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Pineal serotonin is resistant to depletion by serotonergic neurotoxins in rats

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Abstract: Administration of 3,4-methylenedioxymethamphetamine (MDMA) or para-chloroamphetamine (pCA) to adult rats is neurotoxic to serotonin (5HT) nerve terminals and cell bodies. MDMA ( ) mg/kg) reduces 5HT levels in the frontal cortex, medial basal hypothalamus, and striatum acutely and 17 days after a series of multiple injections. The acute reductions occur within 1–2 hr after injection of doses greater than 3 mg/kg. A single injection of pCA reduces 5HT levels in the above mentioned brain regions as well as in the brain stem. However, none of these treatments are able to alter 5HT levels in the pineal gland. It appears that the pineal does not contain the 5HT reuptake system that is thought to be necessary for the neurotoxicity of MDMA and pCA.

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Introduction

3,4-methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that exhibits both amphetamine-like and hallucinogen-like properties. Recent research into MDMA's effects on brain neurochemistry in rats has determined that a sustained depression in hippocampal, striatal, and cortical serotonin (5HT) levels occurs after acute or chronic MDMA administration [Ali et al., 1989; Commins et al., 1987; Finnegan et al., 1988; Johnson et al., 1988; Mokler et al., 1987; Schmidt et al., 1986]. These authors also reported that MDMA was responsible for destroying 5HT nerve terminals as observed by postmortem histologic examination [Commins et al., 1987; Schmidt et al., 1986]. The long-term neurotoxic effect of MDMA may be due to the endogenous conversion of 5HT to 5,6-dihydroxytryptamine that is taken up into the 5HT neuron by the 5HT reuptake mechanism [Ricaurte et al., 1985; Schmidt et al., 1986; Commins et al., 1987]. MDMA (10 mg/kg) also depressed tryptophan hydroxylase activity, 5HT levels, and the levels of the 5HT metabolite, 5-hydroxyindoleacetic acid (5HIAA), in the striatum, hippocampus, and cortex 3 hr after a single injection and 18 hr after five injections [Stone et al., 1986]. These short-term effects may also require neurotoxin transport by the neuronal 5HT reuptake pump. Several investigators have hypothesized that MDMA has a similar mode of action as the 5HT neurotoxin, p-chloroamphetamine (pCA) [Stone et al., 1986; Schmidt et al., 1986].

The pineal gland contains large quantities of 5HT that serves as a precursor for melatonin synthesis may be utilized for other as yet undetermined functions. Since MDMA is known to produce neurotoxicity at 5HT nerve terminals, it is of interest to determine if MDMA or pCA alters pineal 5HT and 5HIAA levels, thereby compromising normal pineal function.

Materials and methods

Animals

All animals used in these studies were drug naive adult male albino rats *(Rattus rattus)* weighing between 175–225 g and purchased from Harlan Sprague Dawley, Houston, TX. They had food and water available ad libitum. They were housed in standard cages (two per cage) in temperature-controlled (22 ± 2°C) rooms and exposed to 12 hr of light and 12 hr of darkness (lights on at 0600). At the termination of the described experiments, the rats were decapitated within 30 sec of their removal from their home cage. The pineal was removed and immediately frozen on solid CO_2. Likewise, the brain was removed rapidly and rinsed in chilled saline. The medial basal hypothalamus (MBH), medial prefrontal cortex, striatum, and brain stem were removed on a chilled plate, weighed and frozen on solid CO_2 as described previously [Matthews et al., 1989]. The average wet weight of these tissues was 23 mg for the MBH, 61 mg for the medial prefrontal cortex, 28 mg for the striatum, and 102 mg for the brain stem.

