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Neuroendocrine Pharmacology of Three Serotonin Releasers: 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butane (MBDB), 5-methoxy-6-methyl-2-aminoindan (MMAI) and *p*-methylthioamphetamine (MTA)[1]

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ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT)-releasing drugs are important experimental tools to examine the role of serotonergic nerve terminals in the secretion of hormones. The drugs 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butane (MBDB), 5-methoxy-6-methyl-2-aminoindan (MMAI) and *p*-methylthioamphetamine (MTA) have been suggested to be 5-HT releasers. The present study characterized MBDB, MMAI and MTA by using their effects on the secretion of the hormones adrenal corticotrophin (ACTH), corticosterone, prolactin, oxytocin and renin. The time course of the effect of MBDB, MMAI and MTA (5 mg/kg, i.p.) showed that the peak effect on plasma ACTH occurred 10 min after the injection, whereas the prolactin response did not reach a maximum until 30 min after injection. MBDB increased plasma renin concentration within 10 min, whereas the effect of MTA was significant only at 30 min after injection. All three 5-HT releasers decreased HR (within 5 min) and blood pressure (at 15 min after injection). MBDB, MMAI and MTA increased plasma ACTH, corticosterone, prolactin and renin levels in a dose-dependent manner, whereas no changes were observed in plasma vasopressin concentrations. MTA and MMAI, but not MBDB, significantly increased plasma oxytocin concentrations in a dose-dependent manner. Pretreatment of rats with fluoxetine blocked the ACTH response to MBDB and MMAI, but not to MTA. The prolactin response to all three 5-HT releasers was blocked by fluoxetine. The oxytocin response to MTA and MMAI was inhibited by fluoxetine. The renin responses to all three 5-HT releasers were not significantly inhibited by fluoxetine. The results suggest that MBDB, MMAI and MTA can increase the secretion of several hormones, at least in part, through stimulation of serotonergic neurotransmission. However, these three 5-HT releasers seem to have effects on other (and as yet uncharacterized) mechanisms that can stimulate the secretion of some hormones.

Serotonergic pathways innervating the hypothalamus are involved in regulation of the release of several hormones. Many neuroendocrine studies have relied on serotonin-releasing drugs to examine the role of serotonergic neurons in the secretion of several hormones. Most 5-HT-releasing drugs used in the past are amphetamine derivatives, such as *d*-fenfluramine and *p*-chloroamphetamine (Fuller and Snoddy, 1980; Saydoff *et al.*, 1996; Levy *et al.*, 1994b; Park and Cowen, 1995; Puig de Parada *et al.*, 1995). Three amphetamine derivatives, MBDB, MMAI and MTA are also 5-HT releasers (Marona-Lewicka and Nichols, 1994; Huang *et al.*, 1992; Johnson *et al.*, 1991; Rudnick and Wall, 1993). Unlike *p*-chloroamphetamine, MMAI and MTA have a low affinity for norepinephrine and dopamine uptake sites and to monamine receptors (Huang *et al.*, 1992; Johnson *et al.*, 1991; Nichols *et al.*, unpublished observations). Furthermore, MMAI releases 5-HT but does not release dopamine *in vitro* and has a low affinity for monoamine receptors (Nichols *et al.*, 1993; Rudnick and Wall, 1993). Therefore, these 5-HT releasers may be very useful for studies of serotonergic function. The present studies used neuroendocrine parameters to examine the relative selectivity of MBDB, MMAI and MTA as 5-HT-releasing drugs.

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ABBREVIATIONS: ACTH, adrenal corticotrophin; ANOVA, analysis of variance; 5-HT, 5-hydroxytryptamine; MBDB, 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butane; MMAI, 5-methoxy-6-methyl-2-aminoindan; MTA, *p*-methylthioamphetamine; MAO-A, monoamine oxidase A.

The effects of both fenfluramine and *p*-chloroamphetamine on secretion of ACTH, corticosterone, prolactin, oxytocin and renin can be blocked by depletion of 5-HT stores and by destruction of serotonergic pathways (Fuller and Snoddy, 1980; Fuller *et al.*, 1980; McElroy *et al.*, 1984; Van de Kar *et al.*, 1982; Van de Kar and Bethea, 1982; Van de Kar *et al.*, 1985b). These 5-HT releasing drugs gain access into serotonergic terminals *via* the 5-HT transporter (Fuller *et al.*, 1988; Berger *et al.*, 1992; Fuller, 1992; Rudnick and Wall, 1992). Therefore, 5-HT uptake blockers should prevent these drugs from entering serotonergic nerve terminals and thus prevent the release of hormones that are regulated by 5-HT (McElroy *et al.*, 1984; Van de Kar *et al.*, 1995; Van de Kar *et al.*, 1985b). Consequently, the ability of fluoxetine to inhibit the hormone responses to drugs that are proposed to be 5-HT releasers can be used to examine their selectivity.

