EFFECTS OF DOPAMINERGIC AND SEROTONERGIC RECEPTOR BLOCKADE ON NEUROCHEMICAL CHANGES INDUCED BY ACUTE ADMINISTRATION OF METHAMPHETAMINE AND 3,4-METHYLENEDIOXYMETHAMPHETAMINE

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Summary—As dopamine (DA) causes neurochemical changes in the central serotonergic system after an acute injection of methamphetamine, the present study examined the possibility that this response is mediated through dopaminergic receptors. Pretreatment with the DA receptor antagonist, haloperidol, failed to prevent the decreases in the activity of tryptophan hydroxylase and the concentration of serotonin (5-HT) in the frontal cortex, hippocampus and neostriatum 1 hr after a single administration of methamphetamine. Because methamphetamine is also a potent releaser of 5-HT, the possibility that 5-HT receptors mediate the effects of methamphetamine was evaluated. Pretreatment with methiothepin an antagonist of both DA and 5-HT receptors, failed to prevent the decline in activity of tryptophan hydroxylase but did attenuate the decreases in concentrations of 5-HT measured in the frontal cortex and hippocampus. This attenuation is not mediated through 5-HT_2 receptors, as ritanserin failed to interfere with the changes induced by methamphetamine. In addition, DA or 5-HT receptors were apparently not involved in the changes in activity of tryptophan hydroxylase and concentrations of 5-HT induced by another analogue of amphetamine, 3,4-methylenedioxymethamphetamine (MDMA). This study suggests different mechanisms are responsible for the acute and long-term changes observed in the central serotonergic system following a single or multiple doses of methamphetamine.

Key words: methamphetamine, 3,4-methylenedioxymethamphetamine, haloperidol, ritanserin, methiothepin, tryptophan hydroxylase.

The administration of a single large dose of methamphetamine induces a rapid but temporary decrease in the activity of tryptophan hydroxylase and the concentration of serotonin (5-HT) in the CNS of the rat (Bakhit and Gibb, 1981; Peat, Warren, Bakhit and Gibb, 1985). Comparable decreases also occur shortly after acute treatment with amphetamine (Knapp, Mandell and Geyer, 1974), or with other amphetamine-related compounds, such as 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine (MDMA; Schmidt 1987; Stone, Stahl, Hanson and Gibb, 1986). However, multiple large doses of methamphetamine cause qualitatively similar, but quantitatively greater changes in serotonin, which appear to be permanent and reflect the neurotoxicity of this agent (Bakhit, Morgan and Gibb, 1981; Hotchkiss and Gibb, 1980) thus, suggesting that the mechanisms responsible for the neurochemical changes, resulting from acute and multiple doses of this drug, differ.

It is noteworthy that although the effects of acute and multiple doses of methamphetamine on serotonin have some differences, the responses to both types of dose protocols share some characteristics. For example, blockers of the uptake of 5-HT prevent the methamphetamine-induced changes in the serotonergic system in both types of administration of drug (Schmidt and Gibb, 1985a). Moreover, the inhibition of the synthesis of dopamine (DA) with alpha-methyl-*p*-tyrosine prevents the decrease in the activity of tryptophan hydroxylase induced by both drug regimens (Hotchkiss and Gibb, 1980; Schmidt, Ritter, Sonsalla, Hanson and Gibb, 1985; Commins and Seiden, 1986). The role of DA in the mediation of both effects of methamphetamine was confirmed by the observation that destruction of the nigrostriatal dopaminergic pathway, with injections of 6-hydroxydopamine into the nigra, selectively prevented decreases in the activity of tryptophan hydroxylase in the neostriatum, induced by a single (Johnson, Stone, Hanson and Gibb, 1987) or by multiple administrations of this drug (Sonsalla, Gibb and Hanson, 1986a). The precise mechanism by which DA, after single and multiple doses of methamphetamine, alters neurochemical measurements in the serotonergic system remains unclear. It appears that the dopamine D_1 receptor mediates the neurotoxic action of DA released by methamphetamine during the multiple dose treatment, as their blockade with haloperidol and with (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SCH23390) protects the serotonergic system (Hotchkiss and Gibb, 1980; Sonsalla, Gibb and Hanson, 1986b). The purpose of this study was to determine if dopamine receptors also mediate the response induced by a single dose of methamphetamine.

Because methamphetamine and its analog, MDMA, also cause substantial release of 5-HT (Johnson, Hoffman and Nichols, 1986; Schmidt, Levin and Lovenberg, 1987), this study examined the possibility that 5-HT receptors participate in the drug-induced changes in the serotonergic system.

