

Elevation of Serum Prolactin and Corticosterone Concentrations in the Rat after the Administration of 3,4-Methylenedioxymethamphetamine¹

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ABSTRACT

The racemic mixture of 3,4-methylenedioxymethamphetamine (MDMA), which has been reported to produce selective destruction of serotonergic neurons in the central nervous system, was studied to determine its neuroendocrine and temperature effects and mechanism of action. MDMA elevated serum concentrations of corticosterone in doses ranging from 3 to 20 mg/kg administered i.p. Serum corticosterone concentrations were elevated 30 min after the administration of MDMA (10 mg/kg i.p.) and remained elevated 4 hr later. Serum prolactin (PRL) concentrations were elevated by administration of MDMA in doses ranging from 1 to 20 mg/kg i.p., and were maximal 60 min after the injection of 10 mg/kg i.p., declining rapidly over the next 4 hr. MDMA also significantly elevated the body temperature of rats maintained at ambient (23°C) temperature. MDMA-induced corticosterone secretion and hyperthermia were blocked by the 5-hydroxytryptamine (5-HT) antagonists, ketanserin and mianserin, which have a high affinity for 5-HT₂ binding sites. Conversely, neither (–)-pindolol, a β antagonist that also blocks 5-HT_{1A}-mediated

responses, nor the nonspecific 5-HT antagonists, cyproheptadine and metergoline, had an effect on MDMA-induced corticosterone secretion. None of the 5-HT antagonists blocked MDMA-induced PRL secretion. Pretreatment with fluoxetine (10 mg/kg i.p.) 16 hr before MDMA administration significantly blunted the effect of MDMA on corticosterone but not PRL secretion. Pretreatment with *p*-chlorophenylalanine (150 mg/kg i.p.) for 3 days depleted cortical and hypothalamic 5-HT and 5-hydroxyindoleacetic acid by approximately 80% and significantly attenuated MDMA-induced corticosterone and PRL secretion. The effects of *p*-chlorophenylalanine and fluoxetine on MDMA-induced neuroendocrine responses were found to be identical to the effects of these drugs on *p*-chloroamphetamine-mediated responses. These data suggest that MDMA is taken up into axon terminals via a fluoxetine-sensitive 5-HT reuptake system and requires endogenous 5-HT to stimulate corticosterone secretion and produce hyperthermia, which are mediated via 5-HT₂ receptor mechanisms. The mechanisms of the MDMA-induced increase in serum PRL levels require further investigation.

The emergence of MDMA as a popular drug of abuse coupled with anecdotal reports of its potential antidepressant effect has made the examination of its biological effects of considerable interest. It has recently been reported that single or multiple doses of MDMA depletes 5-HT in several brain areas (Schmidt *et al.*, 1986; Stone *et al.*, 1986; Mokler *et al.*, 1987; Schmidt 1987a,b), and selectively destroys 5-HT nerve terminals (Commins *et al.*, 1987). To date, few if any studies have examined the mechanism of action of MDMA.

The majority of studies in which the neurotoxic effects of

chronic MDMA administration have been examined have reported depletion of 5-HT and 5-HIAA as well as a reduction in tryptophan hydroxylase activity (Schmidt *et al.*, 1986; Stone *et al.*, 1986; Commins *et al.*, 1987). Schmidt (1987b) found that coadministration of fluoxetine, a selective 5-HT uptake inhibitor (Fuller *et al.*, 1974), completely blocked the long-term, but not the short-term, depletion of cortical 5-HT induced by MDMA. Commins *et al.* (1987) reported morphological evidence in which degenerating axon terminals and cell bodies were observed in the striatum and somatosensory cortex after MDMA treatment. Collectively, these data suggest that MDMA is taken up into serotonergic axon terminals and that it then selectively destroys these neurons. These actions of MDMA have been suggested to resemble the 5-HT-depleting effects of PCA (Schmidt, 1987b).

The administration of MDMA to laboratory animals has been reported to produce hyperthermia (Anderson *et al.*, 1978)

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; PCA, *p*-chloroamphetamine; DA, dopamine; PRL, prolactin; PCPA, *p*-chlorophenylalanine; 5-HICA, 5-hydroxyindole carboxylic acid; RIA, radioimmunoassay.

and analgesia (Braun *et al.*, 1980), responses in which 5-HT is believed to play a role. The most extensively investigated behavioral effect of MDMA is its discriminative stimulus properties. For the most part, MDMA will partially substitute for amphetamine in an animal trained to discriminate amphetamine from saline in a two-lever paradigm (Evans and Johanson, 1986; Glennon, 1986). However, MDMA does not substitute in animals trained to discriminate the hallucinogens, 1-(2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane or lysergic acid diethylamide, from saline (Glennon *et al.*, 1982; Kamien *et al.*, 1986; Nichols *et al.*, 1986). Interestingly, like amphetamine, the most active enantiomer of MDMA is the S(+) configuration, whereas the R(-) configuration of potent hallucinogens, such as lysergic acid diethylamide and 1-(2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane, is the most biologically active and has the greatest affinity for 5-HT₂ receptors (Glennon *et al.*, 1984). The discriminative stimulus studies suggest that MDMA lacks hallucinogenic effects and that amphetamine-like effects predominate, which is consistent with the effects reported in human subjects (Nichols *et al.*, 1986).

MDMA was reported by Nichols *et al.* (1982) to stimulate the release of [³H]5-HT from rat whole brain synaptosomes. More detailed studies measuring the release of 5-HT as well as DA from rat hippocampal slices suggest that MDMA has a more potent effect on 5-HT release as compared to DA release. Although the most potent effect of MDMA is on the release of 5-HT *in vitro*, it has also been reported to affect other brain amine systems, *e.g.*, norepinephrine and DA both *in vitro* and *in vivo* (Stone *et al.*, 1986; Steele *et al.*, 1987), as well as peptidergic systems such as neurotensin (Merchant *et al.*, 1987). Further support for the hypothesis that MDMA exerts its pharmacological effects by an indirect mechanism is the observation that the binding affinities of both enantiomers of MDMA, as well as the racemic mixture for 5-HT₁ and 5-HT₂ binding sites are relatively weak (Lyon *et al.*, 1986).

