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IMMEDIATE AND LONG-TERM EFFECTS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE ON SEROTONIN PATHWAYS IN BRAIN OF RAT

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Summary—In the rat, administration of the psychoactive analog of amphetamine 3,4-methylenedioxymethamphetamine (MDMA), causes selective, pronouced decreases in markers of central serotonergic function. The time course of these neurochemical changes was examined in several serotonergic nerve terminal regions of the brain. Fifteen min after subcutaneous injection of MDMA (10 mg/kg), the enzymatic activity of tryptophan hydroxylase (the rate-limiting enzyme for the biosynthesis of serotonin) was significantly decreased in the frontal cortex; by 1 hr after the injection, the activity of tryptophan hydroxylase had significantly declined in the neostriatum, hippocampus and hypothalamus as well. Although extensive recovery had occurred by 2 weeks, the activity of the enzyme remained significantly depressed in most regions. Decline of the regional content of 5-hydroxytryptamine (5-HT) closely paralleled, but was usually preceded by, that of the enzyme. Concentrations of the primary metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), were less responsive: in most regions levels of 5-HIAA had significantly decreased by 3 hr, but not by 1 hr, following treatment. Markers of dopamine function were altered transiently but had returned to control values by 24 hr.

Administration of multiple doses of MDMA (5 doses over a 24-hr period) resulted in significant decreases in serotonergic parameters for up to 110 days after treatment. The rate and extent of recovery varied according to both the dose administered and the region examined. The persistence of these serotonergic deficits suggests that MDMA induced the destruction of serotonin-containing axon terminals.

Key words: methylenedioxymethamphetamine, tryptophan hydroxylase, serotonin, neurotoxicity.

Recently, much attention has been focussed on the apparent adverse neurochemical effects of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), a ring-substituted congener of amphetamine which has gained notoriety as both a popular recreational drug and as a controversial psychotherapeutic agent (Adler, Abramson, Katz and Hager, 1985). Systemic administration of MDMA to rats causes selective and pronounced decreases in markers of central serotonergic function. More specifically, the regional activity of tryptophan hydroxylase and corresponding regional concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) are dramatically reduced 3 hr after a single subcutaneous injection of MDMA (10 mg/kg; Stone, Stahl, Hanson and Gibb, 1986) at a dose approximately 3–4 times that of the acute human dose (1–3 mg/kg; Shulgin and Nichols, 1978). Multiple injections of MDMA result in persistent depletion of these serotonergic parameters for at least 2 weeks after treatment, with little sign of recovery (Stone, Johnson, Hanson and Gibb, 1987); additionally, the number of high-affinity uptake sites for serotonin are concomitantly decreased (Yeh, Battaglia, O'Hearn, Kuhar, Molliver and De Souza, 1986). Unlike its ring-unsubstituted analog, methamphetamine, MDMA does not decrease markers of catecholamine function (Stone *et al.*, 1986; Schmidt, Wu and Lovenberg, 1986). These and other studies detailing the selective effect of this compound on serotonergic pathways, have prompted investigators to compare MDMA to the classical serotonergic neurotoxin para-chloroamphetamine (PCA), a single 10 mg/kg dose of which decreases central serotonergic parameters in the rat for up to 4 months (Sanders-Bush, Bushing and Sulser, 1972a).

The possible irreversible or prolonged serotonergic effects of MDMA are of particular concern in view of the widespread human abuse of this compound. The present investigation was undertaken to provide a more detailed account of the time course of the neurochemical changes induced by systemic administration of MDMA; in particular, it was hoped to determine whether MDMA does, as previously suggested, cause long-term, irreversible alterations in central serotonergic pathways. In addition, the immediate, dynamic changes in both serotonergic and catecholaminergic parameters induced by acute administration of MDMA were examined. To this end, regional concentrations of monoamine neurotransmitters and their major metabolites were measured at various times after treatment with MDMA by high-performance liquid chromatography with electrochemical detection. As additional indicators of monoaminergic responses, the regional activity of the biosynthetic enzymes for the

synthesis of dopamine and serotonin, tyrosine hydroxylase and tryptophan hydroxylase, respectively, were assessed by radioenzymatic assay.