METHODS

Treatment and dissection

Male Sprague–Dawley rats (180–250 g) were housed 6 per cage in a room with controlled lighting (12-hr light/dark cycle) and temperature (24°C). They had access to food and water *ad libitum*. The animals were given a single intraperitoneal injection of either haloperidol (in 1% lactic acid), methiothepin (in 1:1 propyleneglycol:saline) or ritanserin (in 0.01 N HCl) 15 min prior to the subcutaneous injection of either methamphetamine, MDMA or vehicle (in 0.9% saline). Concentrations of drugs are expressed in terms of the free base. Rats were sacrificed by decapitation and the brains were quickly removed. Frontal cortex [corresponding to the pregenual part of the anteromedial cortex described by Emson and Lindvall (1979)], neostriatum and hippocampus were removed on a cold plate, frozen on dry ice and stored at −80°C, until used for evaluation of the monoamine systems.

Determination of parameters of monoamines

Frontal cortex, hippocampus and neostriatum were separately homogenized in 80, 200 and 125 μl, respectively, of 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) containing 0.2% (v/v) Triton X-100 and 5 mM dithiothreitol. After centrifugation at 40,000 ***g*** (4°C) for 15 min, duplicate 7.5-μl aliquots were removed and assayed for the activity of tryptophan hydroxylase by a modified CO_2-trapping procedure as described by Hotchkiss, Morgan and Gibb (1979); *dl*-6-methyl-5,6,7,8-tetrahydrobiopterin was used as cofactor for this assay.

Tissues from the contralateral regions were used to measure the concentrations of DA, 5-HT and their metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) using high-performance liquid chromatography (HPLC) and electrochemical detection (model LC-4B, Bioanalytical Systems Inc), according to a modification of the method described by Nielsen and Moore (1982). Briefly, tissues were weighed, homogenized in 0.3–0.5 ml mobile phase buffer (0.15 M monochloroacetic acid buffer, 2 mM ethylene diamine tetraacetate (EDTA) 0.1 mM 1-octanesulfonic acid sodium salt and 12.5% methanol, pH 2.9) and centrifuged at 4000 ***g*** for 15 min (4°C). The supernatant fractions were filtered with a 0.2-μm Microfilter system (Bioanalytical Systems Inc.) and 50 μl were injected onto a 10-cm Microsorb reverse-phase column (Rainin Instrument Co.). The eluent was monitored using a glassy carbon electrode with the potential set at +0.73 V (vs Ag/AgCl reference electrode). Tissue levels were quantified by comparison with standards of known concentration.

Drugs

The *d*-methamphetamine hydrochloride and *dl*-MDMA hydrochloride were generously supplied by the National Institute on Drug Abuse. The authors are grateful to the following pharmaceutical companies for supplying the other drugs: haloperidol (McNeil Pharmaceutical), methiothepin (Hofmann-LaRoche Inc.) and ritanserin (Janssen Pharmaceutica). The *dl*-6-methyl-5,6,7,8-tetrahydrobiopterin was purchased from Sigma Chemical Co.

Statistical analysis

All results were analyzed with an analysis of variance (ANOVA) test, followed by a Student–Newman–Keul test.

RESULTS

The role of DA receptors in methamphetamine-induced changes in the serotonergic system

Figure 1 shows the effects of blockade of dopamine receptors by haloperidol on the methamphetamine-induced (single dose) changes in the activity of tryptophan hydroxylase and the concentrations of 5-HT and 5-HIAA in the frontal cortex (Fig. 1A), hippocampus (Fig. 1B) and neostriatum (Fig. 1C). The only changes induced by pretreatment with haloperidol alone in the serotonergic system were decreases of 20% in the activity of tryptophan hydroxylase and concentration of 5-HT of the frontal cortex (Fig. 1A). A single dose of methamphetamine induced at least a 40% decrease of the activity of tryptophan hydroxylase in all structures of the brain; these changes were unaltered by pretreatment with haloperidol. Methamphetamine induced a 40–60% decrease in the concentrations of 5-HT and haloperidol had no effect on these decreases, except in the neostriatum where haloperidol enhanced the effect of methamphetamine on the concentration of 5-HT. Either drug alone had a minor effect on the concentrations of 5-HIAA and only a slight increase in the concentration of metabolites in the cortex was observed after the combination of haloperidol and methamphetamine.

