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THE EFFECT OF MESCALINE, 3,4-DIMETHOXYPHENETHYLAMINE AND 2,5-DIMETHOXY-4-METHYLAMPHETAMINE ON RAT PLASMA PROLACTIN: EVIDENCE FOR SEROTONERGIC MEDIATION

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Summary

Mescaline, 3,4-dimethoxyphenethylamine (DMPEA) or 2,5-dimethoxy-4-methylamphetamine (DOM) was administered to male Sprague-Dawley rats, alone or in combination with para-chlorophenylalanine (PCPA), an inhibitor of serotonin (5-HT) synthesis, or methysergide, a 5-HT receptor blocker. All three compounds increased plasma prolactin (PRL) levels. These increases were potentiated by PCPA and blocked by methysergide. Pretreatment with alpha-methylparatyrosine (AMPT), an inhibitor of catecholamine synthesis, resulted in an increase in plasma PRL equal to the additive effects of the independent administration of mescaline, DMPEA, or DOM plus the AMPT-induced response. The results suggest that mescaline, DMPEA and DOM may be exerting their effects on rat plasma PRL through direct stimulation of serotonin receptors. These results further demonstrate the ability of PCPA to rapidly induce supersensitivity of the 5-HT receptors which stimulate PRL secretion.

It is now well established that the secretion of PRL from the anterior pituitary of the rat can be stimulated through a serotonergic mechanism (1-9). We have recently demonstrated that several indoleamine hallucinogens are capable of inducing PRL release via a serotonergic action (10); and we have also suggested that a serotonergic agonist action may account for the ability of mescaline (3,4-5-trimethoxyphenethylamine), a phenethylamine hallucinogen, to increase rat plasma prolactin levels (11).

Biochemical, neurophysiological and behavioral studies are consistent with a variety of pre-synaptic and post-synaptic serotonergic effects of mescaline and DOM. Brain 5-HT has been reported to increase following mescaline (12-14) or DOM (15,16) administration. Depending upon the dose, mescaline can increase or decrease brain 5-hydroxyindoleacetic acid (5-HIAA) levels (12). Mescaline inhibits some, but not all, 5-HT neurons in the dorsal raphe nucleus (17,18). It has been demonstrated that central 5-HT antagonists, e.g., cinanserin, methysergide, and cyproheptadine, reduce the discriminability of mescaline from saline, whereas a peripheral 5-HT antagonist, xylamidine toxylate, has no effect on the stimulus properties of mescaline; p-chlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, potentiates the discriminative stimulus properties of mescaline (19,20). Serotonin antagonists have also been shown to block the mescaline-

induced reduction of the effects of punishment on drinking behavior in rats (21). Wallach et al. (16) have reported that the 5-HT antagonists: methysergide, brom-LSD and cyproheptadine, block the behavioral effects of DOM in cats. Since in their study the effect of DOM was not altered by PCPA pretreatment, these authors concluded that DOM produces its effects by a direct stimulation of post-synaptic 5-HT receptors.

The hypothesis that mescaline exerts its major effect through a direct 5-HT receptor stimulation has been challenged. Although Haigler and Aghajanian (17) did observe neuronal inhibition of raphe neurons with mescaline administration, they noted that the specific sites of action differed with intraperitoneal (i.p.) vs. microiontophoretic application. They thus concluded that its mechanism of action is probably indirect. Anden et al. (13) reported that mescaline, and a structurally similar phenethylamine, DMPEA, had only weak 5-HT stimulating effects, based on the failure of both compounds to induce the serotonergically-mediated hindlimb extensor reflex. It has been reported that DMPEA can significantly decrease brain dopamine (DA) and norepinephrine (NE), but has no effect on 5-HT (22). DMPEA administration has been reported to significantly increase rat plasma PRL levels (23). This was attributed to the possible competition between DMPEA and DA for DA receptors since L-DOPA antagonized the DMPEA-induced PRL increase. We suspected that this effect of DMPEA might be serotonergically-mediated, similar to previously reported effects of N,N-dimethyltryptamine, bufotenin, and psilocybin (10). We have therefore used several neurochemical manipulations with predictable effects on 5-HT and catecholamine mechanisms, to further examine the effect of DMPEA, mescaline and DOM on PRL secretion. The results suggest that these drugs increase PRL secretion by a serotonergic mechanism.