METHODS

Male Sprague–Dawley rats (200–250 g) were housed 5 per cage in a temperature-controlled room (26°C) with a 12-hr alternating light–dark cycle. They were allowed free access to standard laboratory food and water. The (±) MDMA was dissolved in 0.9% saline and administered subcutaneously in single or multiple (5 doses, 1 every 6 hr) doses. Treatment groups were injected with either 5 or 10 mg/kg MDMA (expressed as the free base), or with vehicle alone. Rats were sacrificed by decapitation (within a 3-hr period at mid-day) at times ranging from 15 min to 110 days after completion of treatment with drug; groups for each time included both MDMA-treated and saline-treated (control) rats. Immediately after decapitation, the whole brain was placed on ice, and the neostriata, hippocampi and frontal cortex regions were removed bilaterally and stored at −80°C until assayed. The hypothalamus was subsequently excised from a coronal slice of the frozen brain (A 5660 to A 2970; Konig and Klippel atlas, 1967) with a dissecting knife.

Enzyme assays

One of the paired tissues from each region was weighed and homogenized in 80–180 μl of 50 mM HEPES buffer (Sigma Chemical Co., St. Louis, Missouri) (pH 7.4), containing 0.2% Triton X-100 (v:v) and 5 mM dithiothreitol (Calbiochem-Behring Corp., San Diego, California). After centrifugation at 27,000 × *g* for 15 min, duplicate 7.5-μl aliquots of the supernatant were removed and assayed for the activity of trytophan hydroxylase by a modified $^{14}CO_2$-trapping procedure (Ichiyama, Nakamura, Nishizuka and Hayaishi, 1970; Sitaram and Lees, 1978) as described by Hotchkiss, Morgan and Gibb (1979). In some cases, identical 7.5-μl aliquots (neostriatal tissue only) were diluted to 50 μl with glass-distilled water and analyzed for the activity of tyrosine hydroxylase, according to the method of Nagatsu, Levitt and Udenfriend (1964). The incubation medium in each tube (100 μl total volume) contained 500,00 cpm of L-[3,5-^{3}H]-tyrosine (54.2 Ci/mmol, New England Nuclear, Boston, Massachusetts), 10 nmol tyrosine, 100 nmol ferrous ammonium sulfate, 320 nmol *dl*-6-methyl-5,6,7,8-tetrahydro-biopterin (Sigma chemical Co., St. Louis, Missouri), 10 nmol 2-mercaptoethanol and 20 μmol sodium acetate.

Determination of concentrations of monoamines and metabolites of monoamines

Regional concentrations of 5-HT and its primary metabolite, 5-HIAA, and neostriatal concentrations of dopamine (DA) and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured in the contralateral tissue by high-performance liquid chromatography. Tissues were homogenized in 0.3–0.5 ml mobile phase buffer (0.15 M monochloroacetic acid, 2.0 mM EDTA, 0.1 mM 1-octanesulfonic acid and 12.5% methanol, pH 2.9) and centrifuged at 4080 × *g* for 15 min. The resulting supernatant fraction was filtered through a 0.2-μm microfilter system (Bioanalytical Systems Inc., West Lafayette, Indiana) and 50 μl were injected onto a 10-cm Microsorb reverse-phase column (Rainin Instrument Co., Woburn, Massachusetts). The eluent was monitored with an amperometric electrochemical detector (model LC-4B; Bioanalytical Systems, Inc., West Lafayette, Indiana), with the potential set at +0.73 V. The concentrations of monoamines and their metabolites in tissue were quantified by comparison with a calibration curve, derived from standards of known concentration, prepared in mobile phase buffer.

RESULTS

Within 15 min of a single injection of MDMA (10 mg/kg), the activity of tryptophan hydroxylase had begun to decline in all regions examined (Fig. 1, left panel), yet was statistically less than the control activity only in the frontal cortex. By 1 hr after the injection, enzymatic activity had also significantly decreased in the neostriatum, hippocampus and hypothalamus. The regional decrease in the activity of tryptophan hydroxylase appeared to precede that of 5-HT or its metabolite, 5-HIAA. Examination of Figure 1 (left panel) reveals that regional concentrations of 5-HT were not significantly decreased 15 min after injection of the drug; however, by 1 hr after the injection concentrations of this transmitter had declined to less than 60% of controls in the neostriatum, hippocampus and frontal cortex and to 74% of control in the hypothalamus. The decline in 5-HIAA generally lagged behind that of its parent compound; by 3 hr after the injection, but not 1 hr, levels of 5-HIAA were significantly decreased in all areas (Fig. 1, left panel).