The role of 5-HT receptors in methamphetamine-induced changes in the serotonergic system

The responses of serotonergic systems to a single dose of methamphetamine, in the presence of methiothepin, an antagonist of both 5-HT and DA receptors, are shown in Figure 2. Treatment with methiothepin alone did not affect the activity of tryptophan hydroxylase in any of the structures of the brain examined; however, methiothepin decreased the concentration of 5-HT in the neostriatum to 78% of control (Fig. 2C) while increasing the concentrations of 5-HIAA in the cortex and hippo-

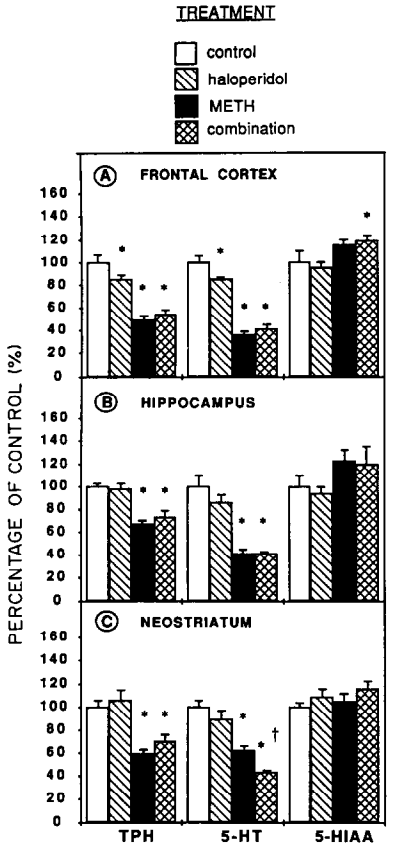

Fig. 1. Effects of haloperidol on the methamphetamine (METH)-induced changes in the serotonergic systems of the frontal cortex (A), hippocampus (B) and neostriatum (C). Haloperidol (3 mg/kg) was administered 15 min before methamphetamine (10 mg/kg) and the animals were sacrificed 1 hr later. Values are the means ± SEM, represented as a percentage of control (n = 5–6 rats). Control values for the activity of tryptophan hydroxylase (in nmol/g tissue/hr) were: frontal cortex (A), 124.4 ± 8.5; hippocampus (B), 74.7 ± 2.2; neostriatum (C), 52.8 ± 3.1. Control values for concentrations of 5-HT (in μg/g tissue) were: frontal cortex (A), 0.66 ± 0.04; hippocampus (B), 0.44 ± 0.04; neostriatum (C), 0.53 ± 0.03. Control values for concentrations of 5-HIAA (in μg/g tissue) were: frontal cortex (A), 0.21 ± 0.02; hippocampus (B), 0.31 ± 0.03; neostriatum (C), 0.44 ± 0.02. *$P < 0.05$ vs corresponding control, †$P < 0.05$ vs corresponding methamphetamine-treated group, by ANOVA analysis, followed by the Student–Newman–Keul test.

campus to 152% (Fig. 2A) and 130% (Fig. 2B) of control, respectively. While a single dose of methamphetamine alone decreased the activity of tryptophan hydroxylase to 60–70% of control in each of the structures of the brain, the presence of methiothepin did not significantly alter these responses to methamphetamine. Treatment with methamphetamine also decreased the concentrations of 5-HT to 40–60% of control in the regions of the brain examined; however, these decreases were significantly attenuated in the frontal cortex and hippocampus by the pretreatment with methiothepin. At this time, treatment with methamphetamine did not affect the concentrations of 5-HIAA except in the frontal cortex where the levels were increased to 130% of control. The presence of methiothepin in the methamphetamine-treated animals increased the concentrations of 5-HIAA to levels similar to those measured in rats treated with methiothepin alone, except in the neostriatum where the levels of 5-HIAA were significantly higher (Fig. 2C). Similar experiments were conducted using a smaller (5 mg/kg) single dose of methamphetamine. Decreasing the dose of methamphetamine did not alter the effects of methiothepin on the methamphetamine-induced decreases in the activity of tryptophan hydroxylase or concentrations of 5-HT (data not shown).