Experiments

In the first experiment, 25 rats were injected with racemic MDMA (10 mg/kg, ip) and five rats were injected with saline (0.9%) between 1200 and 1400. The MDMA-injected rats were subdivided into five groups and killed 15 min, 30 min, 1 hr, 2 hr, or 6 hr after injection. The saline-injected rats were killed 90 min after injection. The tissues were processed as described above. This experiment was repeated and the results of both experiments were combined for a total of ten animals in both the control and experimental groups. In the second experiment, 30 rats were subdivided into five groups and were injected with saline or 1, 3, 10, or 30 mg/kg of MDMA (ip) between 1000 and 1300. All of the rats were killed 2 hr after injection and the tissues were collected as described. In the third experiment, rats were injected with saline (sc), MDMA (10 mg/kg, sc) or pCA (10 mg/kg, ip), with four animals per treatment. The dose of MDMA was determined by the results of the previous experiments as well as previous research by other investigators [Stone et al., 1986]. The dose of pCA is a well established neurotoxic dose for 5HT neurons with little or no long term effects on catecholaminergic neurons [Sanders-Bush et al., 1974]. The saline and MDMA-injected rats received one injection every 12 hr for 4 days (eight injections), while the pCA-injected rats received only one injection. Seventeen days later, the rats were killed between 1500 and 1700 and the tissues collected. This experiment was repeated four times for the saline and MDMA groups with the pCA group included in the last three repetitions. Therefore, there was a total of 16 saline-control rats, 16 MDMA-injected rats, and 12 pCA-injected rats. In all experiments, animals were kept in pairs in their home cages at normal room temperature with no attempt to compensate for drug-induced hyperthermia.

Monoamine assays

The pineal and brain samples were analyzed for dopamine, 3,4-dihydroxyphenylacetic acid, 5HT, and 5HIAA content by high performance liquid chromatography with electrochemical detection [Champney et al., 1985; Matthews et al., 1989]. The dopamine and 3,4-dihydroxyphenylacetic acid data have been reported previously [Matthews et al., 1989]. Just prior to assay, each area was sonicated in a pre-established volume of 0.16 N perchloric acid containing epinine (100 ng/ml) as an internal standard. The samples were centrifuged (2 min at 13,000*g*) and the amines were separated by injecting the supernatant of the centrifuged brain homogenate onto a reversed phase C-18 column. After elution of the amines by a filtered and degassed phosphate buffered mobile phase (0.9465 g Na_2HPO_4: 2.8 g citric acid: 18.6 mg EDTA: 20 mg sodium octyl sulfate in 900 ml double distilled H_2O with 100 ml methanol), they were quantified by electrochemistry using a glassy carbon electrode at a potential of +0.62V. The column was heated to 35°C and the flow rate of the mobile phase was approximately 0.3 ml/min. Sample peak heights were compared to extracted standard peak heights, which were processed in the same manner. The internal standard was utilized to accommodate for changes in extraction efficiency and detector sensitivity. Unextracted standards were also used to rectify any differential loss in amines during extraction. Sample values were expressed as amine content (usually in nanograms) per g wet weight of brain tissue or ng/pineal. The least detectable concentration of the amines was below 50 pg/sample. The interassay variability was less than 5% for each compound.

Statistics

The data were analyzed by one way analysis of variance with significant differences between groups determined by the Student Newman-Keuls test with a homogenous "N".

Results

The time course experiment indicates that the first significant ($P < 0.05$) decline in 5HT after MDMA treatment occurred approximately 1 hr postinjection in the frontal cortex and MBH (Table 1). A decline in striatal 5HT occurred 2 hr after injection ($P < 0.01$) with a slight, but significant increase observed 15 min after injection ($P < 0.05$) (Table 1). The 5HT levels in these three areas were returning to control values by 6 hr after injection (Table 1). Levels of 5HT in the brain stem and the pineal were unaffected by an acute injection of up to 30 mg/kg MDMA over a 6 hr time course (Tables 1 and 2). However, 5HT levels in the frontal cortex, MBH, and striatum were depressed ($P < 0.05$) by an acute dose of 10 mg/kg MDMA or greater (Table 2).

Multiple injections of MDMA or a single injection of pCA reduced ($P < 0.01$) 5HT levels in the frontal cortex, MBH, or striatum seventeen days after the last injection (Fig. 1). A single injection of pCA also reduced 5HT levels in the brain stem ($P < 0.001$), but was ineffective in the pineal (Fig. 1). Multiple injections of MDMA did not alter 5HT levels in the brain stem or the pineal (Fig. 1).