A second way to examine selectivity of the neuroendocrine response to 5-HT releasers is to measure multiple hormones in the same blood samples. The secretion of several hormones can be stimulated by multiple neurotransmitter pathways. For example, activation of dopamine D_2 receptors inhibits prolactin secretion (Crowley *et al.*, 1991; Millan *et al.*, 1995; Ekman and Eriksson, 1993; Burris *et al.*, 1992) but stimulates the secretion of ACTH (Jezova and Vigas, 1988; Fuller and Snoddy, 1984). Therefore, it is important to compare several hormone responses to each 5-HT releaser in order to avoid misinterpreting the results.

Renin secretion can also be stimulated by other peripheral mechanisms, such as decreased blood pressure (renal stretch receptor mechanism) and increased sympathetic activity (Bertolino *et al.*, 1994). Other 5-HT releasers have variable effects on blood pressure. *p*-Chloroamphetamine increases blood pressure, whereas fenfluramine does not alter blood pressure (Alper *et al.*, 1987; Rittenhouse *et al.*, 1994; Van de Kar *et al.*, 1985a). To distinguish between serotonergic mechanisms and peripheral cardiovascular mechanisms that could contribute to the effects of the 5-HT releasers on renin secretion, we also measured the time course of effects of the releasers on blood pressure and HR.

Materials and Methods

Animals. Male Sprague-Dawley rats (225–275 g) were purchased from Harlan (Indianapolis, IN). The rats were housed two per cage in a lighting- (12 hr light/dark; lights on at 7 A.M), humidity- and temperature-controlled room, except for the rats that received i.a. catheters. After surgery, they were singly caged. Food and water were available *ad libitum*. In all the experiments, 8 to 9 rats were used per experimental group. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by the Loyola University Institutional Animal Care and Use Committee.

Surgery. A catheter was inserted only into the rats that were used in the time course study. The procedure was described previously (Rittenhouse *et al.*, 1994). Briefly, catheters (PE 50 tubing) were inserted into the descending aorta of rats anesthetized with pentobarbital (50 mg/kg i.p.), passed through the femoral artery and led s.c. to exit between the scapulas. The rats received an infusion of saline (2 ml/kg i.a.), and the catheters were filled with a 50% sucrose solution containing 1000 U/ml heparin. Arterial blood pressure recordings were made 24 hr after catheter implantation.

Drugs. MMAI, MBDB and MTA were synthesized in the laboratory of Dr. David Nichols (Purdue University, West Lafayette, IN). Fluoxetine was donated by Eli Lilly Co. (Indianapolis, IN). All drugs were dissolved in saline before injection.

Experimental protocol. *Time course study:* The catheterized rats (see surgery above) were used to determine the time course of effects of the 5-HT releasers on hormone secretion, blood pressure and HR. Blood pressure and HR were measured every 5 min, from 15 min before to 30 min after injection of MBDB, MMAI or MTA (all at a dose of 5 mg/kg i.p.) or of saline. Blood samples (0.4 ml) were taken before and 10 min after injection of saline or the 5-HT releasers from the cannulas into tubes containing 0.04 ml of 0.3 M EDTA and, after centrifugation, were saved at −70°C for hormone assays. An equal volume of saline was infused to compensate for volume loss. The rats were decapitated 30 min after injection of the 5-HT releasers, and trunk blood was collected into tubes containing 0.5 ml of 0.3 M EDTA. After centrifugation, the plasma was stored at −70°C for hormone assays.

Dose-response study: MBDB, MMAI, MTA (1, 5 and 10 mg/kg i.p.) or saline was injected 15 min before the rats were decapitated. Trunk blood was collected as described above for hormone assays.

Effect of fluoxetine on the hormone responses to the 5-HT releasers. Rats received an injection of fluoxetine (10 mg/kg i.p.) 3 hr before decapitation. Subsequently, the rats received an injection of saline or MBDB (5 mg/kg i.p.), MMAI (5 mg/kg i.p.) or MTA (10 mg/kg i.p.) and were sacrificed by decapitation 15 min later. Trunk blood was collected as described above for hormone assays.