Methods

Male Sprague-Dawley (Sprague-Dawley, Madison, Wisconsin) rats weighing approximately 150-200 gms. were used throughout these studies. They were housed six animals per cage in a temperature-controlled (T=22°C) room with a 12 hr. light-dark cycle. Food and water were available ad-lib.

All drugs were dissolved in physiological saline immediately before use and injected intraperitoneally (i.p.). PCPA, 300 mg/kg, was injected 24 hrs prior to injection of mescaline, DMPEA or DOM. This dose and time schedule has previously been demonstrated to potentiate 5-HT mediated effects on PRL. Methysergide, 10 mg/kg, alpha-methylparatyrosine methylester (AMPT), 125 mg/kg, and saline were injected 30 min prior to the hallucinogens. At this dose and time AMPT reliably induces increases in plasma PRL while methysergide blocks serotonergic-induced increases in plasma prolactin levels (11). Before sacrifice, rats were anesthetized with ketamine (Vetalar, Parke-Davis, Inc.). 100 mg/kg i.p. Ketamine, 100 mg/kg, i.p., has previously been demonstrated not to affect serum PRL levels (24). We have confirmed this in male rats, comparing plasma PRL levels in ketamine-treated rats and guillotined rats. Heparinized blood was obtained from the inferior vena cava within 5 minutes of ketamine injection. Plasma samples were frozen and assayed later for PRL by a double antibody radioimmunoassay as previously described (25). All samples were assayed in the same assay. The intra-assay variation is less than 5%. PRL is expressed as ng/ml NIH rat PRL-RP-1. The results were analyzed by analysis of variance, followed by t-tests on the individual group's differences.

Results

The temporal pattern of the effects of mescaline (25 mg/kg), DMPEA (75 mg/kg) and DOM (2.5 mg/kg) on rat plasma PRL can be seen in Fig. 1.

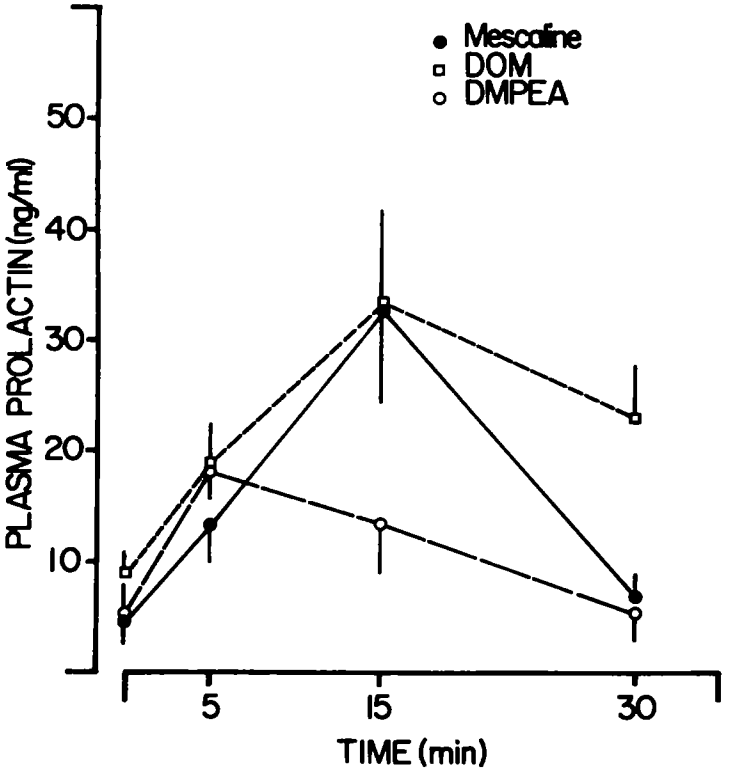

Fig. 1

Effect of mescaline, 25 mg/kg (●——●), DOM, 2.5 mg/kg (□----□) and DMPEA (○——○) 75 mg/kg on rat plasma PRL levels. Each point represents mean ± S.E.M.