TABLE 1. Time course effects of 3, 4-methylenedioxymethamphetamine (MDMA, 10 mg/kg) on brain and pineal serotonin levels (ng/g or ng/pineal).[a]

Brain region	Saline	Minutes after MDMA				
		15	30	60	120	360
Frontal cortex	509 ± 47	640 ± 82	538 ± 69	321[d] ± 39	138[d] ± 10	231[d] ± 17
MBH[e]	843 ± 62	679 ± 77	1043 ± 72	403[c] ± 55	440[c] ± 36	523[c] ± 88
Striatum	604 ± 61	817[b] ± 71	697 ± 56	507 ± 54	286[c] ± 29	369[b] ± 46
Brain stem	784 ± 42	934 ± 56	900 ± 53	779 ± 39	642 ± 48	753 ± 50
Pineal[f]	216 ± 13	205 ± 28	210 ± 18	217 ± 23	217 ± 25	196 ± 19

[a]Male rats were injected with MDMA and killed 15, 30, 60, 120, or 360 min later, or injected with saline and killed 90 min later. Values are means ± standard errors with ten rats per group.
[b]$P < 0.05$ vs. saline; [c]$P < 0.01$ vs. saline; [d]$P < 0.001$ vs. saline; [e]MBH = medial basal hypothalamus; [f]ng/pineal.

The 5HIAA levels declined in parallel with the declines in 5HT observed in the brain regions after MDMA or pCA treatment, although a slight time lag between the two compounds was present (data not presented). In the pineal, a slight, but significant ($P < 0.05$) decline in 5HIAA levels was found 15 min, 30 min, 1 hr, and 2 hr after 10 mg/kg MDMA (saline: 17.45 ± 1.44 ng/pineal; MDMA-15 min: 12.93 ± 1.77; MDMA-30 min: 12.08 ± 0.84; MDMA-1hr: 10.74 ± 0.88; MDMA-2 hr: 10.95 ± 1.14). Likewise, a 30 mg/kg injection of MDMA reduced pineal 5HIAA levels 2 hr later ($P < 0.05$) (saline: 21.75 ± 4.45 ng/pineal; MDMA-30 mg/kg: 11.05 ± 1.81). Multiple injections of MDMA or a single injection of pCA did not alter pineal 5HIAA levels (data not presented).

Discussion

These data are consistent with previous reports that indicate that acute MDMA reduces 5HT levels in brain regions rich in 5HT nerve terminals (frontal cortex, MBH, striatum), while being less effective in brain regions containing 5HT cell bodies and nerve terminals (brain stem) [O'Hearn et al., 1988; Ricaurte et al., 1988a,b; Wilson et al., 1989]. In addition, the present results indicate that the pineal is resistant to the acute depletion of 5HT by MDMA. Both the brain stem and the pineal are also spared the chronic neurotoxic effects of MDMA. Likewise, the more potent 5HT neurotoxin pCA is unable to alter 5HT levels in the pineal, although 5HT is significantly reduced in all other brain regions examined.

It is interesting to note that pinealocytes contain some of the highest concentrations of 5HT per cell [King and Steinlechner, 1985], yet do not respond to 5HT neurotoxins like central 5HT neurons (present results). The most likely difference between 5HT neurons and pinealocytes that could account for our findings is the lack of 5HT nerve terminals and their associated 5HT reuptake mechanism within the pineal. Blockade of 5HT reuptake has been shown to prevent both acute and chronic effects of MDMA on 5HT neurons [Schmidt and Taylor, 1990]. Alternatively, MDMA may inhibit neuronal 5HT synthesis, but not pinealocyte 5HT synthesis. MDMA has been shown to be an effective tryptophan hydroxylase inhibitor in several brain areas [Stone et al., 1986; 1987; Johnson et al., 1989]. The effect of MDMA on this enzyme in the pineal has not been tested but numerous studies have shown that production of 5HT in pinealocytes is identical to synthesis pathways in 5HT neurons [King and Steinlechner, 1985]. Therefore, the lack of a 5HT reuptake mechanism in pinealocytes may

TABLE 2. Dose response effects of 3, 4-methylenedioxymethamphetamine (MDMA) on brain and pineal serotonin levels (ng/g or ng/pineal).[a]

Brain region	Saline	MDMA (mg/kg)			
		1	3	10	30
Frontal cortex	444 ± 25	441 ± 10	382 ± 18	168[d] ± 12	162[d] ± 13
MBH[e]	1070 ± 134	1185 ± 84	1100 ± 79	789[b] ± 52	709[b] ± 40
Striatum	1223 ± 111	1209 ± 00	1083 ± 91	753[c] ± 74	920 ± 81
Brain stem	697 ± 24	651 ± 42	672 ± 23	653 ± 12	617 ± 29
Pineal[f]	254 ± 28	277 ± 29	263 ± 19	259 ± 17	310 ± 38

[a]Male rats were injected with saline or a single dosage of MDMA and killed 2 hr later. Values are means ± standard errors with six rats/group.
[b]$P < 0.05$ vs. saline; [c]$P < 0.01$ vs. saline; [d]$P < 0.001$ vs. saline; [e]MBH = medial basal hypothalamus; [f]ng/pineal.