Abundant evidence exists demonstrating that 5-HT has stimulatory effects in rodents on the hypothalamic neurons that regulate pituitary-adrenocortical function (Fuller, 1981; Holmes *et al.*, 1982). Similarly, the secretion of PRL from the anterior pituitary has been studied extensively and is known to be regulated by tuberoinfundibular dopaminergic neurons, which inhibit PRL secretion, and by a 5-HT-dependent stimulatory mechanism (Gudelsky, 1981; Meltzer *et al.*, 1982). The neuronal mechanisms involved in PRL and corticosterone secretion have been reported to be different, and for the latter largely undetermined (Van de Kar *et al.*, 1985a). Regardless, numerous pharmacological studies demonstrate that drugs which enhance serotonergic activity elevate serum corticosterone and/or PRL concentrations, making these sensitive indicators of 5-HT receptor stimulation (Fuller and Snoddy, 1980; Fuller, 1981).

Multiple binding sites for 5-HT in the rat brain have been identified and classified into two major types: 5-HT₁ and 5-HT₂ (Peroutka and Snyder, 1979). The 5-HT₁ binding site has been subdivided further into 5-HT_{1A}, 5-HT_{1B} (Pedigo *et al.*, 1981) and 5-HT_{1C} (Pazos *et al.*, 1984) subtypes. For the most part, attempts to determine the physiological role of these binding sites have been limited due to the lack of selective agonists and antagonists. However, the development of selective 5-HT₂ antagonists, such as ketanserin and ritanserin, have lead to the identification of specific responses believed to be mediated by 5-HT₂ receptors. Similarly, the ability of (-)-

pindolol to bind to 5-HT_{1A} sites (Nahorski and Willcocks, 1983) and antagonize 8-hydroxy-2-(di-*n*-propylamino)tetralin-induced behaviors (Tricklebank *et al.*, 1985) has facilitated the characterization of putative agonists. For example, stimulation of 5-HT₂ sites has been reported to induce hyperthermia in heat-adapted rats (Gudelsky *et al.*, 1986; Pawlowski, 1984), whereas activation of 5-HT_{1A} sites produces hypothermia in rats (Hjorth, 1985; Goodwin and Green, 1985; Gudelsky *et al.*, 1986). Activation of either 5-HT₂ or 5-HT_{1A} receptors results in an elevation in serum corticosterone concentration (Koenig *et al.*, 1987).

Based on the lack of pharmacological data coupled with the current interest in MDMA, a systematic investigation was undertaken to determine the mechanism of action of MDMA. Given the abundant evidence on the neurotoxic effects of MDMA to 5-HT neurons and the role of 5-HT in neuroendocrine regulation, characterization of MDMA's neuroendocrine effects was initiated.

Materials and Methods

Animals. Male Sprague-Dawley rats weighing 170 to 200 g upon delivery were purchased from Zivic Miller Laboratory (Hillson, PA) and used in all experiments. The animals were housed six per cage in a temperature-controlled room (22–24°C) with a light/dark cycle of 12/12 hr (lights on at 6:00 A.M.). Food (Wayne Lab Blox) and tap water were available *ad libitum*.

Drugs. The racemic mixture of MDMA was generously provided by Dr. David E. Nichols of Purdue University. PCPA hydrochloride and DL-PCA hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO) and Regis Chemical Co. (Morton Grove, IL), respectively. The other drugs were generously provided by the manufacturers: ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium) and fluoxetine hydrochloride (Eli Lilly and Co., Indianapolis IN). All drugs were dissolved in distilled water and injected *i.p.*

Experimental procedure. On the day before an experiment in which serum corticosterone and prolactin concentrations were determined, the animals were housed six per cage and transferred to the experimental room. Between 8:00 A.M. and 9:00 A.M., MDMA in a volume of 1 ml/kg was administered at the doses indicated under "Results." All solutions of drugs were administered *i.p.* with the exception of (-)-pindolol, which was injected *s.c.* Serotonin antagonists were given 1 hr before the administration of MDMA, with the exception of (-)-pindolol, which was injected 30 min before MDMA administration. At designated times after MDMA administration, the animals were sacrificed by decapitation and trunk blood was collected in glass tubes. The blood was allowed to clot, and the serum was obtained after centrifugation, transferred to glass tubes and stored at -20°C until the time of assay.

In the 5-HT depletion studies, either saline or PCPA (150 mg/kg *i.p.*) was administered for 3 consecutive days. Twenty-four hours after the last injection, the animals were challenged with either vehicle, MDMA (3 mg/kg) or PCA (3 mg/kg) and sacrificed 30 min later. Trunk blood was obtained for hormone determinations. The hypothalamus and a section of the frontal cortex were dissected from the brains of saline- and PCPA-treated animals administered vehicle, and homogenized immediately in ice-cold 0.2 N perchloric acid containing 2 ng/ml of 5-HICA, the internal standard. The concentration of 5-HT and 5-HIAA was determined as detailed below.

Fluoxetine (10 mg/kg *i.p.*) was administered 16 hr before challenge with either vehicle, MDMA (3 mg/kg) or PCA (3 mg/kg). The animals were sacrificed 30 min later, and trunk blood was obtained for the analysis of serum corticosterone and PRL concentrations.

Body temperature measurements. On the day in which body temperatures were to be determined, rats were transferred to a temperature-controlled room (23°C) and allowed to acclimate for 2 hr. Body

temperatures were recorded at 30 min, 15 min and immediately before the administration of ketanserin (3 mg/kg i.p.) or vehicle. One hour later body temperatures were measured and MDMA (3 mg/kg i.p.) was administered. A final measurement of body temperature was performed 30 min after the injection of MDMA. Rectal temperature measurements were made using a telethermometer (model 44 TA, Yellow Springs Instrument Co., Yellow Springs, OH) and a thermistor probe. The probe was lubricated with a small amount of petroleum jelly and inserted 5 cm into the rectum of each rat for at least 30 sec, until a stable temperature was obtained.

Assays. Serum concentration of corticosterone was determined by RIA. [3 H]Corticosterone was purchased from Dupont New England Nuclear (Boston, MA), and the antiserum was purchased from Radioassay Systems Laboratories, Inc. (Carson, CA). The unlabeled corticosterone used in preparing the RIA standard was obtained from Steroids, Inc. (Whitler, NH).