Biochemical determinations. Radioimmunoassays were used to determine the concentrations of plasma ACTH, corticosterone, oxytocin, vasopressin, prolactin and renin. These assays were described in detail elsewhere (Li *et al.*, 1993; Brownfield *et al.*, 1988; Saydoff *et al.*, 1991).

Statistical analysis. The data are represented as group means and S.E.M. The hormone data from the dose-response studies were analyzed by a one-way ANOVA, and the data for the effects of fluoxetine on the hormone responses to 5-HT releasers were analyzed by a two-way ANOVA. The data for the time course of the effects of 5-HT releasers on plasma ACTH, prolactin and renin and on blood pressure and HR were analyzed by two-way ANOVA with repeated measures. Group means were compared by Duncan's Multiple Range test (Steel and Torrie, 1960). For all the statistical analyses, we used a computer program (NWA STATPAK, Portland, OR). All the statistical analyses are presented in the figure legends.

Results

Time course of effects of the 5-HT releasers on ACTH and prolactin secretion. As can be seen in figure 1, all three 5-HT releasers increased plasma ACTH with a peak effect seen at 10 min after injection. At 30 min after injection of the 5-HT releasers, plasma ACTH levels were not significantly different from those of saline-injected rats. MBDB and MMAI significantly increased the concentrations of prolactin at 10 min after injection, and the effects were more pronounced at 30 min after the injections (fig. 1). MTA increased plasma prolactin concentration only at 30 min after injection. To avoid hemorrhaging the rats, we limited the volume of blood and the times at which it was withdrawn. Therefore, not enough plasma was obtained for all the hormone assays.

Effect of the 5-HT releasers on blood pressure, HR and plasma renin. MBDB increased plasma renin concentration at 10 and 30 min after injection (fig. 2, upper panel). MTA increased plasma renin concentration only at 30 min and MMAI (5 mg/kg i.p.) had no significant effect at any time (fig. 2, upper panel).

All three 5-HT releasers reduced blood pressure (fig. 2, middle panel). MMAI and MBDB significantly reduced blood pressure at 15 min after injection. The reduction was main-

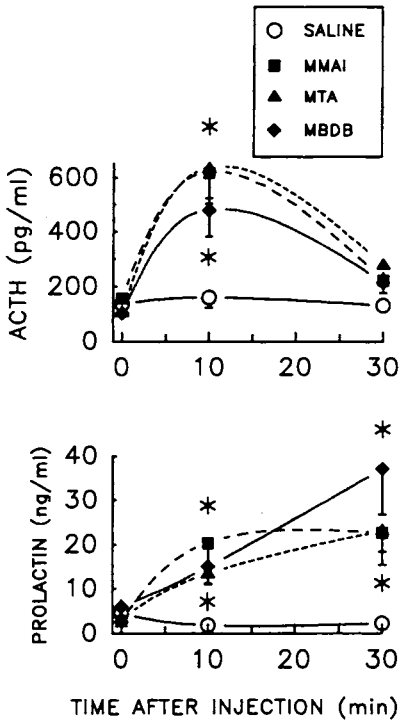

Fig. 1. Time course of effects of MBDB, MMAI and MTA on plasma ACTH and prolactin concentrations. The dose of all three 5-HT releasers was 5 mg/kg i.p. The data represent the mean ± S.E.M. of 8 to 10 rats per group. * Significant difference from saline-injected rats (two-way ANOVA with repeated measures, followed by Duncan's Multiple Range test. For ACTH, drug effect: $F_{(3,36)} = 3.52$, $P < .03$; time: $F_{(2,60)} = 53.11$, $P < .01$; drug × time: $F_{(6,60)} = 6.20$, $P < .01$). For prolactin, drug effect: $F_{(3,40)} = 3.18$, $P < .05$; time: $F_{(2,80)} = 10.85$, $P < .01$; drug × time: $F_{(6,60)} = 3.50$, $P < .01$).

tained until 30 min after injection. The effect of MTA was statistically significant only at 25 min after administration.

Only MMAI and MBDB significantly reduced HR (fig. 2, lower panel). The effects of MMAI and MBDB were statistically significant from 5 min after administration. This reduction of HR had not returned to control values at 30 min after injection. MTA did not significantly reduce HR at any time.

