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Effects of ritanserin on the 3,4-methylenedioxymethamphetamine-induced decrease in striatal serotonin concentration and on the increase in striatal neurotensin and dynorphin A concentrations

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Abstract—The concentration of serotonin (5-HT) measured in rat striatum was reduced to 75% of control 1 week after a single subcutaneous administration of *dl*-3,4-methylenedioxymethamphetamine (MDMA, 20 mg/kg). This decrease was prevented by pretreating the animals with ritanserin. Eighteen hours after MDMA (20 mg/kg), striatal concentrations of neurotensin-like immunoreactivity (NTLI) and of dynorphin A-like immunoreactivity (DLI) were increased to 250 and 487% of control, respectively, but ritanserin failed to prevent these changes. This study supports a role for 5-HT_2 receptors in the mechanism by which a single high dose of MDMA induces neuronal damage to the serotonergic system, but not the MDMA-induced increase in central NTLI and DLI concentrations.

3,4-Methylenedioxymethamphetamine (MDMA*, ecstasy) is a popular drug of abuse capable of inducing neurotoxicity in the central nervous system. A single administration of MDMA produces long-term decreases in the central concentration of serotonin (5-HT), the activity of tryptophan hydroxylase, and the number of 5-HT reuptake sites [1, 2]. Recent evidence suggests that dopamine (DA) released by MDMA mediates these changes [3, 4]. 5-HT may also contribute to the MDMA-induced toxicity by modulating the dopaminergic system. A single dose of MDMA (20 mg/kg) stimulates striatal DA synthesis, which can be blocked with 5-HT_2 receptor antagonists [5, 6]. This blockade of stimulated DA synthesis reduces the amount of DA released by MDMA [7] and prevents the MDMA-induced alterations in the serotonergic system [4, 6]. Interestingly, blockade of 5-HT_2 receptors provides little protection against the decrease in 5-HT induced by multiple MDMA administrations [8]. This suggests that neurochemical alterations induced by a single and multiple injections of MDMA may be governed by different mechanisms.

By releasing DA, MDMA also alters other brain neurotransmitter systems that are regulated by the dopaminergic system. Like methamphetamine, a single administration of MDMA increases striatal and nigral neurotensin and dynorphin A concentrations: these responses are blocked by D_1 receptor antagonists [9]. The elevation in neurotensin and dynorphin A content is not related to the neurotoxic changes induced by amphetamine analogues since their concentration returns to normal within a week [10, 11]. Because these peptide systems are regulated by DA, it is possible that MDMA-induced release of 5-HT also modulates the central peptidergic systems by stimulating DA synthesis via 5-HT_2 receptors. The role of 5-HT_2 receptors in regulating brain transmitter systems is of particular interest in light of reports that ritanserin, a 5-$HT_{2/1c}$ receptor antagonist, may be useful in combating drug addiction [12]. Thus, the purpose of this study was to determine whether ritanserin can prevent the MDMA-induced changes in central neurotensin and dynorphin systems.

Methods and Results

Male Sprague–Dawley rats (Simonsen Laboratories Inc., Gilford, CA) weighing 180–250 g were housed 4–6 per cage in a temperature-controlled room (24°) with a 12-hr alternating light–dark cycle, and allowed access to food and water *ad lib*. In the first experiment, the animals were injected with a single dose of *dl*-MDMA hydrochloride (20 mg/kg acid-free; s.c.) or vehicle (0.9% NaCl) and returned to their cages. Rats treated with ritanserin (1 or 5 mg/kg dissolved in propylene glycol; i.p.) were injected 15 min prior to the administration of MDMA or saline. The animals were killed by decapitation 1 week after treatment and striata were rapidly removed on a cold plate, frozen on dry ice, and stored at −80° until assayed. The concentration of 5-HT was measured by HPLC using electrochemical detection as previously reported [3].