The right panel in Figure 1 presents a time course of the more prolonged regional effects of a single injection of MDMA (10 mg/kg). In both the hippocampus and frontal cortex, a biphasic effect on 5-HT was observed: maximum decreases occurred within the first 3 hr after treatment with drug, after this time the concentrations of 5-HT transiently recovered, then once again declined, so that 2 weeks after treatment regional levels of 5-HT remained significantly depressed. Whereas the activity of tryptophan hydroxylase was also maximally decreased 3–6 hr after MDMA, the pattern of recovery differed from that of 5-HT; enzyme activity remained maximally depressed for 24 hr after treatment with the drug, then exhibited a gradual, prolonged recovery. Complete recovery had not occurred by 2 weeks,

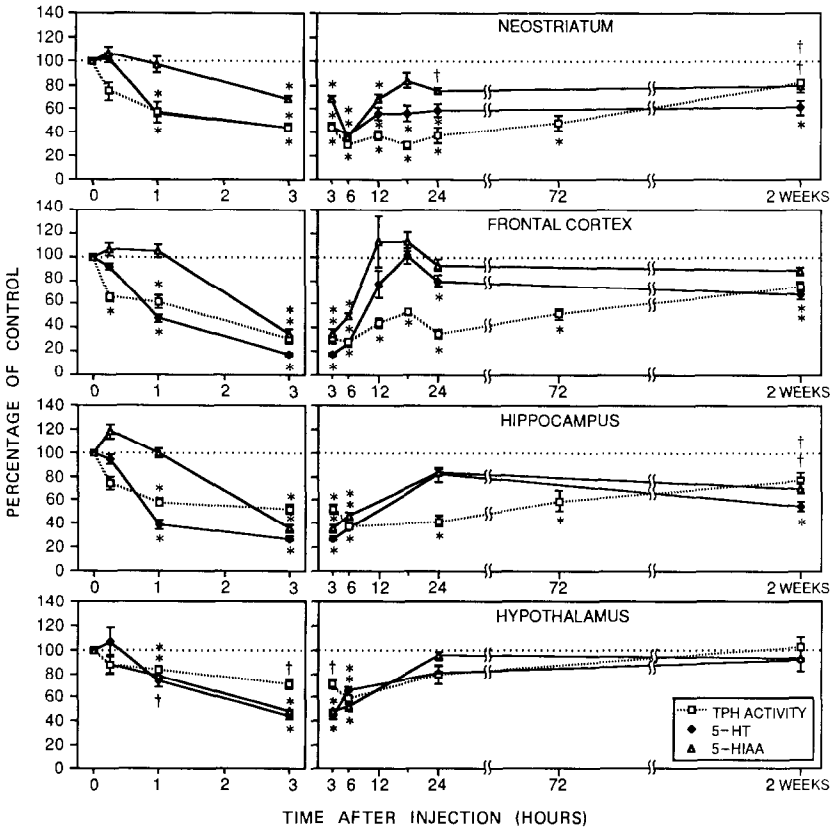

Fig. 1. Time course of the regional serotonergic effects of acute administration of MDMA. A single dose of MDMA (10 mg/kg) or saline (control) was injected subcutaneously; rats were killed at specified times thereafter. Each point represents the mean ± SEM from 4–6 rats, expressed as a percentage of the corresponding control. Immediate effects (up to 3 hr after injection) are represented in the left panel; the right panel diagrams longer-term regional responses (from 3 hr–2 weeks after injection). One hr control values ± SEM for neostriata (n), frontal cortex (fc), hippocampus (h) and hypothalamus (ht) were as follows: activity of tryptophan hydroxylase (TPH) (in nmol/g tissue/hr); n = 39.9 ± 3.6, fc = 80.1 ± 4.9, h = 63.0 ± 2.6, ht = 249.3 ± 7.0; concentrations of 5-HT and 5-HIAA, respectively (in μg/g tissue): n = 0.533 ± 0.019 and 0.557 ± 0.023, fc = 0.518 ± 0.018 and 0.218 ± 0.013, h = 0.359 ± 0.040 and 0.338 ± 0.009, ht = 0.905 ± 0.064 and 0.396 ± 0.018. Control values at other times did not vary significantly from those listed above. †$P < 0.05$, *$P < 0.005$ vs corresponding control by the two-tailed Student's t-test.

however, and enzyme activity remained significantly depressed in the neostriatum, hippocampus and frontal cortex at this time (Fig. 1, right panel). Of the regions examined, only the hypothalamus showed no residual serotonergic effects, at either 24 hr or 2 weeks after MDMA.