PRL was determined by RIA using reagents and antiserum that were generously provided by the NIDDK. Rat PRL antiserum (S-9) was used at a final dilution of 1:12,500. Rat PRL RP-3 served as the reference preparation. Iodinated rat PRL was purchased from Dupont New England Nuclear (Boston, MA).

The concentration of 5-HT and 5-HIAA in the hypothalamus and a section of the frontal cortex were determined by high pressure liquid chromatography with electrochemical detection. Chromatographic analyses were performed on an Ultrasphere C₁₈ reverse phase column (Beckman Instruments, Berkeley, CA) connected to an electrochemical detector (model 5100 A, ESA, Inc., Bedford, MA). The mobile phase consisted of 0.1 M sodium acetate (pH 4.5), 5% methanol and 0.02% w/v EDTA. The amount of 5-HT and 5-HIAA in each sample was determined by comparing the ratio of peak heights (5-HT/5-HICA, 5-HIAA/5-HICA) with that of a known amount of standard.

Statistical analysis. The data were analyzed using one-way and two-way analysis of variance as indicated. Differences between treatment means were assessed with the Student-Newman-Keuls' test and were considered significant at $P < .05$.

Results

The time course for the elevation in serum corticosterone and PRL concentrations after the administration of MDMA (10 mg/kg) is presented in figure 1, A and B. The serum concentration of both hormones was significantly ($P < .05$) elevated 30 min after MDMA administration. Serum corticosterone remained elevated throughout the 240-min test period with no significant differences between the 30- to 240-min time points (fig. 1A). However, serum PRL concentrations began to decline after 60 min and were not different from base-line levels at 240 min postadministration (fig. 1B).

A dose-response study was conducted by administering different doses of MDMA and collecting blood samples 60 min later. Figure 2, A and B, illustrates the effect of various doses of MDMA on serum corticosterone and PRL concentrations. MDMA, 3 to 20 mg/kg, produced a significant, dose-dependent elevation of serum corticosterone (fig. 2A). Serum levels of PRL were significantly elevated by MDMA at doses ranging from 1 to 20 mg/kg (fig. 2B), but there were no significant differences between doses.

Table 1 presents the effect of pretreatment with 5-HT antagonists on MDMA-induced corticosterone and PRL secretion. The animals were sacrificed 30 min after MDMA (3 mg/kg) administration and 90 min after the administration of the antagonist. None of the 5-HT antagonists used in this study significantly altered basal corticosterone or PRL concentrations when administered alone (data not shown). Ketanserin blocked MDMA-induced corticosterone secretion in a dose-

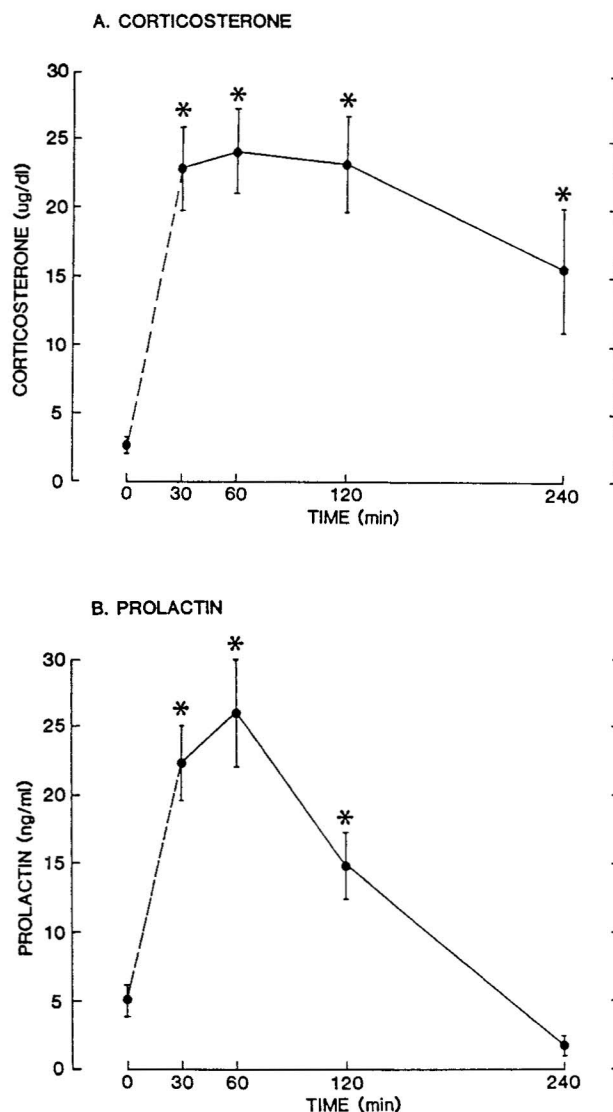


Fig. 1. Effect of MDMA (10 mg/kg) on serum corticosterone (A) and PRL (B) levels. Rats were sacrificed by decapitation at the designated time points. Each value is the mean $\pm$ S.E. of 6 rats. Significant differences ($P < .05$) between basal hormone levels (T_0) and the other time points are designated by *.

dependent manner. Similarly, mianserin blocked the increase in serum corticosterone levels after MDMA administration. In contrast, neither (–)-pindolol, cyproheptadine nor metergoline significantly attenuated MDMA-induced corticosterone secretion at the doses tested. None of the 5-HT antagonists tested significantly antagonized MDMA-induced PRL secretion (table 1).

The effect of pretreatment with the specific 5-HT uptake inhibitor, fluoxetine, on MDMA- or PCA-induced hormone responses is presented in table 2. Two-way analysis of variance revealed that both MDMA and PCA significantly ($P < .05$) elevated serum corticosterone and PRL levels. Fluoxetine (10 mg/kg) pretreatment significantly elevated basal serum PRL levels but had no effect on serum corticosterone concentration. The secretion of corticosterone elicited by either MDMA or PCA was significantly ($P < .05$) attenuated by fluoxetine pretreatment. Interestingly, as was the case with the 5-HT antagonists, fluoxetine had no effect on MDMA- or PCA-induced PRL secretion.