As shown in Fig. 1, striatal 5-HT concentration was reduced to 75% of control 1 week after a single dose of MDMA (20 mg/kg) (5-HT concentrations expressed in μg/g tissue: control, 0.48 ± 0.01; MDMA, 0.36 ± 0.02), and ritanserin administered at a dose of 1 and 5 mg/kg prevented this change. The remaining studies were performed with 1 mg/kg ritanserin since this dosage blocked the long-term

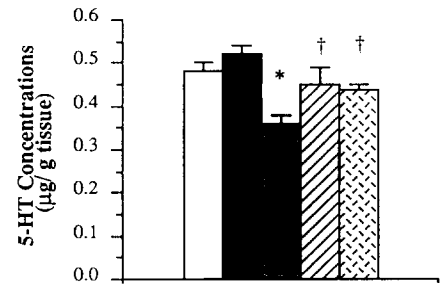

Fig. 1. Effect of ritanserin on the MDMA-induced decrease in striatal 5-HT concentration. Rats were injected with MDMA (20 mg/kg; i.p.) or vehicle (0.9% NaCl) with or without ritanserin (1 or 5 mg/kg; i.p.) and killed 7 days later. The results are expressed as means ± SEM. Each mean represents the average from 6 animals. Columns: (□) control; (■) ritanserin (5 mg/kg). (■) MDMA; (▨) MDMA + ritanserin (1 mg/kg); and (▨) MDMA + ritanserin (5 mg/kg). Statistical analysis was performed with a one-way analysis of variance (ANOVA) followed by a Fisher multiple comparison test. Key: (*) $P < 0.05$ versus control group; and (†) $P < 0.05$ versus MDMA group.

* Abbreviations: DA, dopamine; DLI, dynorphin A-like immunoreactivity; 5-HT, serotonin; MDMA, 3,4-methylenedioxymethamphetamine; and NTLI, neurotensin-like immunoreactivity.

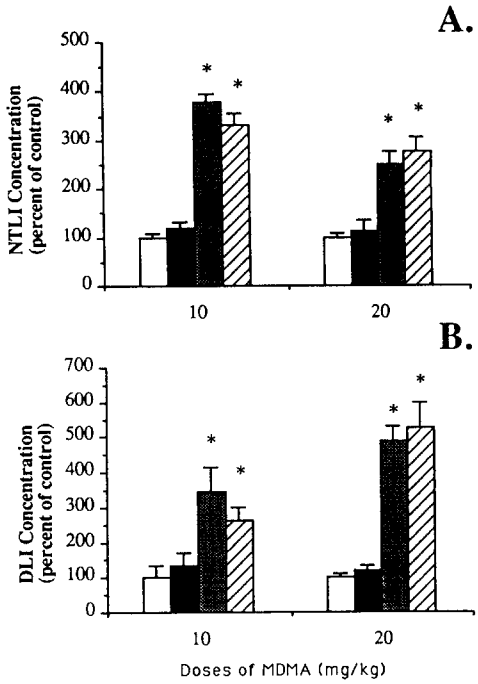

Fig. 2. Effect of ritanserin on the MDMA-induced increase in striatal NTLI (A) and DLI (B) concentrations. The rats were treated as described in Fig. 1 except that tissues were collected 18 hr after drug administration. Means are expressed as a percentage of control (NaCl-treated group) ± SEM. All groups contained 5–6 rats. Concentrations of NTLI and DLI from control animals were 353 and 337 pg/mg protein, respectively. Columns: (□) control; (■) ritanserin (1 mg/kg); (▩) MDMA; and (▨) MDMA + ritanserin (1 mg/kg). Statistical analysis was performed with a one-way analysis of variance followed by a Fisher multiple comparison test. Key: (*) $P < 0.05$ versus control group.

alterations in the serotonergic system induced by MDMA and this dosage is within the biologically effective range of ritanserin [13].

Rats were given MDMA (10 or 20 mg/kg, s.c.) with or without ritanserin (1 mg/kg, i.p.) and the animals were killed 18 hr later. This time was selected in order to measure a maximal increase in the concentration of neurotensin and dynorphin A [10, 14]. The striatal concentrations of neurotensin-like immunoreactivity (NTLI) and dynorphin A-like immunoreactivity (DLI) were measured by radioimmunoassay according to the methods described by Letter *et al.* [14] and Hanson *et al.* [15], respectively.

Figure 2A demonstrates that striatal NTLI concentration was raised to 379% of control 18 hr after the administration of 10 mg/kg MDMA. Pretreatment with ritanserin failed to alter these changes. Since the release of 5-HT stimulates DA synthesis when a dose of 20 mg/kg MDMA is used but not with a 10 mg/kg dose [5], the experiment was repeated with a higher dose of MDMA. As shown in Fig. 2A, a 20 mg/kg dose of MDMA increased NTLI concentration to 250% of control in the striatum but ritanserin again failed to alter these changes.