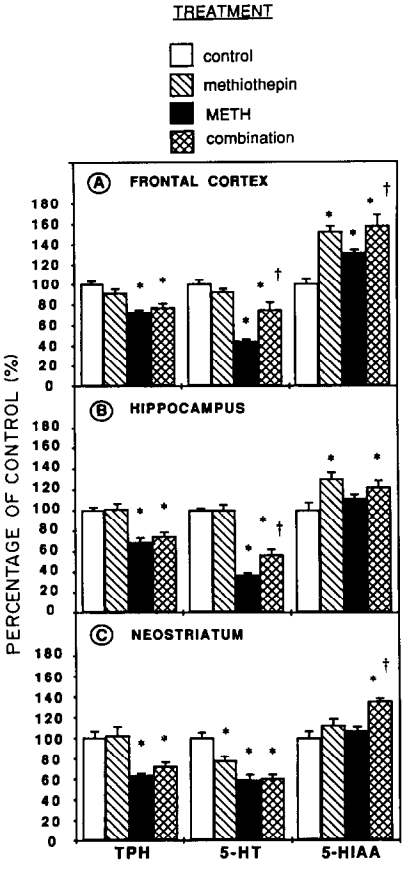

Fig. 2. Effects of methiothepin on the methamphetamine (METH)-induced changes in the serotonergic system of the frontal cortex (A), hippocampus (B) and neostriatum (C). Methiothepin (20 mg/kg) was administered 15 min before methamphetamine (10 mg/kg) and the animals were sacrificed 1 hr later. Values from 2 separate experiments are the means ± SEM, represented as a percentage of control (n = 9–11 rats). Control values for activity of tryptophan hydroxylase (in nmol/g tissue/hr) were: frontal cortex (A), 86 ± 2.9; hippocampus (B), 74.5 ± 2.9; neostriatum (C), 53 ± 3.2. Control values for concentrations of 5-HT (in μg/g tissue) were: frontal cortex (A), 0.61 ± 0.03; hippocampus (B), 0.39 ± 0.01; neostriatum (C), 0.55 ± 0.03. Control values for concentrations of 5-HIAA (in μg/g tissue) were: frontal cortex (A), 0.18 ± 0.01; hippocampus (B), 0.27 ± 0.02; neostriatum (C), 0.47 ± 0.03. *$P < 0.05$ vs corresponding control, †$P < 0.01$ vs corresponding methamphetamine-treated group, by ANOVA analysis, followed by the Student–Newman–Keul test.

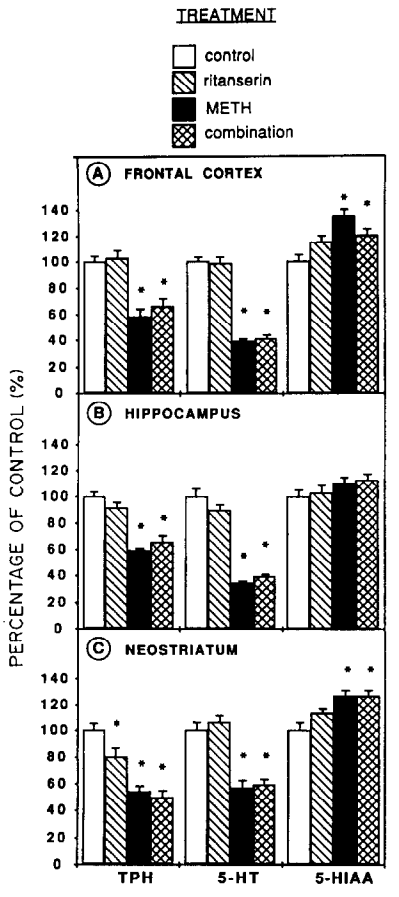

Fig. 3. Effects of ritanserin on the methamphetamine (METH)-induced changes in the serotonergic system of the frontal cortex (A), hippocampus (B) and neostriatum (C). Ritanserin (1 mg/kg) was administered 15 min before methamphetamine (10 mg/kg) and the animals were sacrificed 1 hr later. Values are the means ± SEM, represented as a percentage of control ($n = 5$–6 rats). Control values for activity of tryptophan hydroxylase (in nmol/g tissue/hr) were: frontal cortex (A), 94.8 ± 4.3; hippocampus (B), 75.5 ± 3.5; neostriatum (C), 57.7 ± 3.2. Control values for concentrations of 5-HT (in μg/g tissue) were: frontal cortex (A), 0.56 ± 0.02; hippocampus (B), 0.38 ± 0.03; neostriatum (C), 0.49 ± 0.03. Control values for concentrations of 5-HIAA (in μg/g tissue) were: frontal cortex (A), 0.20 ± 0.01; hippocampus (B), 0.31 ± 0.02; neostriatum (C), 0.48 ± 0.03. *$P < 0.05$ vs corresponding control, by ANOVA analysis, followed by the Student–Newman–Keul test.

The role of 5-HT$_2$ receptors in methamphetamine-induced changes in the serotonergic system

The effects of the specific 5-HT$_2$ receptor blocker, ritanserin, on the changes in serotonin induced by a single administration of methamphetamine were evaluated next (Fig. 3). Treatment with ritanserin alone had no significant effects on the activity of tryptophan hydroxylase or concentrations of 5-HT and 5-HIAA in the brain of the rat, except for a slight decrease in the activity of tryptophan hydroxylase in the neostriatum (Fig. 3C). Methamphetamine-induced changes in the serotonergic system were similar to those shown in the previous figures: the simultaneous administration of ritanserin did not significantly alter the changes related to methamphetamine.