The maximum effect of this dose of mescaline occurs at 15 min with a return to baseline level by 30 min. DMPEA had its maximum effect on PRL levels at 5 min with a return to baseline level by 30 min. DMPEA has been demonstrated to have low penetrability through the blood-brain-barrier (26), which may be why high doses were required to produce a measurable change in plasma PRL concentration. The peak increase in plasma PRL levels produced by DOM was at 15 min; however, plasma PRL levels were still markedly increased at 30 min after injection.

Fig. 2 shows the effects of various doses of mescaline, DMPEA or DOM on rat plasma PRL concentrations at the time of peak effect for each drug. PRL secretion was most sensitive to DOM but mescaline produced the largest increase in plasma PRL levels. The slightly smaller increases in PRL secretion with doses of DOM greater than 0.75 mg/kg were not significantly less than those produced by the higher doses of DOM. The two highest doses of mescaline studied produced increases in plasma PRL that were not significantly different from each other. The same was true for DMPEA. To compare the three drugs, we determined the doses which raised plasma PRL levels five fold over baseline from the data in Fig. 2. For DOM, this was 0.46 mg/kg; for mescaline, 16.2 mg/kg; and for DMPEA, 125 mg/kg.

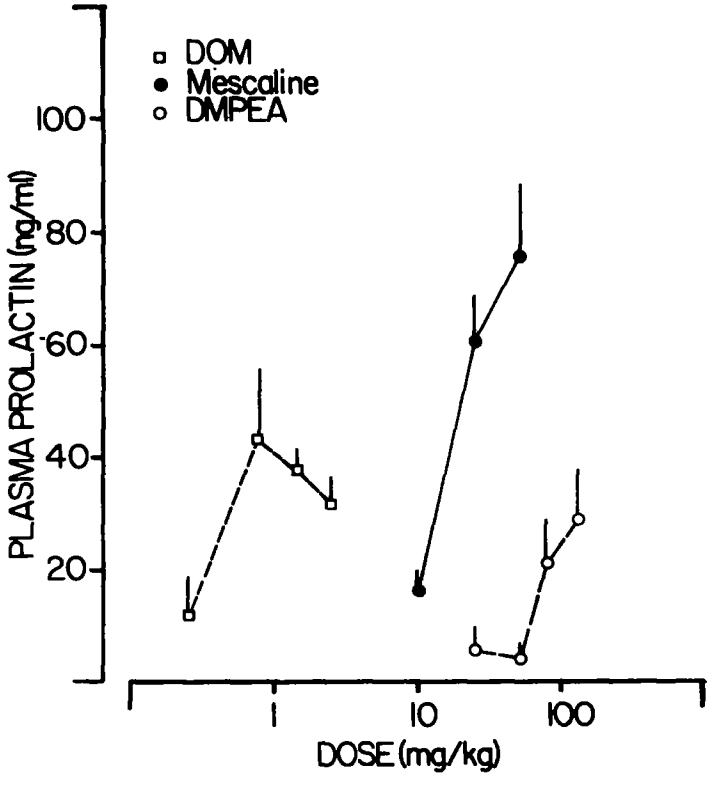

Fig. 2

Dose response curves for the effects of mescaline (●——●), DOM (□----□), and DMPEA (○— —○) on rat plasma prolactin.

