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Effect of Flunarizine and Nimodipine on the Decrease in Tryptophan Hydroxylase Activity Induced by Methamphetamine and 3,4-Methylenedioxymethamphetamine[1]

MICHEL JOHNSON, KATARZYNA MITROS, DONNA M. STONE,[2] RAY ZOBRIST, GLEN R. HANSON and JAMES W. GIBB

Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah (M.J., K.M., D.M.S., G.R.H., J.W.G.) and Marion Merrell Dow Inc., Cincinnati, Ohio (R.Z.)

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ABSTRACT

The effect of calcium channel blockers on the decrease in central tryptophan hydroxylase (TPH) activity and serotonin (5-HT) concentration induced by repeated large doses of methamphetamine (METH) or 3,4-methylenedioxymethamphetamine (MDMA) was evaluated. Rats received four or five injections of METH (10 or 15 mg/kg) or MDMA (10 mg/kg) at 6-h intervals, and were sacrificed 18 to 20 h or 1 week after the last administration. Flunarizine (30 mg/kg) prevented the decline in cortical and neostriatal TPH activity induced by MDMA, but failed to alter the effect of METH. The effect of flunarizine on the METH- and MDMA-induced changes in cortical 5-HT and 5-hydroxyindoleacetic acid concentrations paralleled the changes in enzyme activity. Nimodipine, diltiazem or TA-3090 failed to prevent the MDMA- and the METH-induced decline in TPH activity or in 5-HT and 5-hydroxyindoleacetic acid content. Because haloperidol failed to mimic the protective action of flunarizine, it is unlikely that flunarizine exerts its action by blocking the dopamine D-2 receptors. This study suggests that calcium influx may participate in the MDMA-induced decline in central TPH activity, and that the mechanism by which MDMA and METH decreases TPH activity differs.

Repeated administration of large doses of METH or MDMA destroys serotonergic nerve terminals (Commins *et al.*, 1987; O'Hearn *et al.*, 1988), which is reflected by a long-lasting decline in central TPH activity, in the concentration of 5-HT and 5-HIAA (Hotchkiss and Gibb, 1980; Stone *et al.*, 1987) and in the number of 5-HT uptake sites (Battaglia *et al.*, 1987). The mechanism mediating these changes remains unknown, although several findings indicate that central DA participates in this response. For instance, depletion of this transmitter attenuates the decrease in TPH activity after treatment with either drug (Hotchkiss and Gibb, 1980; Stone *et al.*, 1988), whereas blocking the D_1 dopamine receptor with SCH 23390 attenuates the METH-induced changes in the serotonergic system (Sonsalla *et al.*, 1986).

Recent evidence suggests that excitatory amino acids contribute to the METH-induced changes in the dopaminergic and serotonergic systems by stimulating NMDA receptors. Sonsalla *et al.* (1989) reported that the noncompetitive NMDA receptor antagonist, MK-801, as well as other glutamate receptor antagonists protect the mouse neostriatal dopaminergic system from neuronal degeneration induced by METH. We also reported that MK-801 attenuates the METH effects on the serotonergic system in rat neostriatum, but observed that blockade of the NMDA receptors did not alter the MDMA-induced changes (Johnson *et al.*, 1989).

The NMDA receptor is a subclass of the glutamate receptor family linked to a calcium channel which, upon stimulation, allows calcium influx into the neuronal cell (MacDermott and Dale, 1987). Calcium overload is detrimental to neurons and is thought to contribute to ischemia-related damage (Siesjö, 1986). Interestingly, both ischemia-induced neuronal death and METH-induced changes in the serotonergic system are prevented by treatment with MK-801 or by inhibiting DA synthesis with α-methyl-*p*-tyrosine (Clemens and Phebus, 1988; Hotchkiss and Gibb, 1980; Simon *et al.*, 1984; Weinberger *et al.*, 1985). These observations suggest that similar mechanisms involving calcium and DA might be responsible for these two degenerative processes.

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[2] Current address: The Burke Rehabilitation Center, Laboratory of molecular neurobiology of the Cornell University Medical Center, 785 Marmaroneck Ave, White Plains, NY 10605.

ABBREVIATIONS: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high-performance liquid chromatography; 5-HT, serotonin; HVA, homovanillic acid; METH, methamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; NMDA, N-methyl-D-aspartate; TPH, tryptophan hydroxylase.