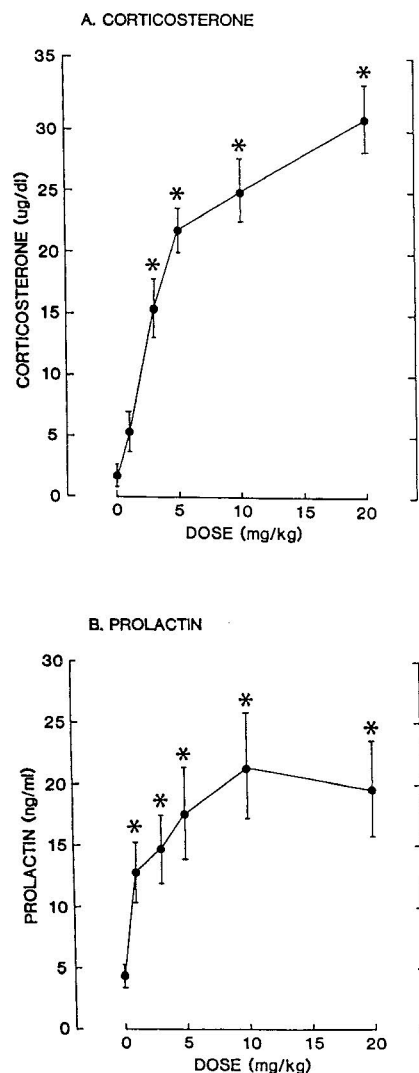


Fig. 2. Effect of different doses of MDMA on serum corticosterone (A) and PRL (B) levels. Rats were sacrificed by decapitation 60 minutes after the i.p. administration of MDMA. Each value represents the mean $\pm$ S.E. of 6 rats. Significant differences ($P < .05$) from vehicle (0) dose are designated by *.

TABLE 1

Effect of 5-HT antagonists on MDMA-induced corticosterone and PRL secretion

Ketanserin, cyproheptadine, metergoline and mianserin were administered 60 min before MDMA. (–)Pindolol was injected s.c. 30 min before MDMA. The animals were sacrificed 30 min after MDMA or vehicle administration. Each value is the mean $\pm$ S.E. of 6 rats.

Treatment	Dose of Antagonist mg/kg	Corticosterone μ g/dl	Prolactin ng/ml
Vehicle + vehicle		7.7 $\pm$ 0.9*	6.6 $\pm$ 0.9*
Vehicle + MDMA		28.0 $\pm$ 1.4	16.0 $\pm$ 1.4
Ketanserin + MDMA	0.3	23.1 $\pm$ 1.7	11.3 $\pm$ 1.9
Ketanserin + MDMA	1.0	13.9 $\pm$ 2.0*	11.7 $\pm$ 0.3
Ketanserin + MDMA	3.0	12.6 $\pm$ 2.7*	14.1 $\pm$ 5.0
(–)Pindolol + MDMA	0.3	22.5 $\pm$ 3.7	13.0 $\pm$ 4.6
Cyproheptadine + MDMA	10.0	20.0 $\pm$ 3.9	13.2 $\pm$ 3.3
Metergoline + MDMA	0.5	23.1 $\pm$ 1.1	12.8 $\pm$ 2.3
Mianserin + MDMA	10.0	13.9 $\pm$ 1.7*	12.6 $\pm$ 3.1

* $P < .05$ vs. the vehicle + MDMA group.

TABLE 2

Effect of MDMA or PCA administration on serum corticosterone and PRL levels in rats pretreated with vehicle or fluoxetine

Rats were injected with vehicle or fluoxetine (10 mg/kg i.p.) 16 h before the administration of MDMA (3 mg/kg), PCA (3 mg/kg) or the vehicle; animals were sacrificed 30 min later. Each value is the mean $\pm$ S.E. of 6 rats.

Treatment	Corticosterone μ g/dl	Prolactin ng/ml
Vehicle + vehicle	9.0 $\pm$ 1.0*	8.0 $\pm$ 1.9*
Fluoxetine + vehicle	7.1 $\pm$ 2.0*	13.7 $\pm$ 1.3
Vehicle + MDMA	32.7 $\pm$ 3.3	18.2 $\pm$ 2.0
Fluoxetine + MDMA	20.2 $\pm$ 2.4*	19.0 $\pm$ 5.3
Vehicle + PCA	36.3 $\pm$ 1.9	13.2 $\pm$ 3.3
Fluoxetine + PCA	19.4 $\pm$ 3.6*	15.4 $\pm$ 2.3

* $P < .05$ vs. the treatment (vehicle + MDMA, vehicle + PCA) groups.

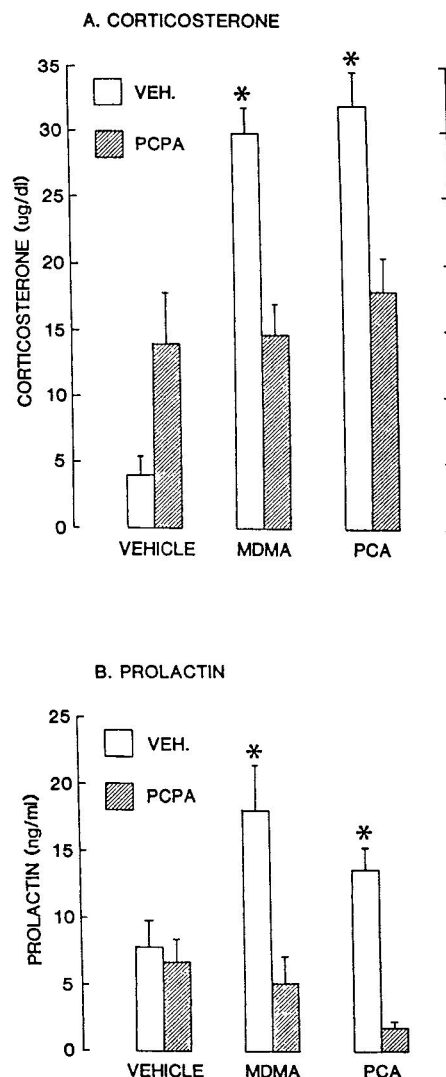


Fig. 3. Effects of vehicle, MDMA (3 mg/kg) or PCA (3 mg/kg) on serum corticosterone (A) and PRL (B) levels in rats pretreated with vehicle or PCPA (150 mg/kg). Rats were injected with either vehicle or PCPA for 3 consecutive days and 24 hr after the last injection challenged with either vehicle, MDMA or PCA and sacrificed 30 min later. Each value represents the mean $\pm$ S.E. of 6 rats. Significant differences ($P < .05$) from corresponding vehicle-pretreated group are designated by *.