Dose-response effects of the 5-HT releasers on plasma hormones. All three 5-HT releasers increased plasma ACTH and corticosterone concentrations in a dose-dependent manner (fig. 3). MBDB and MMAI, but not MTA, dose-dependently increased plasma prolactin concentrations (fig. 4). MMAI and MTA, but not MBDB, stimulated oxytocin release (fig. 4). MBDB and MMAI increased plasma renin concentration in a dose-dependent manner. Only the highest dose of MTA (10 mg/kg i.p.) increased plasma renin concentration (fig. 4). Plasma vasopressin was not altered by any of the doses of the 5-HT releasers (saline: 0.7 ± 0.2; MMAI: 0.7 ± 0.2, 0.6 ± 0.2, 1.0 ± 0.4 resp; MTA: 0.9 ± 0.2, 0.8 ± 0.3, 0.9 ± 0.3 resp; MBDB: 0.6 ± 0.1, 0.6 ± 0.2, 0.5 ± 0.1 resp).

The lack of statistically significant effects of MTA on prolactin is probably attributable to the hormone responses to the 5-HT releasers being measured 15 min after the injections. At this time-point, the effect of MTA on prolactin is not statistically significant, but from the time course experiment, it is clear that it would have been significant at 30 min. Because the ACTH response had already declined at 30 min after injection, we were limited to measuring all hormones at 15 min after injection.

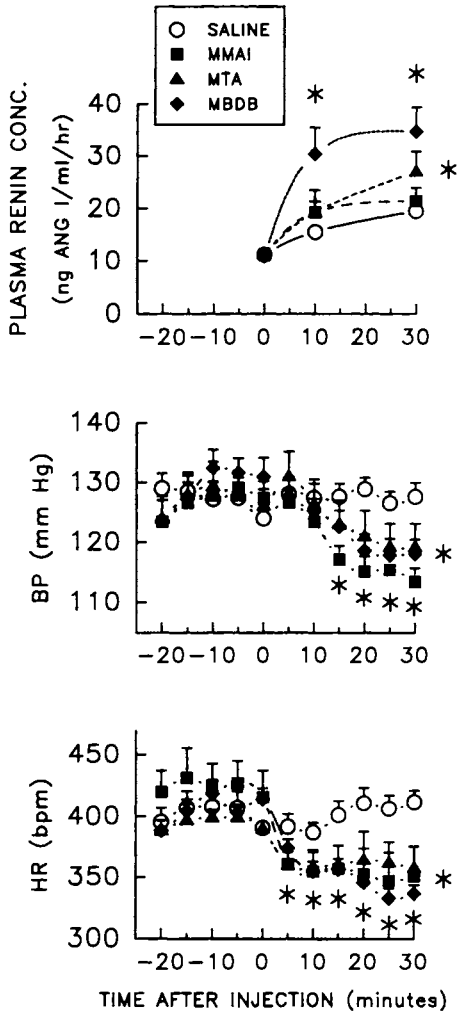

Fig. 2. Time course of effects of MBDB, MMAI and MTA on plasma renin concentration, mean blood pressure (BP) and HR. The dose of all three 5-HT releasers was 5 mg/kg i.p. The data represent the mean ± S.E.M. of eight rats per group. * Significant difference from saline-injected rats (two-way ANOVA with repeated measures, followed by Duncan's Multiple Range test. For renin: drug effect: $F_{(3,40)} = 2.99$, $P < .05$; time: $F_{(2,77)} = 37.04$, $P < .01$; drug × time: $F_{(6,77)} = 1.94$, NS). For BP, drug: $F_{(3,28)} = .60$, NS; time: $F_{(10,280)} = 18.76$, $P < .01$; drug × time: $F_{(30,280)} = 3.42$, $P < .01$). For HR, drug effect: $F_{(3,28)} = 1.36$, NS; time: $F_{(10,280)} = 23.04$, $P < 0.01$; drug × time: $F_{(30,280)} = 3.91$, $P < .01$).

Effect of fluoxetine on the hormone responses to the 5-HT releasers. Pretreatment with the 5-HT uptake blocker fluoxetine inhibited the ACTH responses to MBDB and MMAI, but not to MTA (fig. 5). Only the MBDB-induced increase in corticosterone secretion was inhibited by fluoxetine (fig. 5). The lack of effect of fluoxetine on the MMAI-induced increase in corticosterone release may be due to incomplete blockade of the ACTH response. The levels of ACTH probably were sufficient to increase corticosterone secretion maximally.