DLI concentrations were also measured in these same experiments in order to determine if 5-$HT_{2/1c}$ receptors participate in the mechanism by which MDMA alters the concentration of this neuropeptide. As shown in Fig. 2B, 10 and 20 mg/kg of MDMA increased striatal DLI concentration to 346 and 487% of control, respectively, and ritanserin failed to alter these MDMA-induced changes.

Discussion

The increases in striatal NTLI and DLI content induced by a single injection of MDMA are dependent on DA released by this amphetamine analogue [9]. In this study, we examined the possibility that a 5-HT_2 receptor antagonist could alter the MDMA-induced changes in brain neuropeptides because these antagonists block or attenuate (1) the MDMA-induced increase in DA synthesis, (2) the MDMA-induced increase in DA release, and (3) the MDMA-induced decline in central 5-HT concentration [4–7]. The results presented in this study support a role for 5-HT_2 receptors in the mechanism by which a single high dose of MDMA induces neuronal damage to the serotonergic system (Fig. 1), but not in the MDMA-induced increase in central NTLI and DLI concentrations (Fig. 2).

It has been demonstrated that DA mediates the MDMA-induced neurotoxicity in serotonergic nerve terminals [3, 4]. The exact DA-mediated dysfunction leading to neurotoxicity remains unknown but serotonergic and dopaminergic uptake carriers have been implicated since blockers of these transporters prevent the alterations in the serotonergic system [2, 3]. Interestingly, ketanserin, another 5-HT_2 receptor antagonist, completely abolishes the MDMA-induced stimulation of DA synthesis but only partially blocks MDMA-stimulated DA release [5, 7]. Moreover, ritanserin and other 5-HT_2 receptor antagonists prevent the MDMA-induced decrease in 5-HT [Fig. 1; Refs. 4–6], but the toxicity returns if DA synthesis is stimulated with the DA precursor, 3,4-dihydroxyphenylalanine (DOPA) [6]. This suggests that 5-HT_2 receptor antagonists protect the serotonergic system by reducing the amount of DA released by MDMA below the threshold required to induce toxicity. However, the present findings demonstrate that the MDMA-induced increased in striatal NTLI and DLI was not prevented by ritanserin (Fig. 2, A and B). To understand this discrepancy it is critical to note that (1) MDMA stimulates DA synthesis at a dose of 20 mg/kg but not at a dose of 10 mg/kg [5], (2) DA released by a single dose of 10 mg/kg MDMA is not affected by 5-HT_2 receptor antagonists [7], (3) a 20 mg/kg dose of MDMA releases more DA than a dose of 10 mg/kg and DA release is reduced to a level comparable to that seen with 10 mg/kg MDMA in the presence of 5-HT_2 receptor antagonists [7], and (4) a maximal, or near maximal, increase in striatal neuropeptide levels was seen with 10 mg/kg MDMA (Fig. 2). Thus, the results presented in this study indicate that the elevation of striatal NTLI and DLI induced by MDMA is mediated by a pool of DA released selectively by the lower dose of MDMA, which is not regulated by the serotonergic 5-HT_2 receptor.

The large increase in striatal NTLI and DLI induced by the lower dosage of MDMA suggests that these systems respond to lower fluctuations in DA release. On the other hand, the serotonergic neurotoxic effect induced by a single MDMA administration occurs when large quantities of DA are released in an uncontrolled fashion. The observation that ritanserin affected the MDMA-induced decrease in 5-HT, which is the result of abnormal DA release, but not the elevation in neuropeptides, suggests that ritanserin may be useful in preventing the detrimental response induced by an overstimulated dopaminergic system. It is also noteworthy that ritanserin has been reported to reverse the preference of rats for cocaine, indicating that ritanserin may block the rewarding stimulus induced by high abnormal DA stimulation [12].

In brief, this study demonstrates that ritanserin prevented the long-term decrease in central 5-HT concentrations induced by MDMA, but not the temporary increases in striatal concentrations of NTLI and DLI. This difference

suggests that distinct pools of DA may be involved in regulating peptide systems and in mediating neuronal toxicity.

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