The role of DA and 5-HT receptors in methamphetamine-induced changes in dopamine

To compare the effects of a single dose of methamphetamine on serotonergic systems with the response of dopamine to the same treatment, the concentrations of DA and its principal metabolites in the neostriatum were determined after acute treatment with methamphetamine, with and without haloperidol, methiothepin and ritanserin (Fig. 4). At this time, methamphetamine, by itself, did not significantly alter the concentration of DA, but tended to decrease and increase the concentrations of DOPAC and HVA, respectively. Treatment with haloperidol alone increased the concentrations of DOPAC and HVA while simultaneous treatment with methamphetamine resulted in further increases in these metabolites of DA (Fig. 4A). Methiothepin produced changes in the dopaminergic system similar to those induced by haloperidol (Fig. 4B). In contrast, ritanserin alone had no significant impact on the neostriatal dopaminergic system nor on the methamphetamine-induced decrease in the concentration of DOPAC and increase in HVA (Fig. 4C).

The role of 5-HT receptors in MDMA-induced changes in the serotonergic system

Data presented in Figure 5 demonstrate the effect of blocking DA and 5-HT receptors with methiothepin on the changes in the serotonergic system induced by an acute treatment with MDMA. As shown in Figure 2, methiothepin alone had no significant effect on the activity of tryptophan hydroxylase, significantly decreased the concentration of 5-HT in the neostriatum (Fig. 5C), and significantly increased the concentrations of 5-HIAA in all regions of the brain examined. In contrast to Figure 2, the increase in the concentration of 5-HIAA in the neostriatum was significant in the experiment, but as in Figure 2, the greatest increase in this metabolite occurred in the frontal cortex (Fig. 5A), followed by the hippocampus (Fig. 5B) while the smallest effect was in the neostriatum. A single administration of MDMA induced a 20–30% decrease in the activity of tryptophan hydroxylase neostriatum and hippocampus as well as a decrease in the concentrations of 5-HT. The pretreatment with methiothepin did not significantly alter MDMA-induced decreases in activity of tryptophan hydroxylase or concentrations of 5-HT, whereas, the levels of 5-HIAA approached the concentrations measured after treatment with methiothepin alone.

Effect of 5-HT receptor blockade on the recovery of changes in serotonergic systems induced by amphetamine-like drugs

In order to determine if blockade of 5-HT recep-

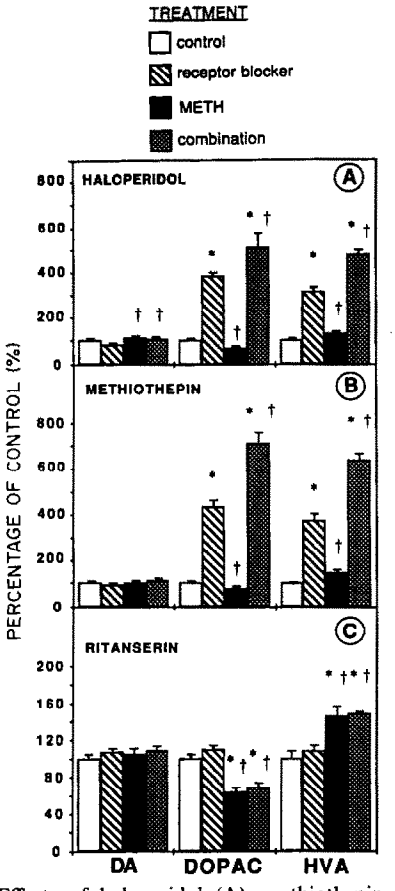

Fig. 4. Effects of haloperidol (A), methiothepin (B), or ritanserin (C) on the methamphetamine (METH)-induced changes in the neostriatal dopaminergic system. Haloperidol (3 mg/kg) (A), methiothepin (20 mg/kg) (B) and ritanserin (1 mg/kg) (C) were administered 15 min before methamphetamine (10 mg/kg) and the animals were sacrificed 1 hr later. Values are the means ± SEM represented as a percentage of control ($n = 5$–11 rats). Control values for concentrations of DA (in μg/g tissue) were: haloperidol (A), 10.7 ± 0.5; methiothepin (B), 9.8 ± 0.7; ritanserin (C), 8.9 ± 0.3. Control values for concentrations of DOPAC (in μg/g tissue) were: haloperidol (A), 0.82 ± 0.03; methiothepin (B), 0.69 ± 0.05; ritanserin (C), 0.72 ± 0.04. Control values for concentrations of HVA (in μg/g tissue) were: haloperidol (A), 0.74 ± 0.05; methiothepin (B), 0.59 ± 0.04; ritanserin (C), 0.66 ± 0.05. *$P < 0.01$ vs corresponding control, †$P < 0.05$ vs corresponding receptor blocker, by ANOVA analysis followed by the Student–Newman–Keul test.