The effect of pretreatment with PCPA, methysergide, or AMPT on the plasma PRL response to mescaline (10 mg/kg i.p.), DOM (2.5 mg/kg i.p.), and DMPEA (75 mg/kg i.p.) are shown in Table 1. Separate ANOVA's for each of the main treatments revealed that some of the pre-treatments

TABLE 1

Effect of Pretreatment on Rat Plasma PRL Response to Hallucinogens

Pretreatment	Plasma PRL (ng/ml)			
	Saline	Mescaline (10 mg/kg)	DOM (2.5 mg/kg)	DMPEA (75 mg/kg)
Saline	7.8±3.1	15.5±4.6	29.3±3.3	13.4±2.2
PCPA(300 mg/kg)	4.3±0.7	46.5±12.0**	62.0±11.0**	47.2±13.9*
Methysergide (10 mg/kg)	3.2±0.5+	4.0±0++	6.1±1.9+++	7.0±1.3++
AMPT (125 mg/kg)	16.4±5.6	36.9±12.7*	42.3±16.4**	30.8±1.5*

All groups consisted of 5 rats. Results are mean ± S.D. Time of pretreatment is given in Methods. Rats were sacrificed 15 min after the last injection. *Significantly greater than saline-pretreated rats at <.05 level; **or <.01 level; +significantly less than saline-pretreated rats at <.025 level; ++at <.01 level; +++at <.001 level.

did affect the plasma PRL levels following mescaline ($F=7.10$, $p<.005$), DOM ($F=6.98$, $p<.005$) and DMPEA ($F=5.79$, $p<.01$). Subsequent t-tests were then performed between individual groups to determine the nature of these effects. Pretreatment with PCPA resulted in a significant potentiation of the PRL increase produced by mescaline, DOM and DMPEA. This dose of PCPA significantly depleted brain 5-hydroxyindoleacetic acid levels by 85% and brain 5-HT concentrations by 41% (Fessler and Meltzer, unpublished data). PCPA did not affect plasma PRL levels in the saline-group. Methysergide completely blocked the PRL increase following mescaline, DOM and DMPEA and significantly diminished plasma PRL levels in the saline group. AMPT (125 mg/kg i.p.) elevated plasma PRL levels in the saline-treated rats and when given prior to mescaline, DOM, or DMPEA produced a plasma PRL concentration approximately equal to the additive effects of AMPT and each drug. All AMPT-pretreated groups had plasma PRL levels which were significantly greater than the corresponding saline-pretreated group. AMPT(125 mg/kg) did not significantly change striatal dopamine concentrations at 45 min, the time of sacrifice (Fessler and Meltzer, unpublished data).

Discussion

Administration of mescaline, DOM and DMPEA to saline-pretreated male rats produces consistent and reproducible increases in plasma PRL. The most likely causes of these increases are increased release of 5-HT, direct 5-HT receptor stimulation or DA receptor blockade. Pretreatment with the tryptophan hydroxylase inhibitor, PCPA, 24 hrs prior to each drug, results in a strong potentiation of the PRL response. Since PCPA was shown to deplete brain 5-HT extensively while affecting brain DA only slightly (27) and since PCPA alone has no significant effect on plasma PRL (see Table 1) (10), it is unlikely that release of 5-HT by the three drugs could account for their ability to increase plasma PRL levels; such an effect would be expected to diminish after PCPA pretreatment. We have also found no effect of PCPA pretreatment on the increase in PRL produced by dopamine receptor blockers, such as halo-

peridol (Meltzer, H.Y., unpublished data). Because the effects of mescaline, DOM, and DMPEA are all increased by PCPA pretreatment, this suggests that these drugs are not achieving their increase in PRL by blocking dopamine receptors. Thus, the increase in plasma PRL levels produced by mescaline, DOM and DMPEA is most likely due to direct 5-HT receptor stimulation. The most reasonable explanation for the potentiating effect of PCPA on mescaline-, DOM-, or DMPEA-induced increases in plasma PRL is that decreased availability of 5-HT at the synapse caused by PCPA, results in the development of 5-HT receptor supersensitivity (28). Similar potentiation has been found with quipazine (28) and indole hallucinogens (10), all of which are thought to be 5-HT agonists. PCPA has also been reported to potentiate the disruptive effects of quipazine and mescaline on a conditioned behavior in the rat (29).