The effect of PCPA-induced depletion of 5-HT on MDMA- and PCA-induced corticosterone and PRL secretion is illustrated in figure 3, A and B. Administration of PCPA (150 mg/kg i.p., daily for 3 days) alone significantly ($P < .05$) elevated

serum corticosterone concentrations. PCPA pretreatment completely blocked the subsequent stimulatory effects of MDMA and PCA on corticosterone secretion (fig. 3A). PCPA pretreatment did not elevate basal serum PRL concentrations, but did block the stimulatory effects of MDMA and PCA on PRL secretion (fig. 3B). Table 3 presents the effect of PCPA administration on 5-HT and 5-HIAA concentrations in the hypothalamus and cortex. The concentration of 5-HT and 5-HIAA was reduced by approximately 80% in PCPA-treated animals.

The effect of MDMA on body temperature is presented in table 4. Both doses of MDMA (3 and 10 mg/kg) significantly ($P < .05$) elevated body temperatures at 30 min after its administration as compared to vehicle-treated animals. Pretreatment with ketanserin (3 mg/kg i.p.) significantly attenuated the increase in body temperature produced by MDMA (3 mg/kg) administration. Ketanserin administration produced a slight hypothermia, which did not differ significantly from vehicle-treated animals (data not shown).

Discussion

MDMA produced dose- and time-related increases in serum corticosterone and PRL concentrations and a marked increase in body temperature. The effects of MDMA on these two hormones and body temperature are consistent with previously published studies on other substituted amphetamine analogs. For example, PCA and fenfluramine have been reported to stimulate corticosterone and PRL secretion in male rats (McElroy *et al.*, 1984; Van de Kar *et al.*, 1985a,b), as well as produce hyperthermia (Quock and Weick, 1979). Although the precise mechanisms by which PCA and fenfluramine elicit these responses may be different (Quock and Weick, 1979; Sloviter *et al.*, 1980; McElroy *et al.*, 1984), it is generally agreed that the pharmacological effects of these drugs are mediated by 5-HT. Thus, given the evidence for serotonergic stimulation of corticosterone and PRL secretion (for review see Tuomisto and

Mannisto, 1985), serotonergic receptor function in thermoregulatory responses (Gudelsky *et al.*, 1986) and the evidence for neurotoxic effects of MDMA on 5-HT neurons, it seemed of interest to determine if the neuroendocrine and hyperthermic effects of this compound were mediated by a 5-HT-dependent mechanism. The present results suggest that MDMA does act *via* 5-HT mechanisms to stimulate the secretion of corticosterone and possibly PRL, as well as elevated body temperature.

Antagonism of MDMA-induced corticosterone and hyperthermia by ketanserin and mianserin, but not by the putative 5-HT_{1A} antagonist (-)-pindolol, is suggestive that these responses are mediated *via* 5-HT₂ receptor mechanisms. However, it is unlikely that these responses are the result of a direct activation of 5-HT₂ sites, because MDMA has been shown to have a relatively low affinity for 5-HT₂ as well as 5-HT₁ receptors (Lyon *et al.*, 1986).

The ability of fluoxetine to antagonize MDMA-induced corticosterone secretion is consistent with reports in which fluoxetine pretreatment blocked the 5-HT-depleting effects of MDMA (Schmidt, 1987b). More recently, Battaglia *et al.* (1987) found that repeated MDMA administration resulted in a significant reduction in [³H]paroxetine-labeled 5-HT uptake sites in several brain regions. These data suggest that MDMA enters the axon terminal *via* the 5-HT uptake system and the subsequent release of endogenous 5-HT, in turn, stimulates postsynaptic 5-HT₂ receptors.

In the present study some, but not all of the MDMA-mediated responses (*e.g.*, PRL elevation) were blocked by 5-HT₂ antagonists, suggesting that MDMA may elicit different responses at various brain sites through a variety of mechanisms. This concept appears to be supported by the results of Rosecrans and Glennon (1987), who reported that the 5-HT₂ antagonist, pirenpirone, also failed to block the behaviorally disruptive effects of MDMA in mice. Collectively, these data are suggestive that the pharmacological effects of MDMA involve more than one neurotransmitter system and may be site selective.

The effect of MDMA on PRL secretion is more complex than its effect on corticosterone secretion. Presumably, MDMA-induced PRL secretion is mediated *via* 5-HT receptor stimulation. This hypothesis is supported by the fact that depletion of 5-HT by PCPA pretreatment abolished the effect of MDMA, as well as PCA, on PRL secretion. However, the 5-HT antagonists used in these studies failed to block MDMA-induced PRL secretion. In this regard, Meltzer *et al.* (1983) found that the doses of ketanserin (5–25 mg/kg) needed to antagonize 5-methoxy-N,N-dimethyltryptamine- and quipazine-induced PRL secretion were 5 to 10 times those which block other 5-HT-mediated behaviors such as wet-dog shakes (Lucki *et al.*, 1984), hyperthermia (Gudelsky *et al.*, 1986) or corticosterone secretion (Koenig *et al.*, 1987). Moreover, the PRL response after administration of the 5-HT precursor, 5-HTP, was only partially antagonized by ketanserin even at a dose of 25 mg/kg (Meltzer *et al.*, 1983). Thus, the type of 5-HT receptor mediating PRL secretion may be a novel one, neither 5-HT_{1A} nor 5-HT₂. Finally, a nonspecific stress effect could also account for the antagonist-resistant rise in serum PRL.

The effect of MDMA on PRL secretion was short-lived and dose-independent as compared to its effect on corticosterone secretion. The failure of MDMA to elicit a dose-dependent PRL response may be due to direct or indirect effects of MDMA on dopaminergic mechanisms. For example, MDMA has been

TABLE 3

Effect of vehicle or PCPA pretreatment on 5-HT and 5-HIAA concentrations in the hypothalamus and cortex

Vehicle or PCPA (150 mg/kg) was administered for 3 consecutive days and the animals were sacrificed 24 hr after the last injection. Each value is the mean $\pm$ S.E. of 6 rats. The values are presented as nanograms per milligrams of protein.