tors would alter the recovery of the activity of tryptophan hydroxylase from treatment with methamphetamine or MDMA, activity of tryptophan hydroxylase was measured in rats sacrificed 1, 3 and 6 hr after either a single dose of methamphetamine (Fig. 6A) or MDMA (Fig. 6B), with and without pretreatment with methiothepin. At the 3- and 6-hr periods, treatment with methiothepin alone tended to increase activity of tryptophan hydroxylase; however pretreatment with methiothepin did not significantly alter methamphetamine- or MDMA-induced decreased activity of tryptophan hydroxylase at these times.

DISCUSSION

The purpose of this study was to determine whether DA antagonists, which block the neurotoxic effects of methamphetamine in the dopaminergic and serotonergic systems, also prevented the acute methamphetamine-induced decrease in central activity of tryptophan hydroxylase and concentration of 5-HT. The dopaminergic or serotonergic receptor blockers used in this study did not alter significantly the neurochemical responses of the serotonergic system to a single administration of either methamphetamine or MDMA. These findings suggest that DA and 5-HT receptors do not play a major role in mediating the changes in serotonergic systems induced by acute treatments with analogs of am-

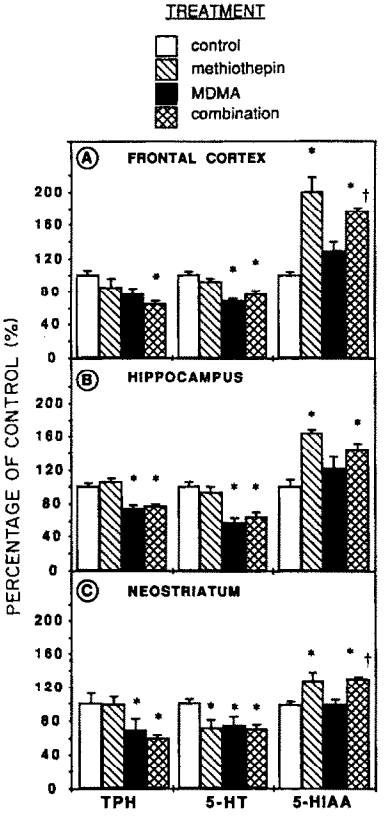

Fig. 5. Effects of methiothepin on the MDMA-induced changes in the serotonergic system of the frontal cortex (A), hippocampus (B) and neostriatum (C). Methiothepin (20 mg/kg) was administered 15 min before MDMA (5 mg/kg) and the animals were sacrificed 1 hr later. Values are the means ± SEM, represented as a percentage of control ($n = 5$–6 rats). Control values for activity of tryptophan hydroxylase (in nmol/g tissue/hr) were: frontal cortex (A), 90.4 ± 5.6; hippocampus (B), 79.5 ± 2.9; neostriatum (C), 62.5 ± 5.6. Control values for concentrations of 5-HT (in μg/g tissue) were: frontal cortex (A), 0.52 ± 0.02; hippocampus (B), 0.43 ± 0.02; neostriatum (C), 0.58 ± 0.03. Control values for concentrations of 5-HIAA (in μg/g tissue) were: frontal cortex (A), 0.17 ± 0.01; hippocampus (B), 0.23 ± 0.02; neostriatum (C), 0.41 ± 0.01. *$P < 0.05$ vs corresponding control, †$P < 0.01$ vs corresponding methamphetamine-treated group, by ANOVA analysis followed by the Student–Newman–Keul test.