When a smaller dose of MDMA was administered (5 mg/kg), regional concentrations of 5-HT and 5-HIAA had recovered by 24 hr, whereas the activity of tryptophan hydroxylase was still significantly depressed in the neostriatum, hippocampus and frontal cortex (to 69, 74 and 69% of control, respectively; data not shown). Two weeks after this smaller dose, however, no residual serotonergic effects remained.

Figure 2 presents the concurrent effects of MDMA on the concentrations of DA and metabolites of DA in the neostriatum. The levels of DOPAC declined significantly within the first 15 min, then returned toward control values. Conversely, DA and HVA were significantly elevated after MDMA; while neither concentration had been altered by 15 min, levels of DA were increased to 143% ($P < 0.005$) and 138% ($P < 0.02$) of control, at 1 and 3 hr, respectively, and HVA in the striatum significantly increased, to 140% of control, by 3 hr after the injection. Twenty four hr after treatment, however, the concentrations of dopamine and its metabolites were not significantly different from control values (Fig. 2). In agreement with a previous report (Stone *et al.*, 1986), MDMA had no effect on the activity of tyrosine hydroxylase in the neostriatum, at either 15 min, 1 hr, 3 hr or 24 hr after the injection (data not shown).

To assess the recovery of the serotonergic systems after repeated exposure to MDMA, rats were given multiple doses of drug (5 injections over a 24-hr period) and sacrificed at times ranging from 18 hr–110 days after the cessation of treatment. Figure 3 shows the initial decline and subsequent recovery, of the regional activity of tryptophan hydroxylase in response to multiple injections of 5 or

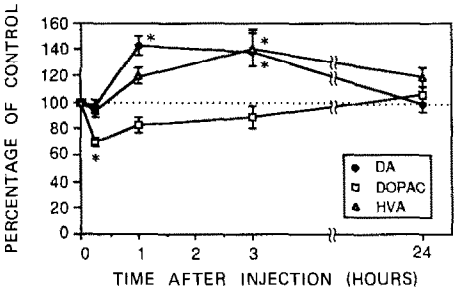

Fig. 2. Effect of acute MDMA on concentrations of dopamine and metabolites of dopamine in the neostriatum. Experimental details are described in Figure. 1. Control values ± SEM (in μg/g tissue) at 25, 1, 3 and 24 hr, respectively, were: dopamine (DA): 9.91 ± 0.44, 8.97 ± 0.59, 8.50 ± 0.45 and 10.45 ± 0.48; dihydroxyphenylacetic acid (DOPAC): 1.02 ± 0.04, 0.81 ± 0.11, 1.09 ± 0.06 and 0.92 ± 0.04; homovanillic acid (HVA): 0.79 ± 0.08, 0.66 ± 0.06, 0.62 ± 0.03 and 0.69 ± 0.04. *$P < 0.02$ vs corresponding control by the two-tailed Student's t-test.

10 mg/kg of MDMA. Eighteen hr after the cessation of treatment with either dose, enzyme activity had declined dramatically in all areas except the hypothalamus, in which a significant decrease was observed at the 10 mg/kg, but not the 5 mg/kg, dose.

The rate and extent of the recovery of the activity of tryptophan hydroxylase appeared to vary according to both the magnitude of the dose and the region of the brain examined. At the 10 mg/kg dose,