	Hypothalamus		Cortex	
	5-HT	5-HIAA	5-HT	5-HIAA
Vehicle	7.39 $\pm$ 0.30	5.91 $\pm$ 0.41	2.04 $\pm$ 0.25	1.23 $\pm$ 0.04
PCPA	1.77 $\pm$ 0.08 (-76%) ^a	1.07 $\pm$ 0.08 (-82%)	0.38 $\pm$ 0.10 (-81%)	0.29 $\pm$ 0.10 (-76%)

^a Percent changes are compared to corresponding vehicle group.

TABLE 4

Effect of MDMA on body temperature following pretreatment with vehicle or ketanserin (3 mg/kg)

Each value is the mean $\pm$ S.E. of 6 rats. Rats were injected with either ketanserin or vehicle 60 min before MDMA (3 mg/kg or 10 mg/kg i.p.) administration. Changes in body temperature were recorded before and 30 min after MDMA administration.

Treatment	Dose of MDMA mg/kg	Δ ° Body Temperature °C
Vehicle + vehicle		-0.1 $\pm$ 0.1
Vehicle + MDMA	10	1.8 $\pm$ 0.2*
Vehicle + MDMA	3	1.1 $\pm$ 0.2*
Ketanserin + MDMA	3	0.3 $\pm$ 0.1

* $P < .05$, compared to vehicle + vehicle group.

^a Indicates change (Δ) in body temperature.

reported to possess dopaminergic releasing properties *in vitro* as well as *in vivo* (Johnson *et al.*, 1986; Kulmala *et al.*, 1987). Such a DA-releasing effect of MDMA would offset serotonergic mediated stimulation of PRL secretion, and thereby contribute to a nondose-related increase in serum PRL concentration.

The dissociation between serotonergic-mediated corticosterone and PRL secretion has been reported previously. For example, Van de Kar *et al.* (1985b) found that the effects of fenfluramine on PRL secretion were mediated *via* 5-HT release, whereas the effect on corticosterone secretion was not. Furthermore, lesions of the mediobasal hypothalamus completely abolished PCA-induced PRL secretion but did not completely block PCA-induced corticosterone secretion (Van de Kar *et al.*, 1985a). These studies suggest that the neural mechanisms that control the secretion of PRL and corticosterone are different. Moreover, these data support the hypothesis that the indirect agonist, MDMA, stimulates site-specific areas of the brain.

The inability of the nonspecific 5-HT antagonists, metergoline and cyproheptadine, to block MDMA-induced PRL and corticosterone secretion is difficult to understand. The doses used in this study were sufficient to block corticosterone secretion induced by direct-acting 5-HT agonists such as quipazine and MK-212 (Fuller and Snoddy, 1979; Koenig *et al.*, 1987). Furthermore, the antagonism of MDMA-induced corticosterone secretion by the specific 5-HT₂ antagonists, ketanserin and mianserin, suggests that the nonspecificity of the other antagonists may, in fact, account for their inability to block the effects of MDMA on hormone secretion.

PCPA administration significantly reduced the levels of 5-HT and 5-HIAA concentrations in both the cortex and hypothalamus. The depletion of 5-HT by PCPA completely abolished MDMA-induced corticosterone and PRL secretion. PCPA administration, alone, elevated corticosterone levels in vehicle-treated animals. Thus, it could be argued that elevated basal levels of corticosterone could inhibit further stimulation. However, as demonstrated by Fuller and Snoddy (1980), PCPA-pretreatment did not block the ability of the direct acting agonist, quipazine, from stimulating corticosterone secretion. Furthermore, these authors demonstrated that PCA-induced corticosterone secretion was completely blocked by PCPA pretreatment. In the present study, the results unequivocally establish that MDMA requires endogenous 5-HT to stimulate the secretion of PRL and corticosterone.

The neurotoxic effects of MDMA clearly illustrate the potentially dangerous consequences of its use in man. However, two lines of evidence question the functional consequences of these depletions. The first relates to the depleting effects of the frequently used anorexic agent, fenfluramine. Harvey *et al.* (1975) and Harvey and McMaster (1977) reported that fenfluramine, as well as other halogenated amphetamines, induces long-term depletion of 5-HT. Fenfluramine, which has been used for the management of obesity for several years, also has been reported to destroy 5-HT nerve cell bodies. It is possible that this neurotoxicity is subtle and predisposes individuals to chronic diseases; however, to date, there are little data to support this hypothesis. The second line of evidence includes the clinical reports of van Praag *et al.* (1970) and van Praag and Korf (1973) in which PCA, a thoroughly studied 5-HT-depleting agent, was reported to possess antidepressant effects. Therefore, contrary to what might be predicted in these studies, PCA was an effective antidepressant, which apparently did not result in serious medical consequences as a result of any 5-HT-

depleting effects. These studies may add insight into the anecdotal suggestions that MDMA has antidepressant and euphoric effects. More importantly, as suggested by Nichols *et al.* (1986), MDMA may represent a unique pharmacologic agent.

To our knowledge, these data are the first to demonstrate 5-HT receptor-mediated responses elicited by MDMA. For the most part, MDMA exhibits a pharmacologic profile similar to that of PCA. MDMA is taken up by a fluoxetine-sensitive uptake mechanism, requires endogenous 5-HT and indirectly stimulates 5-HT₂ postsynaptic receptors, resulting in the secretion of corticosterone. Furthermore, research into the neurotoxic effect of MDMA, as well as its action on other neurotransmitter systems, may provide insight into the complex pharmacology of it and other substituted phenethylamines.