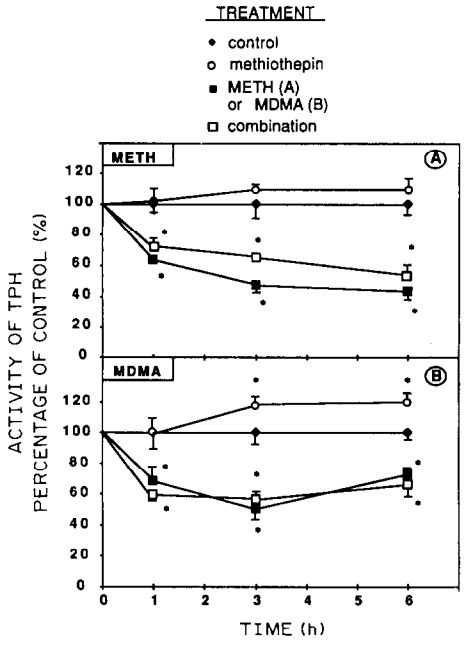

Fig. 6. Effects pretreatment with methiothepin on the methamphetamine (METH)- (A) or MDMA- (B) induced changes in neostriatal activity of tryptophan hydroxylase 1, 3 and 6 hr after the administration of methamphetamine or MDMA. Methiothepin (20 mg/kg) was administered 15 min before methamphetamine (10 mg/kg) or MDMA (5 mg/kg) administration. Values are the means ± SEM, represented as a percentage of control ($n = 3$–11 rats). Control values for activity of tryptophan hydroxylase at the 1 hr time (in nmol/g tissue per hr) are presented in the legend of Fig. 2 for methamphetamine (A), and in the legend of Fig. 5 for MDMA (B). $^*P < 0.05$ vs corresponding control.

phetamine. Because blockade of DA receptors by haloperidol prevented the actions of multiple doses of methamphetamine on the serotonergic system (Hotchkiss and Gibb, 1980), the present data demonstrate that the acute effects of methamphetamine are distinct from those resulting from multiple administrations of this drug. As multiple doses of methamphetamine (but not shortly after a single dose) induce long-lasting degeneration of serotonergic neurons (Hotchkiss and Gibb, 1980; Ricaurte, Schuster and Seiden, 1980; Commins and Seiden, 1986), the present results suggest that haloperidol can prevent only the changes associated with neurotoxicity.

The lack of involvement of DA receptors in the mediation of the acute serotonergic response to a single dose of methamphetamine was demonstrated by the inability of haloperidol or methiothepin to affect this response. These two DA receptor antagonists are potent neuroleptics which increase the turnover of DA (Lloyd and Bartholini, 1974; Zivkovic, Guidotti, Revuelta and Costa, 1975) as shown in Figure 4. Because these findings suggest that DA mediates the acute effects of methamphetamine without interacting with its receptor, its action is perhaps produced after entering into the serotonergic terminals through the uptake carrier for 5-HT. The participation of this carrier is necessary for the development of the acute effects of methamphetamine (Schmidt and Gibb, 1985a). This uptake of DA would cause the release of 5-HT (Schmidt and Lovenberg, 1985), which in turn could activate serotonergic receptors, leading to some of the acute changes induced by methamphetamine. It is likely that the blockers of the uptake carrier for 5-HT prevent the acute effects of methamphetamine by preventing the entry or exit of a substance, other than amphetamine-like drugs, as these drugs appear to diffuse freely into neuronal terminals (Fuller and Snoddy, 1979; Schmidt and Gibb, 1985b; Schmidt *et al.*, 1987).

In order to test if 5-HT receptors played a role in the acute effects of methamphetamine, these receptors were blocked with methiothepin prior to the treatment with methamphetamine. Radioligand binding studies indicate the existence of several binding sites for 5-HT (Peroutka and Snyder, 1979; Peroutka, 1986). Methiothepin acts as an antagonist for 5-HT_1 and 5-HT_2 receptors (Hibert and Middlemiss, 1986). The lack of an effect by methiothepin on the response of tryptophan hydroxylase to acute treatment with methamphetamine and MDMA suggests that 5-HT receptors do not participate in this response. In support of this conclusion, it was also found that the specific 5-HT_2 antagonist, ritanserin (Leysen, Gommeren, Van Gompel, Wynants, Janssen and Laduron, 1985), had no effect on methamphetamine-induced alterations in any of the neurochemical parameters of the serotonergic system (Fig. 3). This lack of effect of these 5-HT antagonists on methamphetamine- and MDMA-mediated changes in the activity of tryptophan hydroxylase was somewhat unexpected as Ross and Frödén (1977) reported that the 5-HT antagonist, metergoline, protected this enzyme in the cortex from the effects of *p*-chloroamphetamine (PCA), an analog of amphetamine which produces alterations similar to those of MDMA (Stone *et al.*, 1986). As this protection was measured 4 hr after treatment with the drug, the possibility that the blockade of the 5-HT receptors could induce a faster recovery of the activity of the enzyme rather than a blockade of the initial decline was investigated. However, no influence by methiothepin on the effects of either methamphetamine or MDMA on the serotonergic system was observed up to 6 hr after acute treatment with drug (Fig. 6). This indicates that the mechanism by which PCA produces the decrease in central activity of tryptophan hydroxylase differs from that of methamphetamine and MDMA, or that the protection of the activity of the enzyme by metergoline observed by Ross and Frödén (1977) is not related to the 5-HT receptors.