Fluoxetine abolished the prolactin response to all three 5-HT releasers (fig. 6). Fluoxetine reduced the oxytocin response only to MTA and MMAI (fig. 6). Because MBDB did not significantly increase plasma oxytocin, the effect of fluoxetine on the oxytocin response could not be examined statistically. The renin response to all three 5-HT releasers was not influenced by pretreatment with fluoxetine (fig. 6).

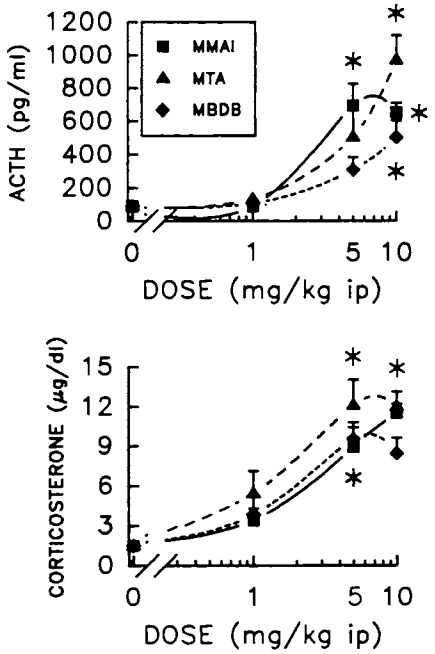

Fig. 3. Dose-response curves for the effect of MBDB, MMAI and MTA on plasma ACTH and corticosterone. Blood samples were obtained 15 min after injection. The data represent the mean ± S.E.M. of 7 to 8 rats per group. * Significant difference from saline-injected rats (one-way ANOVA followed by Duncan's Multiple Range test. For ACTH: $F_{(9,70)} = 10.7$, $P < .01$; for corticosterone: $F_{(9,66)} = 8.77$, $P < .01$).

Discussion

The results of this study indicate that with few exceptions, MMAI, MBDB and MTA stimulate the secretion of ACTH, corticosterone, prolactin, oxytocin and renin in a dose-dependent manner. The exceptions were that MTA and MBDB had little effect on prolactin and oxytocin secretion, respectively, and that none of these drugs increased plasma vasopressin concentrations. Fluoxetine was used to test the specificity of these 5-HT releasing drugs. Fluoxetine blocks 5-HT reuptake by inhibiting the 5-HT transporter (Wong *et al.*, 1983; Wong *et al.*, 1985). Other 5-HT-releasing drugs (*i.e.*, *p*-chloroamphetamine and *d*-fenfluramine) need the 5-HT transporter to enter into the serotonergic nerve terminal, where they induce the release of 5-HT and hence the release of hormones (Fuller and Snoddy, 1980; McElroy *et al.*, 1984; Kuhn *et al.*, 1985; Levy *et al.*, 1994b; Van de Kar *et al.*, 1995). In addition to using the 5-HT transporter to enter serotonergic nerve terminals, *d*-fenfluramine and *p*-chloroamphetamine may also invert the direction of the transporter and use it to release 5-HT (Berger *et al.*, 1992; Rudnick and Wall, 1992; Rudnick and Wall, 1993). The inhibition of many of the hormone responses to MBDB, MMAI and MTA by fluoxetine suggests that all three 5-HT releasers need the 5-HT transporter in serotonergic nerve terminals to exert their effects on the secretion of hormones. By inference, these results suggest that MBDB, MMAI and MTA increase the secretion of hormones by activating serotonergic neurotransmission. However, the inhibition of hormone responses was not uniform for all the tested drugs or for all the hormones measured. This suggests that additional mechanisms are activated by these drugs.