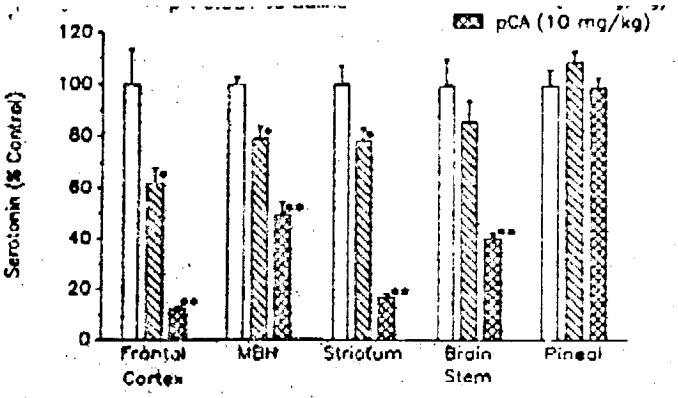

Fig. 1. Chronic effects of 3,4-methylenedioxymethamphetamine (MDMA) or parachloroamphetamine (pCA) on brain and pineal serotonin levels. Male rats were injected with saline or MDMA twice daily for 4 days or received a single injection of pCA. Seventeen days after the last injection, brain regions and pineals were collected. Values are means ± standard errors with 16 rats in the saline and MDMA groups and 12 rats in the pCA group. Serotonin values for the saline controls are: 1256 ng/g in the frontal cortex, 960 ng/g in the medial basal hypothalamus (MBH), 723 ng/g in the striatum, 2419 ng/g in the brain stem, and 215 ng/pineal in the pineal gland.

protect tryptophan hydroxylase from inhibition by MDMA. Other possible differences between 5HT neurons and pinealocytes, such as storage mechanisms of 5HT, 5HT catabolism, or MDMA metabolism, may also account for our data. Regardless of the mechanism involved, our data suggest that 5HT neurotoxins such as MDMA and pCA do not compromise serotonin-dependent pineal biochemistry.

Other investigators have recently found that depletion of circulating tryptophan levels reduces brain tryptophan and 5HT content, but does not alter pineal 5HT or melatonin levels [Daya et al., 1989]. These results suggest that pineal 5HT levels are conserved during treatments that affect other 5HT systems, and indirectly support our present findings. The pineal appears to be insensitive to many treatments that reduce central 5HT levels. This could lead to the suggestion that the pineal 5HT system is tightly regulated and/or protected. However, it should be emphasized that in our study the effects of MDMA and pCA were only examined during the daytime when pineal 5HT levels are increased. Also, pineal melatonin levels were not determined. The ability of these neurotoxins to alter the diurnal rhythm of pineal 5HT and melatonin should be investigated.

Finally, it is worthy to note that MDMA acutely and reversibly decreased pineal 5HIAA levels, suggesting that MDMA may acutely decrease 5HT pineal. In the past, it has been hypothesized that 5HT from pinealocytes is metabolized in the nor-adrenergic nerve terminals surrounding the pinealocytes [King and Steinlechner, 1985]. Our data would indirectly support this hypothesis in that MDMA may inhibit 5HT uptake into norepinephrine terminals resulting in reduced production of 5HIAA without changing pineal 5HT levels. Others have shown that MDMA blocks reuptake of 5HT and DA into neurons [Schmidt and Taylor, 1990; Schmidt et al., 1991].

In conclusion, MDMA is unable to alter 5HT levels in the pineal even though it has neurotoxic effects on 5HT nerve terminals in the central nervous system. Likewise, pineal 5HT levels are not modified by pCA administration, which reduces 5HT levels in all other brain regions examined. The pinealocyte appears to be resistant to the effects of these toxins due to its lack of a 5HT reuptake mechanism.

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