It is noteworthy that while methiothepin did not alter the changes in activity of tryptophan hydroxylase due to acute administrations of methamphetamine and MDMA, it did attenuate the

methamphetamine-induced decreases in concentrations of 5-HT in the cortex and hippocampus (Fig. 2). The mechanism of this attenuation is not clear, although the lack of an effect by haloperidol or ritanserin under similar conditions suggests that the effects were not mediated by DA or 5-HT_2 receptors. It might be that this effect of methiothepin is not due to the action of this compound on 5-HT receptors but to the blockade of the uptake carrier for 5-HT; however, the fact that methiothepin did not block the methamphetamine-induced changes in the activity of tryptophan hydroxylase argue against the possibility. Methiothepin had no effect on the MDMA-related decline in the concentrations of 5-HT (Fig. 5): the discrepancy between treatment with methamphetamine and MDMA could result from the difference in doses, as methiothepin did not alter the effects of a smaller dose of methamphetamine (5 mg/kg; data not shown), while producing an attenuation of the decrease induced by MDMA when administered at 10 mg/kg (Schmidt and Taylor, 1987).

The 5-HT autoreceptors are reported to control the rate of release of 5-HT from serotonergic nerve terminals (Pettibone and Pflueger, 1984; Middlemiss, 1985; Moret, 1985; Hibert and Middlemiss, 1986) and the firing rate of the serotonergic cells (Sprouse and Aghajanian, 1987). Consequently, the blockade of the 5-HT autoreceptors with methiothepin increases the release of 5-HT from cortical preparations (Middlemiss, 1984), which could account for the elevation of concentrations of 5-HIAA observed in the frontal cortex and hippocampus after the administration of this agent (Figs 2 and 5). However, these changes in the concentrations of 5-HIAA occurred in the same structures of the brain where methiothepin attenuated methamphetamine-induced decreases in concentrations of 5-HT. This suggests that in these regions of the brain, 5-HT autoreceptors contribute to the decline in levels of 5-HT caused by methamphetamine, although postsynaptic receptors might also be involved.

The decrease and increase observed in the concentrations of DOPAC and HVA respectively, in the neostriatum after administration of amphetamine, have been reported previously (Roffler-Tarlov, Sharman and Tegerdine, 1971). Although the increase in the concentration of HVA can be explained by the ability of methamphetamine to release DA (Schmidt *et al.*, 1987), it is yet unclear why methamphetamine caused a decrease in the concentration of DOPAC. The ability of methamphetamine to inhibit the activity of monoamine oxidase (Fellows and Bernheim, 1950) and to stimulate the transport of DOPAC out of the brain (Westerink and Kikkert, 1986), suggests that the decrease in the concentration of DOPAC might result from a variety of factors. As reported in previous studies, an increase in the concentrations of DOPAC and HVA in the neostriatum was observed after the treatment with haloperidol or methiothepin, probably due to an increase in the turnover of DA perhaps mediated by a compensatory feedback system in response to blockade DA receptors (Lloyd and Bartholini, 1974; Wilk, Watson and Stanley, 1975; Zivkovic *et al.*, 1975). However, while acute treatment with methamphetamine alone induced a decrease or increase in the concentrations of DOPAC and HVA, respectively, simultaneous administration of haloperidol or methiothepin resulted in substantial increases in the levels of DOPAC and HVA above those which occurred after treatment with the receptor antagonists alone. This observation is similar to the report by Sonsalla *et al.* (1986b), that the simultaneous administration of multiple doses of methamphetamine, together with the specific D_2 antagonist, sulpiride, substantially enhanced the effect of sulpiride to elevate the concentrations of DOPAC and HVA in the striatum. A comparison of these findings with those of Sonsalla *et al.* (1986b) suggests that there is a similarity between the response of the DA system to acute and multiple administrations of methamphetamine and that the effects of a haloperidol and methiothepin on the levels of DOPAC and HVA are mediated by D_2 receptors.

In summary, this study demonstrates that dopaminergic and serotonergic receptors do not play a major role in mediating methamphetamine- and MDMA-induced changes in the 5-HT system after an acute treatment with these agents. This is in contrast to the neurotoxic responses of the serotonergic system to multiple doses of methamphetamine, where DA receptors are important. Thus, the mechanisms whereby DA contributes to the decrease in activity of tryptophan hydroxylase and in concentrations of 5-HT induced by an acute treatment with methamphetamine remains unclear. If DA does not produce its action by interacting with its receptor, it is then possible that DA must be taken up into the serotonergic neurons by the uptake carrier for 5-HT and mediates its effects at an intraneuronal site.

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