Although increased release of 5-HT will increase the secretion of ACTH, corticosterone, prolactin, oxytocin and renin, other neurotransmitters, such as norepinephrine and dopamine, also influence the secretion of these hormones (Levy *et al.*, 1994a). As mentioned in the introduction, activation of dopamine D_2 receptors increases plasma ACTH concentration (Jezova and Vigas, 1988; Fuller and Snoddy, 1984) but decreases plasma prolactin concentration (Crowley *et al.*, 1991; Millan *et al.*, 1995; Ekman and Eriksson, 1993; Burris *et al.*, 1992). Therefore, it is important to compare several hormone responses to a 5-HT releaser in order to determine its selectivity. In the present studies, fluoxetine inhibited the prolactin response to all three 5-HT releasers, whereas the ACTH response to MTA was not inhibited by pretreatment with fluoxetine. One possible interpretation of our data is that MTA could activate dopamine D_2 receptors (directly or indirectly) in addition to inducing the release of 5-HT. This could also explain why the efficacy of MTA on the secretion of prolactin is lower than for MMAI or MBDB (see fig. 4). Alternatively, MTA might increase the secretion of ACTH through more than one mechanism, and only the effect of 5-HT release could be blocked by fluoxetine, whereas the

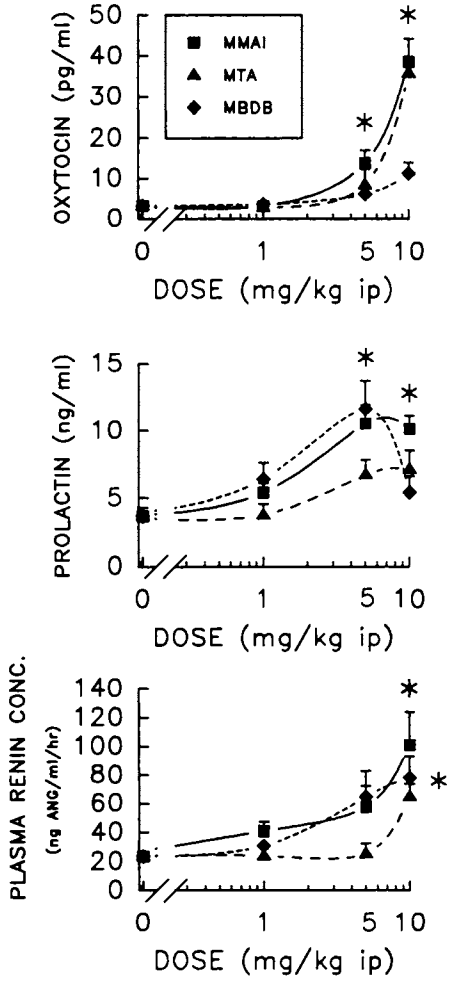

Fig. 4. Dose-response curves for the effect of MBDB, MMAI and MTA on plasma oxytocin, prolactin and renin concentrations. Blood samples were obtained 15 min after injection. The data represent the mean ± S.E.M. of 7 to 8 rats per group. * Significant difference from saline-injected rats (one-way ANOVA followed by Duncan's Multiple Range test. For oxytocin: $F_{(9,70)} = 22.11$, $P < .01$. For prolactin: $F_{(9,69)} = 5.27$, $P < .01$. For renin: $F_{(9,63)} = 4.32$, $P < .01$).

