of 5-HT concentrations were found in the frontal cortex, hippocampus and hypothalamus. These results are comparable to those reported by others who also administered single doses of MDMA and studied the animals 1–2 weeks later (Commins et al. 1987; Schmidt 1987b; Stone et al. 1987; Johnson et al. 1989; Gibb et al. 1990; Schmidt and Kehne 1990; Lehman et al. 1992; Schmidt et al. 1992). Few studies have evaluated the effects of a single dose of MDMA past 2 weeks. The exception is the study by Commins et al. (1987), where regional brain 5-HT concentrations were measured 2 months after a single dose of MDMA (20 and 40 mg/kg). In that study, significant reductions of 5-HT were found in the hippocampus and cortex following both doses of the drug, as well as in the hippocampus following the lower dose, with a similar trend occurring in the cortex. There was a non-significant reduction of 5-HT in the hypothalamus following the 40 mg/kg dose, with 5-HT levels being somewhat higher in this region following the 20 mg/kg dose. In the present study, no significant differences of 5-HT were found at any time point beyond 2 weeks. However, some trends for reductions as well as increases were observed which differed according to brain region and time. To the best of our knowledge, no data are available on the effects of a single dose of MDMA past 2 months.

The multiple dose MDMA regimen produced significant reductions of 5-HT in the frontal cortex at 4 and 12 months, with a similar trend observed at 8 months. Using the identical multiple-dose MDMA administration regimen, Battaglia et al. (1990) reported reductions of cortical 5-HT concentrations of approximately 30% at 1 year, whereas the reduction of cortical 5-HT reported herein was 35.7%. More recently, Sabol et al. (1996), also using the same dosage

regimen, found significant reductions of 5-HT in frontal cortex at 4 months and 8 months with a trend at 1 year. We also found trends for the reduction of 5-HT in the hippocampus at 4 and 8 months, results which also are consistent with those found by Sabol et al. (1996).

Although no significant differences in hypothalamic 5-HT concentrations were observed past 2 weeks, 5-HT concentrations were lower at 8 months following both dosage regimens. This is in contrast to previous studies which have found that 5-HT neurons in the hypothalamus were less affected by MDMA than other regions of the brain (Commins et al. 1987; Stone et al. 1987). However, it is possible that the reduction of 5-HT was due, at least in part, to the progressive increase in hypothalamic 5-HT levels which occurred from 4 to 12 months in the saline controls (see Table 2). For the control rats, hypothalamic 5-HT concentrations increased from 731 ± 64 ng/g at 4 months, to 929 ± 92 ng/g at 8 months, and to 995 ± 46 ng/g at 1 year. The MDMA treated animals did not show increases until 1 year. Consequently, the reductions of hypothalamic 5-HT at 8 months in the MDMA treated rats would appear due to the 5-HT increase in the control animals and not necessarily to a fall in 5-HT concentrations in the MDMA treated rats. Sabol et al. (1996) also observed progressive and significant increases in 5-HT over time in a number of brain regions, although not in the hypothalamus. Differences in experimental design might account for the discrepant findings in the hypothalamus. In the latter study, MDMA treatment was staggered so that all animals were studied within an 8-week period. In the present study, treatment time was fixed and assessments were made over the course of 1 year. In addition to the reductions of 5-HT, trends for an increase in both the hypothalamus and hippocampus were observed at 1 year, as well as in the hippocampus at 8 months. This "overshoot" of 5-HT concentrations has been observed previously and might be related to hyperreinnervation (Ricaurte et al. 1992; Fischer et al. 1995; Sabol et al. 1996).

It should be noted that the neuroendocrine responses to FEN were abnormal even when 5-HT concentrations were not. However, brain 5-HT levels were not determined in the same animals from which the hormone responses were elicited because tissue obtained from these animals would not have reflected "basal" levels of 5-HT. Since considerably variability exists in regard to the degree of recovery among animals following MDMA (Scanzello et al. 1993), it is not surprising that no relationship between neurochemistry and function was discerned. It also is possible that the abnormal neuroendocrine response to FEN might be linked to a different aspect of neuronal function, such as 5-HT reuptake sites, on which FEN acts to influence 5-HT neurotransmission. Following multiple doses of MDMA, De Souza and Battaglia (1989) reported complete recovery of 5-HT reuptake sites in the cortex at 1 year, whereas Scanzello et al. (1993) found 5-HT reuptake sites remained reduced at 1 year in the hippocampus. More recently, Sabol et al. (1996) found 5-HT reuptake sites significantly decreased in a number of brain regions at 12 months. Although no significant reductions were found in the hypothalamus, a trend did occur in the ventromedial hypothalamus.

In summary, the ACTH response to FEN was blunted for up to 1 year following exposure to multiple doses of MDMA, and up to 8 months after exposure to a single dose. The augmented prolactin response to FEN was shorter lived, but still abnormal at 8 months, in the multiple dose group. There was no obvious relationship between brain 5-HT concentrations and the abnormal neuroendocrine responses to FEN. Whether the abnormal hormone responses in the MDMA treated animals solely reflect a dysregulation of 5-HT systems or are due to 5-HT neurodegeneration remains to be determined. In addition, based upon these data, the assessment of the neuroendocrine response profile to FEN in humans previously exposed to MDMA might help to clarify the controversy surrounding the issue of MDMA use and 5-HT neurotoxicity (Liester et al. 1992; Grob and Poland 1997).

Acknowledgements This research was supported in part by the National Institutes of Health grants NIDA DA06863, NIMH Research Scientist Development Award MH00534 (to R.E.P.) and NIMH Physician Scientist Award MH00722 (J.T.M.). We thank Jeffrey Lee, DVM, for his help with the implementation of this study, and Scott McGeoy, Mary Magdaline Que and Mary Price for excellent technical assistance. We thank Debbie Hanaya for expert administrative assistance.

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