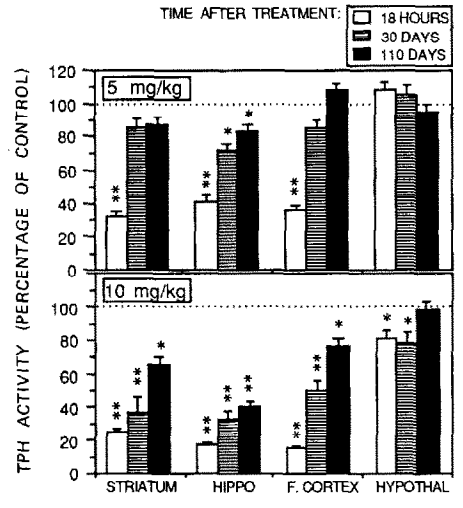

Fig. 3. Regional recovery of the activity of tryptophan hydroxylase (TPH) after multiple doses of MDMA. Rats were given 5 doses of saline or MDMA (one dose every 6 hr; 5 or 10 mg/kg) and killed at either 18 hr, 30 days or 110 days after cessation of treatment. Results are presented as the means ± SEM from 6–10 rats, and expressed as a percentage of corresponding controls. Mean control activities ± SEM (in nmol/g tissue/hr) at 18 hr, 30 days and 110 days, respectively, were: 45.09 ± 3.5, 42.8 ± 2.9 and 45.1 ± 3.6 in the neostriatum; 50.6 ± 1.4, 57.6 ± 2.9 and 66.5 ± 3.4 in the hippocampus; 75.4 ± 3.6, 64.3 ± 3.9 and 90.0 ± 6.0 in the frontal cortex; 222.3 ± 5.0, 230.2 ± 9.1 and 245.1 ± 14.1 in the hypothalamus. *$P < 0.02$, **$P < 0.001$ vs corresponding control by the two-tailed Student's t-test.

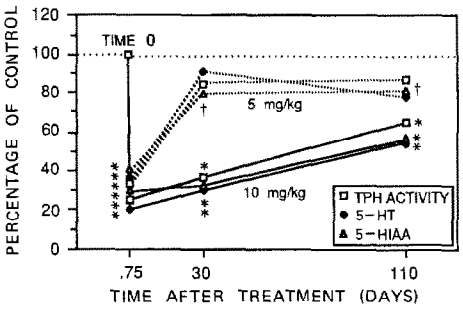

Fig. 4. Long-term recovery of serotonergic parameters in the neostriatum after multiple doses of MDMA. Experimental details are described in Figure 3. Each point represents the mean value (error bars have been omitted for the sake of clarity) from 6–10 rats, expressed as a percentage of the corresponding control. Doses of 5 mg/kg (dotted lines) and 10 mg/kg (solid lines) are represented. Control enzyme activities are listed in Figure 3. Control concentrations, of 5-HT and 5-HIAA respectively, (in μg/g tissue) were: 0.469 ± 0.024 and 0.362 ± 0.023 at 18 hr; 0.365 ± 0.061 and 0.381 ± 0.034 at 30 days; and 0.527 ± 0.047 and 0.453 ± 0.032 at 110 days. †$P < 0.05$ (5-HIAA only), *$P < 0.005$ vs corresponding control, by the two-tailed Students t-test.

significant recovery from the intial decline (18-hr time point) in enzyme activity had occurred in all regions by 110 days; however, only in the hypothalamus was this recovery complete. In contrast, after treatment with 5 mg/kg, all regions except the hippocampus had recovered by 30 days after treatment (Fig. 3). Of the regions of the brain examined, the hippocampus appeared to be the most sensitive (in which, even at the smaller dose, serotonergic parameters were still markedly depressed at 110 days) and the hypothalamus the least sensitive, to the long-term effects of MDMA.

All serotonergic parameters recovered more or less simultaneously from the multiple dose regimen, i.e. regional concentrations of 5-HT and 5-HIAA, and the corresponding activity of tryptophan hydroxylase, were decreased to similar degrees at each time examined. Figure 4 shows such a relationship in the neostriatum; other regions exhibited a similar response. However, at 110 days after treatment the mean concentration of 5-HT was, in all regions, decreased to a greater extent than the corresponding regional enzyme activity ($P < 0.05$; data not shown).

Other than a modest elevation of the activity of tyrosine hydroxylase in the striatum ($P < 0.05$ vs control, by the two tailed Student's t-test; data not shown) at the 30-day time (both dose levels), dopamine systems were not affected at either 30 or 110 days after treatment.