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References

- ANDERSON, G. M., BRAUN, G., BRAUN, U., NICHOLS, D. E. AND SHULGIN, A. T.: Absolute configuration and psychotomimetic activity. In: Quantitative Structure Activity Relationships of Analgesics, Narcotic Antagonists and Hallucinogens, ed. by G. Barnett, M. Trsic and R. E. Willette, pp. 8-15, U.S. Government Printing Office, Washington, D.C., 1978.
- BATTAGLIA, G., YEH, S. Y., O'HEARN, E., MOLLIVER, M. E., KUHA, M. J. AND DE SOUZA, E. B.: 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [³H]paroxetine-labeled serotonin uptake sites. *J. Pharmacol. Exp. Ther.* **242**: 911-916, 1987.
- BRAUN, U., SHULGIN, A. T. AND BRAUN, G.: Prüfung auf zentrale aktivität und analgesie von N-substituierten analogen des amphetamin-derivates 3,4-methylenedioxyphenylisopropylamin. *Arzneim. Forsch.* **30**: 825-830, 1980.
- COMMINS, D. L., VOSMER, G., VIRUS, R. M., WOOLVERTON, W. L., SCHUSTER, C. R. AND SEIDEN, L. S.: Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. *J. Pharmacol. Exp. Ther.* **241**: 338-345, 1987.
- EVANS, S. M. AND JOHANSON, C. E.: Discriminative stimulus properties of (±)-3,4-methylenedioxymethamphetamine and (±)-3,4-methylenedioxyamphetamine in pigeons. *Drug Alcohol Depend.* **18**: 159-164, 1986.
- FULLER, R. W.: Serotonergic stimulation of pituitary-adrenocortical function in rats. *Neuroendocrinology* **32**: 118-127, 1981.
- FULLER, R. W., PERRY, K. W., SNOODY, H. D. AND MOLLOY, B. B.: Comparison of the specificity of 3-(p-trifluoromethylphenoxy)-n-methyl-3-phenyl propylamine and chlorimipramine as amine uptake inhibitors in mice. *Eur. J. Pharmacol.* **28**: 233-236, 1974.
- FULLER, R. W. AND SNOODY, H. D.: The effects of metergoline and other serotonin receptor antagonists on serum corticosterone in rats. *Endocrinology* **105**: 923-928, 1979.
- FULLER, R. W. AND SNOODY, H. D.: Effect of serotonin-releasing drugs on serum corticosterone concentration in rats. *Neuroendocrinology* **31**: 96-100, 1980.
- GLENNON, R. A.: Discriminative stimulus properties of phenylisopropylamine derivatives. *Drug Alcohol Depend.* **17**: 119-134, 1986.
- GLENNON, R. A., TITLER, M. AND MCKENNEY, J. D.: Evidence for 5-HT₂ involvement in the mechanism of action of hallucinogenic agents. *Life Sci.* **35**: 2505-2511, 1984.
- GLENNON, R. A., YOUNG, R., ROSECRANS, J. A. AND ANDERSON, G. M.: Discriminative stimulus properties of MDA analogs. *Biol. Psychiatry* **17**: 807-814, 1982.
- GOODWIN, G. M. AND GREEN, A. R.: A behavioral and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT₁ and 5-HT₂ receptors. *Br. J. Pharmacol.* **84**: 743-753, 1985.
- GUDELSKY, G. A.: Tuberoinfundibular dopamine neurons and the regulation of prolactin secretion. *Psychoneuroendocrinology* **6**: 3-16, 1981.
- GUDELSKY, G. A., KOENIG, J. I. AND MELTZER, H. Y.: Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat: Evidence for opposing roles of 5-HT₂ and 5-HT_{1A} receptors. *Neuropharmacology* **25**: 1307-1313, 1986.
- HARVEY, J. A. AND MCMASTER, S. E.: Fenfluramine: Cumulative neurotoxicity after chronic treatment with low dosages in the rat. *Commun. Psychopharmacol.* **1**: 3-17, 1977.
- HARVEY, J. A., MCMASTER, S. E. AND YUNGER, L. M.: p-Chloroamphetamine: Selective neurotoxic action in brain. *Science (Wash. DC)* **187**: 841-843, 1975.
- HJORTH, S.: Hypothermia in the rat induced by the potent serotonergic agent 8-OH-DPAT. *J. Neural Transm.* **61**: 131-135, 1985.
- HOLMES, M. C., DiRENZO, G. D., BECKFORD, B., GILLHAM, B. AND JONES, M. T.: Role of serotonin in the control of secretion of corticotropin releasing factor. *J. Endocrinol.* **93**: 151-160, 1982.
- JOHNSON, M. P., HOFFMAN, A. J. AND NICHOLS, D. E.: Effects of the enantiomers