Calcium enters neuronal cells through three types of voltage-dependent calcium channels (Tsien *et al.*, 1988). Nimodipine, an L-type calcium channel antagonist, sometimes protects neuronal damage from ischemia (Scriabine *et al.*, 1989; Van Reempts *et al.*, 1986). Flunarizine, a calcium overload blocker (Holmes *et al.*, 1984), also protects neuronal cells from ischemic damage (Poignet *et al.*, 1989). Thus, blocking calcium influx through non-NMDA calcium channels can provide protection where excitatory amino acids are believed to induce neuronal damage. The possibility that flunarizine and nimodipine prevent the changes induced by METH and MDMA in the central serotonergic system is examined in this study.

Methods

Animals. Male Sprague-Dawley rats (Simonsen Laboratories Inc., Gilroy, CA) weighing 180 to 250 g were housed four to six per cage in a temperature-controlled room (24°C) with a 12-h alternating light/dark cycle, and access to food and water *ad libitum*.

Drugs. *dl*-MDMA hydrochloride and *d*-METH hydrochloride were kindly donated by the National Institute on Drug Abuse. Flunarizine dihydrochloride, nimodipine and haloperidol were purchased from Sigma Chemical Co. (St. Louis, MO), and diltiazem hydrochloride and TA 3090 were kindly donated by Marion Merrell Dow Inc. METH, MDMA, diltiazem and TA 3090 were dissolved in 0.9% NaCl, haloperidol in 1% lactic acid, flunarizine and nimodipine in propylene glycol.

Experimental procedures. The rats received four or five doses of METH (10 or 15 mg/kg, s.c.), MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-h intervals. The animals receiving the calcium antagonists were administered either flunarizine (30 mg/kg, i.p.), nimodipine (1 or 5 mg/kg, i.p.), diltiazem (50 mg/kg, i.p.), TA 3090 (20 mg/kg, i.p.) or haloperidol (2 mg/kg, i.p.) 15 min before the injection of METH, MDMA or vehicle (drug concentrations are expressed as the free base). The animals were sacrificed by decapitation 18 to 20 h or 1 week after the last drug treatment, and the frontal cortex (corresponding to the pregenual part of the anteromedial cortex described by Emson and Lindvall, 1979) and neostriatum were rapidly removed on a cold plate; all tissues were stored at −80°C until assayed.

Assay. TPH activity was assayed by a modified $^{14}CO_2$ trapping procedure (Stone *et al.*, 1987) using *dl*-6-methyl-5,6,7,8-tetrahydrobiopterin (Sigma Chemical Co.) as cofactor. Tissues contralateral to those used for TPH activity were assayed for 5-HT and 5-HIAA content by HPLC using an amperometric electrochemical detector as previously described (Stone *et al.*, 1987). The same procedure was used to measure the concentrations of DA and its metabolites, DOPAC and HVA, in the neostriatum.

Statistical analysis. The data were analyzed using a one-way analysis of variance. The differences between the means were considered significant at $P < .05$ following a Scheffe F test. Student's t test was used to analyze the results from figure 5.

Results

Effects of flunarizine on the METH- and MDMA-induced changes in TPH activity. The treatment with METH reduced cortical (fig. 1A) and neostriatal (fig. 1B) TPH activity to 53 and 64% of their respective controls 18 h after the last drug administration, whereas the treatment with MDMA reduced TPH activity in these brain structures to 31 and 38% of control, respectively. The administration of flunarizine (30 mg/kg, i.p.) 15 min before each injection of METH failed to alter significantly the METH-induced decline in TPH activity, although a trend toward a greater decline in TPH activity appeared in all brain structures surveyed after the drug combination. In contrast, partial protection against the MDMA-induced decline in TPH activity was observed in flunarizine-pretreated animals as cortical and neostriatal TPH activity were reduced to only 67 and 78% of their respective controls. Flunarizine alone had no significant effect on central TPH activity.