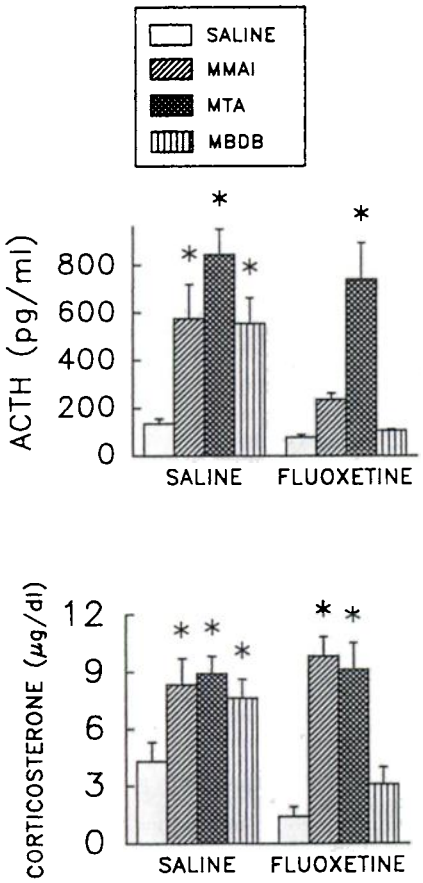

Fig. 5. Effect of pretreatment with the 5-HT uptake blocker fluoxetine (10 mg/kg i.p.) 3 hr before injection of the 5-HT releasers on the concentration of ACTH and corticosterone. The dose of MMAI and MBDB was 5 mg/kg i.p., whereas the dose of MTA was 10 mg/kg i.p. The data represent the mean ± S.E.M. of eight rats per group. The two-way ANOVA indicated that for ACTH, the fluoxetine effect was $F_{(1,56)} = 13.67$ $P < .01$; for 5-HT releasers: $F_{(3,56)} = 19.72$, $P < .01$; and for the fluoxetine × 5-HT releasers interaction: $F_{(3,56)} = 2.12$, NS). For corticosterone, the two-way ANOVA indicated that the fluoxetine effect was $F_{(1,56)} = 4.143$ $P < .05$; for 5-HT releasers: $F_{(3,56)} = 13.96$, $P < .01$; and for the fluoxetine × 5-HT releasers interaction: $F_{(3,56)} = 1.98$, NS). * Significant difference from corresponding saline-injected (*i.e.*, 0 dose of 5-HT releasers) rats, $P < .05$, by Duncan's Multiple Range test.

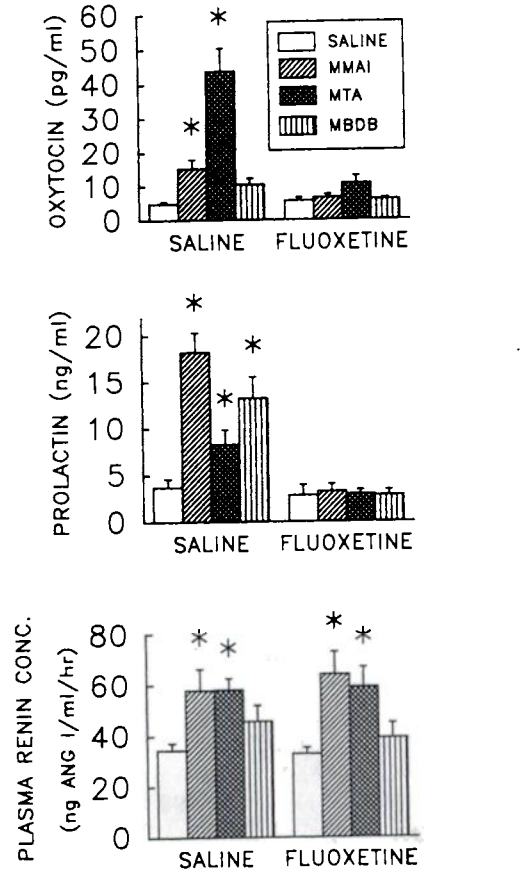

Fig. 6. Effect of pretreatment with the 5-HT uptake blocker fluoxetine (10 mg/kg i.p.) 3 hr before injection of the 5-HT releasers on the concentration of oxytocin, prolactin and renin. The dose of MMAI and MBDB was 5 mg/kg i.p., whereas the dose of MTA was 10 mg/kg i.p. The data represent the mean ± S.E.M. of eight rats per group. The two-way ANOVA indicated that for oxytocin, the fluoxetine effect was $F_{(1,55)} = 15.43$, $P < .01$; for 5-HT releasers: $F_{(3,55)} = 13.48$, $P < .01$; and for the fluoxetine × 5-HT releasers interaction: $F_{(3,55)} = 9.07$, $P < .01$). For prolactin, the two-way ANOVA indicated that the fluoxetine effect was $F_{(1,53)} = 60.46$, $P < .01$; for 5-HT releasers: $F_{(3,53)} = 9.91$, $P < .01$; and for the fluoxetine × 5-HT releasers interaction: $F_{(3,53)} = 9.11$, $P < .01$). For renin, the two-way ANOVA indicated that the fluoxetine effect was $F_{(1,56)} = 0.07$ NS; for 5-HT releasers: $F_{(3,56)} = 6.07$, $P < .01$; and for the fluoxetine × 5-HT releasers interaction: $F_{(3,56)} = 0.36$, NS). * Significant difference from corresponding saline-injected (*i.e.*, 0 dose of 5-HT releasers) rats, $P < .05$, by Duncan's Multiple Range test.

other effects might not involve serotonergic mechanisms. For example, after completion of the experiments, it was discovered that MTA is a relatively potent and reversible inhibitor of rat brain MAO-A, having an IC_{50} value of 370 nM (Nichols *et al.*, unreported observations). This could indirectly increase dopaminergic activity. It cannot be ruled out that MTA could alter the secretion of hormones by inhibiting MAO-A. If it could enter serotonergic nerve terminals through a transporter-independent mechanism, MTA could increase the concentration of 5-HT and allow for 5-HT release through a fluoxetine-independent mechanism.