- of MDA, MDMA and related analogues on [3 H]serotonin and [3 H]dopamine release from superfused rat brain slices. *Eur. J. Pharmacol.* **132**: 269-276, 1986.
- KAMIEN, J. B., JOHANSON, C. E., SCHUSTER, C. R. AND WOOLVERTON, W. L.: The effects of ($\pm$)-methylenedioxymethamphetamine and ($\pm$)-methylenedioxymphetamine in monkeys trained to discriminate ($\pm$)-amphetamine from saline. *Drug Alcohol Depend.* **18**: 139-147, 1986.
- KOENIG, J. I., GUDELSKY, G. A. AND MELTZER, H. Y.: Stimulation of corticosterone and β -endorphin secretion in the rat by selective 5-HT receptor subtype activation. *Eur. J. Pharmacol.* **137**: 1-8, 1987.
- KULMALA, H. K., BOJA, J. W. AND SCHECHTER, M. D.: Behavioral suppression following 3,4-methylenedioxymethamphetamine. *Life Sci.* **41**: 1425-1429, 1987.
- LUCKI, I., NOBLER, M. S. AND FRAZER, A.: Differential actions of serotonin antagonists on two behavioral models of serotonin receptor activation in the rat. *J. Pharmacol. Exp. Ther.* **228**: 133-139, 1984.
- LYON, R. A., GLENNON, R. A. AND TITELER, M.: 3,4-Methylenedioxymethamphetamine (MDMA): Stereoselective interactions at brain 5-HT₁ and 5-HT₂ receptors. *Psychopharmacology* **88**: 525-526, 1986.
- MCELROY, J. F., MILLER, J. M. AND MEYER, J. S.: Fenfluramine, *p*-chloroamphetamine and *p*-fluoroamphetamine stimulation of pituitary-adrenocortical activity in rat: Evidence for differences in site and mechanism of action. *J. Pharmacol. Exp. Ther.* **228**: 593-599, 1984.
- MELTZER, H. Y., SIMONOVIC, M. AND GUDELSKY, G. A.: Effects of pirenperone and ketanserin on rat prolactin secretion *in vivo* and *in vitro*. *Eur. J. Pharmacol.* **92**: 83-89, 1983.
- MELTZER, H. Y., WHITA, B., TRICOU, B. J., SIMONOVIC, M., FANG, V. AND MANOU, G.: Effect of serotonin precursors and serotonin agonists on plasma hormone levels. In *Serotonin in Biological Psychiatry*, ed. by B. T. Ho, pp. 117-138, Raven Press, New York, 1982.
- MERCHANT, K. M., LETTER, A. A., JOHNSON, M., STONE, D. M., GIBB, J. W. AND HANSON, G. R.: Effects of amphetamine analogs on neurotensin concentrations in rat brain. *Eur. J. Pharmacol.* **138**: 151-154, 1987.
- MOKLER, D. J., ROBINSON, S. E. AND ROSECRANS, J. A.: 3,4-Methylenedioxymethamphetamine (MDMA) produces long-term reductions in brain 5-hydroxytryptamine in rats. *Eur. J. Pharmacol.* **138**: 265-268, 1987.
- NAHORSKI, S. R. AND WILLCOCKS, A. L.: Interactions of β -adrenoreceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. *Br. J. Pharmacol.* **78**: 107P, 1983.
- NICHOLS, D. E., HOFFMAN, A. J., OBERLENDER, R. A., JACOB, P., III AND SHULGIN, A. T.: Derivatives of 1(1,3-benzodioxol-5-yl)-2-butanamine: Representatives of a novel therapeutic class. *J. Med. Chem.* **29**: 2009-2015, 1986.
- NICHOLS, D. E., LLOYD, D. H., HOFFMAN, A. J., NICHOLS, M. B. AND YIM, G. K. W.: Effects of certain hallucinogenic amphetamine analogues on the release of [3 H]-serotonin from rat brain synaptosomes. *J. Med. Chem.* **25**: 530-535, 1982.
- PAWLOWSKI, L.: Amitriptyline and femoxetine, but not clomipramine or citalopram, antagonize hyperthermia induced by directly acting 5-hydroxytryptamine-like drugs in heat adapted rats. *J. Pharm. Pharmacol.* **36**: 197-199, 1984.
- PAZOS, A., HOYER, D. AND PALACIOS, J. M.: The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.* **106**: 539-546, 1984.
- PEDIGO, N. W., YAMAMURA, H. I. AND NELSON, D. L.: Discrimination of multiple [3 H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **36**: 220-226, 1981.
- PEROUTKA, S. J. AND SNYDER, S. H.: Multiple serotonin receptors: Differential finding of [3 H]-5-hydroxytryptamine, [3 H]-lysergic acid diethylamide and [3 H]-spiperidol. *Mol. Pharmacol.* **16**: 687-699, 1979.
- QUOCK, R. M. AND WEICK, B. G.: *p*-Chloroamphetamine-induced hyperthermia pharmacologically distinct from fenfluramine-induced hyperthermia. *J. Pharm. Pharmacol.* **31**: 27-37, 1979.
- ROSECRANS, J. A. AND GLENNON, R. A.: The effect of MDA and MDMA ("Ecstasy") isomers in combination with pirenperone on operant responding in mice. *Pharmacol. Biochem. Behav.* **28**: 39-42, 1987.
- SCHMIDT, C. J.: Acute administration of methylenedioxymethamphetamine: Comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs. *Eur. J. Pharmacol.* **136**: 81-88, 1987a.
- SCHMIDT, C. J.: Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. *J. Pharmacol. Exp. Ther.* **240**: 1-7, 1987b.
- SCHMIDT, C. J., WU, L. AND LOVENBERG, W.: Methylenedioxymethamphetamine: A potentially neurotoxic amphetamine analogue. *Eur. J. Pharmacol.* **124**: 175-178, 1986.
- SLOVITER, R. S., CONNOR, J. D., DAMIANO, B. P. AND DRUST, E. G.: Parahalo-genated phenethylamines: Similar serotonergic effects in rats by different mechanisms. *Pharmacol. Biochem. Behav.* **13**: 283-286, 1980.
- STEELE, T. D., NICHOLS, D. E. AND YIM, G. K. W.: Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [3 H]monoamines into synaptosomes from different regions of rat brain. *Biochem. Pharmacol.* **36**: 2297-2303, 1987.
- STONE, D. M., STAHL, D. C., HANSON, G. R. AND GIBB, J. W.: The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxymphetamine (MDA) on monoaminergic systems in the rat brain. *Eur. J. Pharmacol.* **128**: 41-48, 1986.
- TRICKLEBANK, M. D., FORLER, C. AND FOZARD, J. R.: The involvement of subtypes of the 5-HT₁ receptor and of catecholaminergic systems in the behavioral response to 8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat. *Eur. J. Pharmacol.* **106**: 271-282, 1985.
- TUOMISTO, J. AND MANNISTO, P.: Neurotransmitter regulation of anterior pituitary hormones. *Pharmacol. Rev.* **37**: 251-332, 1985.
- VAN DE KAR, L. D., KARTESZI, M., BETHEA, C. L. AND GANONG, W. F.: Serotonergic stimulation of prolactin and corticosterone secretion is mediated by different pathways from the mediobasal hypothalamus. *Neuroendocrinology* **41**: 380-384, 1985a.
- VAN DE KAR, L. D., URBAN, J. H., RICHARDSON, K. D. AND BETHEA, C. L.: Pharmacological studies on the serotonergic and nonserotonin-mediated stimulation of prolactin and corticosterone secretion by fenfluramine. *Neuroendocrinology* **41**: 283-288, 1985b.
- VAN PRAAG, H. M. AND KORF, J.: 4-Chloroamphetamines. Chance and trend in the development of new antidepressants. *J. Clin. Pharmacol.* **13**: 3-14, 1973.
- VAN PRAAG, H. W., KORF, J. AND VAN WOUDEBERG, F.: Investigation into the possible influence of chlorinated amphetamine derivatives on 5-hydroxytryptamine synthesis in man. *Psychopharmacology* **18**: 412-420, 1970.

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