That the ability of mescaline, DOM and DMPEA to enhance PRL secretion is the result of 5-HT receptor stimulation is also supported by the demonstration that methysergide, a 5-HT receptor blocker, blocked the effects of each drug. Moreover, methysergide also blocked the effect of each drug following pretreatment with PCPA (data not presented). Methysergide only slightly blocked the increase in plasma PRL produced by chlorpromazine (Meltzer, Fessler, Simonovic, unpublished data), an effect probably related to the dopamine agonist properties of methysergide. We have recently found that methysergide can reverse the increase in plasma prolactin levels produced by AMPT and reserpine, similar to DA agonist such as apomorphine and bromcryptine although methysergide is less potent (Meltzer and Fang, in preparation). Similar conclusions have been reached by other investigators (30). Nevertheless, it is still likely all 3 drugs act via a common mechanism. It is possible that the dopamine agonist effects of methysergide also contributes to its ability to block the 5-HT related increase in plasma PRL produced by mescaline, DOM and DMPEA.

Administration of the tyrosine hydroxylase inhibitor, AMPT in combination with either mescaline, DOM, or DMPEA, resulted in a PRL response approximately equal to the additive effects of AMPT and the hallucinogens when administered independently. It is of interest to compare these results with the effects of d-amphetamine on reserpine and AMPT-induced increase in plasma PRL levels. We have reported that d-amphetamine lowers plasma PRL in reserpine-treated rats, and in rats treated with the same dose of AMPT used in this study (31). These effects are consistent with amphetamine having indirect DA agonist effect. DOM, though a substituted amphetamine, apparently lacks the ability of amphetamine to promote DA release.

The results reported in Fig. 2 indicate that DOM is about 35 times more potent than mescaline which in turn is about 8 times more potent than DMPEA. However, the magnitude of the PRL response was greater with mescaline than with DMPEA or DOM whereas it was more prolonged with DOM compared to the other two drugs. Factors such as penetration into the brain, tissue destruction and rates of metabolism as well as interaction with 5-HT-like receptors probably play a role in these variations in response. Mescaline is approximately as potent as N,N-dimethyltryptamine, while DOM appears to be somewhat more potent than bufotenin, the most potent of the indole hallucinogens we studied, in terms of ability to stimulate plasma prolactin in male rats. The concentration of these agents in the brain would have to be determined before these results could be directly related to their serotonin agonist properties.

Our observation that DMPEA administration results in increases in rat plasma PRL concentrations is in agreement with the previous report of Smythe and Lazarus (23). They concluded that the plasma PRL increase resulted from DA receptor blockade, which could be overcome by the administration of low doses of L-DOPA, 8 mg/kg. However, we have recently demonstrated that subthreshold doses of DA receptor agonists are capable of suppressing the PRL response following 5-HT receptor stimulation (32). Thus, the Smythe and Lazarus report that low doses of L-DOPA inhibit the effect of DMPEA on PRL reaction is consistent with our hypothesis that the effect of DMPEA (and DOM and mescaline) is due to a serotonergic agonist effect. It is of interest that Smythe and Lazarus (23) reported that 5-HTP, 10 mg/kg i.p., which had no effect on rat PRL secretion, potentiated the effect of DMPEA on PRL secretion. This might indicate that DMPEA can also promote 5-HT activity by an indirect mechanism, e.g., increased 5-HT release or decreased 5-HT uptake. However, we have been unable to replicate their finding (Meltzer, unpublished data). We also found that the effect of mescaline 25 mg/kg i.p. on rat plasma prolactin levels was not potentiated by 5-HTP 10 mg/kg i.p. (Meltzer, unpublished data).

In summary, we have demonstrated that mescaline, DOM and DMPEA can produce consistent and reproducible increases in rat plasma PRL concentrations, which can be potentiated by PCPA pretreatment and blocked by methysergide. AMPT pretreatment results in a PRL response equal to the independent effects of the hallucinogens plus AMPT. It is likely that mescaline, DOM and DMPEA increase plasma PRL levels by direct 5-HT receptor stimulation.

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