DISCUSSION

The present study clearly demonstrates that systemic administration of MDMA caused long-term, irreversible changes in several serotonergic nerve terminal regions of the brain of the rat. Such findings

were not unexpected, due to the similarity of the short-term serotonergic effects of MDMA and certain other analogs of amphetamine such as methamphetamine or *p*-chloroamphetamine (PCA) (Peat, Warren Bakhit and Gibb, 1985) which also cause long-term decreases in markers of central serotonergic function (Bakhit, Morgan, Peat and Gibb, 1981; Sanders-Bush *et al.*, 1972a). However, the doses of methamphetamine required (10–15 mg/kg; repeated doses) to produce similar "toxic "effects are much greater than the effective human dose (0.1–0.5 mg/kg; Weiner, 1985) and the halogenated derivatives of amphetamine possess a smaller human abuse potential (Smith, 1979; Woods and Tessel, 1974; Verster and van Praag, 1970). The present results are disturbing in view of the fact that MDMA is, currently, a popular drug of abuse among young people and the doses of MDMA used in these experiments were only 2–4 times the acute human dose (mg/kg basis; however, interspecies differences in metabolism may influence the response).

The mechanism of the serotonergic effects of MDMA is a subject of much debate and speculation. The similarity of the neurochemical responses to both MDMA and PCA suggests that the two act in a similar fashion. However, the precise mechanism of the serotonin-depleting effect of PCA remains to be defined. Fuller, Perry and Molloy (1975) have identified both an immediate, reversible phase and a longer-term, irreversible phase, of effects induced by PCA. The initial decline of both 5-HT and tryptophan hydroxylase induced by PCA could be completely reversed by the concurrent administration of an inhibitor of the uptake of 5-HT such as fluoxetine. Partial reversal of the effects were obtained with the injection of fluoxetine from 6–32 hr after the administration of PCA; after this time, fluoxetine was of no benefit (Fuller *et al.*, 1975). In a similar manner, fluoxetine appeared to prevent or reverse the effects of MDMA (Schmidt, 1987a). Two alternative mechanisms have been proposed for this protective effect of blockade of the uptake of 5-HT: inhibition of carrier-mediated uptake of the drug into serotonin-containing terminals (Fuller, 1980) or, in light of the potent 5HT-releasing capability of MDMA *in vitro* (Johnson, Hoffman and Nichols, 1986), inhibition of the drug-induced carrier-mediated efflux of endogenous releasable transmitter (Ross and Froden, 1977). Either of these processes could conceivably play an integral role in the development of toxicity induced by the drug. While indirect experimental evidence favors the former mechanism (Fuller, 1980), direct evidence of a carrier-mediated accumulation of PCA (Ross, 1976) or MDMA (Schmidt, Levin and Lovenberg, 1987b) into synaptosomes from the brain of the rat has not been forthcoming.

Several investigators (Sanders-Bush, Bushing and Sulser, 1975; Fuller *et al.*, 1975; Schmidt, 1987a) have presented evidence to suggest that the immediate control serotonergic effects of PCA or MDMA are distinct from the residual, longer-term effects. The transient recovery of the regional content of 5-hydroxyindoles observed 1 day after administration of MDMA (see Fig. 1, right panel) is consistent with such an interpretation. However, unlike that reported for PCA (Sanders-Bush *et al.*, 1975), no intermediate recovery of the regional activity of tryptophan hydroxylase was observed after MDMA (Fig. 1, right panel). The lack of strict correlation between these two parameters (i.e. 5-HT and its biosynthetic enzyme) suggests that changes in 5-HT at this early time were not a direct consequence of drug-induced alterations in the activity of tryptophan hydroxylase, or that the activity of tryptophan hydroxylase as measured *in vitro* may not reflect the enzyme activity *in vivo*.

While the initial decline in 5-HT after MDMA, or related analogs, is usually attributed to drug-induced release of 5-HT and blockade of its reuptake, possibly coupled with a drug-mediated inhibition of the synthesis of 5-HT, the cause of the rapid decline in enzyme activity has remained elusive. Most likely, the initial decrease in the activity of tryptophan hydroxylase produced by MDMA is due to an inactivation of existing enzyme molecules, mediated by the drug, since a hypothetical inhibition of enzyme synthesis or blockade of axonal transport would result in a much more delayed response (Meek and Neff, 1972). In this regard, however, neither methamphetamine (Hotchkiss and Gibb, 1980) nor PCA (Sanders-Bush *et al.*, 1972b) which, when administered acutely cause similar decreases in enzyme activity (Peat *et al.*, 1985), competitively inhibit tryptophan hydroxylase *in vitro*. These observations suggest the occurrence of an intermediary process *in vivo* such as active formation of metabolites, or autoregulatory feedback inhibition resulting from drug-induced release of monoamines.