MBDB is less efficacious than MMAI and MTA in its ability to increase the secretion of some hormones, particularly oxytocin and ACTH. The ACTH, prolactin and oxytocin responses to MMAI were significantly inhibited by fluoxetine. Our results are consistent with those of another study indicating that fluoxetine also inhibits MMAI-induced behaviors (Callaway *et al.*, 1993). However, the ACTH response to MMAI was not completely blocked by fluoxetine. This is probably not due to an insufficient dose of fluoxetine, because the dose used in the present study blocks the effects of *d*-fenfluramine and *p*-chloroamphetamine on the secretion of several hormones (Fuller and Snoddy, 1980; McElroy *et al.*, 1984; Levy *et al.*, 1994b; Van de Kar *et al.*, 1995). The incomplete blockade, by fluoxetine, of the ACTH response to MMAI suggests that MMAI also activates another mechanism to stimulate the secretion of ACTH. Because MMAI is a very weak uptake blocker or releaser of norepinephrine and dopamine (Rudnick and Wall, 1993; Johnson *et al.*, 1991), it is unlikely that increased release of norepinephrine or dopamine is involved in MMAI-induced ACTH secretion. The additional mechanism through which MMAI increases the secretion of ACTH remains to be determined.

The renin data are puzzling. In the present studies, fluoxetine did not inhibit the renin responses to MMAI, MBDB or MTA. This is in contrast to our findings with fenfluramine, in which the same dose of fluoxetine completely blocked the renin response (Van de Kar *et al.*, 1985a). This lack of inhi-

bition of the renin response to MMAI, MBDB and MTA by fluoxetine suggests that additional mechanisms could be triggered by these drugs to increase renin release. It is not likely that a reflex response to a reduction in blood pressure, induced by all three 5-HT releasers, is involved, because the increase in renin seems to occur before a significant reduction in blood pressure is seen (see fig. 2). A reduction in blood pressure normally would result in increased renin release through activation of renal baroreceptors (Bertolino *et al.*, 1994). Fenfluramine does not alter blood pressure, and *p*-chloroamphetamine increases blood pressure in conscious rats (Alper *et al.*, 1987; Rittenhouse *et al.*, 1994; Van de Kar *et al.*, 1985a). Therefore, the present observation suggests that new mechanisms need to be explored.

Vasopressin secretion was not stimulated by any of the three serotonin releasers used in this study. This negative finding is difficult to explain, because a variety of neuropharmacological studies have established that brain serotonin neurons stimulate vasopressin secretion in the rat. Evidence in favor of serotonergic stimulation of vasopressin secretion is provided by studies showing that two other 5-HT releasers, *p*-chloroamphetamine and *d*-fenfluramine, stimulate vasopressin secretion (Iovino and Steardo, 1985; Brownfield *et al.*, 1988). Vasopressin is also released by peripheral administration of the 5-HT agonists quipazine (Iovino and Steardo, 1985), MK-212 (Brownfield *et al.*, 1988), and m-CPP (Bagdy *et al.*, 1992) and by i.c.v. injection of the selective 5-$HT_{2A/2C}$ agonist DOI (Brownfield *et al.*, 1992) or 5-HT (Montes and Johnson, 1990; Saydoff *et al.*, 1992; Brownfield *et al.*, 1993; Pergola *et al.*, 1993). Perhaps other, nonserotonergic effects of MBDB, MMAI and MTA mask their stimulation of vasopressin release.

In conclusion, our results suggest that MBDB, MMAI and MTA increase the secretion of several hormones by stimulating 5-HT release. However, none of these drugs is as sensitive to inhibition of 5-HT uptake as are *d*-fenfluramine and *p*-chloroamphetamine. It should be noted that *p*-chloroamphetamine is not more pharmacologically specific than the new drugs tested here; it also has a high affinity for dopamine and norepinephrine transporters and affects the metabolism of dopaminergic and noradrenergic neurons (Iyer *et al.*, 1994; Fattaccini *et al.*, 1991; McKenna *et al.*, 1991; Huang *et al.*, 1992; Costa *et al.*, 1971). However, in terms of its effects on the secretion of several hormones, *p*-chloroamphetamine appears to be more useful. MMAI, MBDB and MTA may have other effects that will become apparent when the responses of several hormones are compared with one another.

Acknowledgments

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