The quantitative differences in the regional response to MDMA could be due to several factors. With regard to serotonin pathways, those areas containing primarily axon terminals, i.e. the striatum, hippocampus and frontal cortex, showed a more complete, and more persistent, depletion of 5-HT and the activity of tryptophan hydroxylase than the hypothalamus (see Fig. 3), a region containing not only axon terminals but larger fibers of passage (i.e. the median forebrain bundle) and cell bodies as well (Steinbusch, 1984). Immunocytochemical techniques utilizing antibodies specific for 5-HT have revealed a loss of 5-HT in small varicosities and fine terminals, induced by MDMA, with a concomitant increase in the diameter and staining intensity of larger preterminal axon fibers (O'Hearn, Battaglia, De Souza, Kuhar and Mollivar, 1986). Summation of these two opposing effects, namely a decrease of 5-HT in hypothalamic terminal areas and a buildup of 5-HT in neighboring preterminal areas, might account for the decreased responsiveness of the hypothalamic region to MDMA. Alternatively, regional differences

in the disposition of MDMA could account for the quantitative variability in the regional response, such that the concentrations of MDMA reached in certain nuclei may be too small to cause substantial toxicity.

A decreased number of central uptake sites for 5-HT 1 week after administration of MDMA (Schmidt, 1987a) has been cited as evidence of drug-induced destruction of serotonergic nerve terminals. In the present study, the persistence (up to 110 days) of the MDMA-induced decreases in 5-HT, 5-HIAA and the activity of tryptophan hydroxylase in serotonin-containing terminal regions, supports this conclusion. Such long-term changes might result from a prolonged drug-induced inactivation of enzyme synthesis or transport, or a more general proteolysis or membrane disruption due perhaps to a reactive metabolic product or intermediate. In this regard, histological evidence of cytotoxic damage (Harvey, McMaster and Yunger, 1975) and degeneration of axon terminals in response to PCA (McGeer, Hattori and McGeer, 1975) or MDA (Ricaurte, Bryan, Strauss, Seiden and Schuster, 1985), the *N*-desmethyl analog of MDMA, has been presented. Contradictory results, however, have been reported (Lorez, Saner, Richerds and Da Prada, 1976; Funderburk, 1978).

The nature of the neurochemical changes induced by MDMA, i.e. the extremely prolonged deficit in markers of serotonergic function and the gradual recovery of these parameters over a period of 1–4 months, is comparable to the long-term effects of chemical axotomy induced by the cytotoxic agent 5,6-dihydroxytryptamine (Wiklund, Bjorklund and Nobin, 1978). By analogy, the return of serotonergic parameters after administration of MDMA might involve regenerative growth of 5-HT neurons into and around the damaged terminal areas, as has been observed after 5,6-dihydroxytryptamine-induced axonal damage (Björklund, Nobin and Stenevi, 1973). In regard to the ultrastructural location of the drug-induced damage, however, the two compounds appear to differ. Whereas the "neurotoxic" derivatives of amphetamine effect selective degeneration of axon terminals (Massari, Tizabi and Sanders-Bush, 1977; O'Hearn *et al.*, 1986), the indoleamine compounds result in degeneration of preterminal axons as well. The sparing of larger axon figers and cell bodies (O'Hearn *et al.*, 1986) by MDMA may allow a more successful reinnervation of damaged terminal areas to eventually occur.

In conclusion, the illicit amphetamine derivative, MDMA, caused rapid alterations in markers of both serotonergic and dopaminergic function in the brain of the rat. While changes in catecholamine pathways were transient and had disappeared by 24 hr, decreases in serotonergic markers were still apparent in most terminal areas 2 weeks after a single injection of drug; these serotonergic parameters were still significantly depressed 110 days after multiple doses of MDMA. The pattern observed after treatment with MDMA, i.e an intial drug-induced decline in the concentration of 5-HT and the activity of tryptophan hydroxylase, followed by a gradual, delayed recovery of these serotonergic parameters, is suggestive of MDMA-induced destruction of nerve terminals and subsequent regenerative axonal regrowth. Possible toxicological implications with regard to human abusers of MDMA should be considered.

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