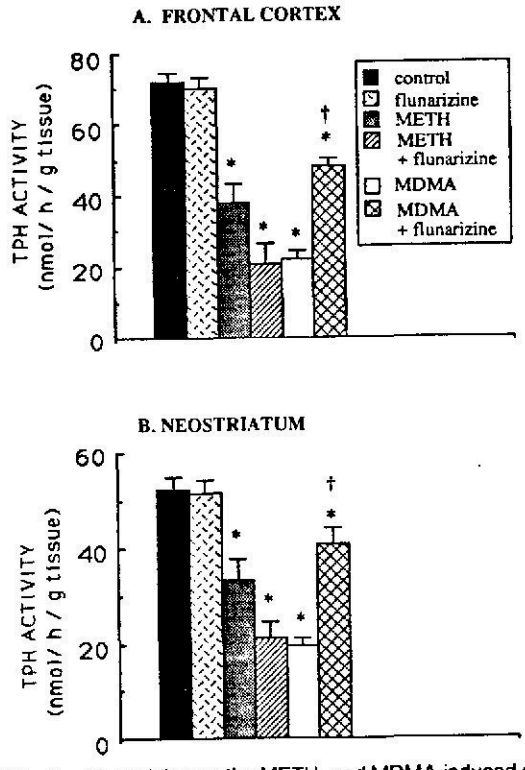

Fig. 1. Effects of flunarizine on the METH- and MDMA-induced decrease in TPH activity measured in the frontal cortex (A) and in the neostriatum (B). The rats received four doses of METH (15 mg/kg, s.c.), MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-h intervals. The flunarizine-treated animals received a dose of 30 mg/kg i.p. 15 min before the administration of METH, MDMA or vehicle. The animals were sacrificed by decapitation 18 to 20 h after the last drug administration. Means ± S.E. of TPH activity are expressed as nmol/h/g of tissue (n = 5–10 per group). Statistical analyses were performed with a one-way analysis of variance followed by a Scheffe multiple comparison test. *$P < .05$ *vs.* control group; †$P < .05$ *vs.* respective MDMA-treated group.

Flunarizine altered the METH- and MDMA-induced changes in cortical 5-HT and 5-HIAA concentrations in a manner similar to cortical TPH activity (fig. 2). Flunarizine blocked the MDMA-induced decline in 5-HT (fig. 2A) and 5-HIAA (fig. 2B) while having no significant effect on the METH-induced decline in 5-HT concentration.

Flunarizine blocked the MDMA-induced decline in TPH activity and in the concentrations of 5-HT and 5-HIAA when measured 1 week after the drug treatment (fig. 3). MDMA reduced cortical and neostriatal TPH activity to 33 and 35% of control, respectively (fig. 3, A and B). Cortical TPH activity measured in the MDMA-treated animals receiving flunarizine was not different than control (fig. 3A), whereas neostriatal TPH activity was attenuated to only 65% of control (fig. 3B). The decline in 5-HT and 5-HIAA concentrations measured in both brain structures of MDMA-treated animals was prevented by flunarizine (fig. 3, A and B). The flunarizine treatment alone significantly increased neostriatal TPH activity (fig. 3B).

Effects of nimodipine on the METH- and MDMA-induced changes in TPH activity. The administration of nimodipine 15 min before each of the five injections of METH or MDMA failed to alter the decline in neostriatal TPH activity

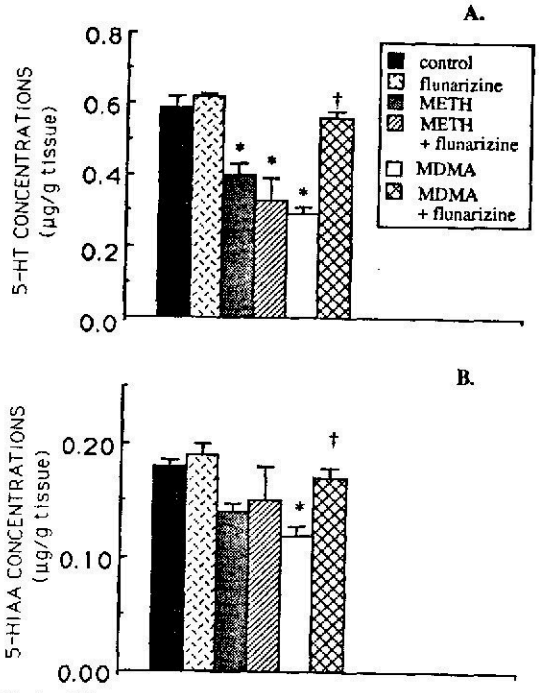

Fig. 2. Effects of flunarizine on the METH- and MDMA-induced decrease in 5-HT (A) and 5-HIAA (B) concentrations measured in the frontal cortex. The animals were treated as described in figure 1. Results are expressed in μg/g of tissue and represent means ± S.E. (n = 5–10 per group). Statistical analyses were performed with a one-way analysis of variance followed by a Scheffe multiple comparison test. *P < .05 *vs.* control group; †P < .05 *vs.* respective MDMA-treated group.

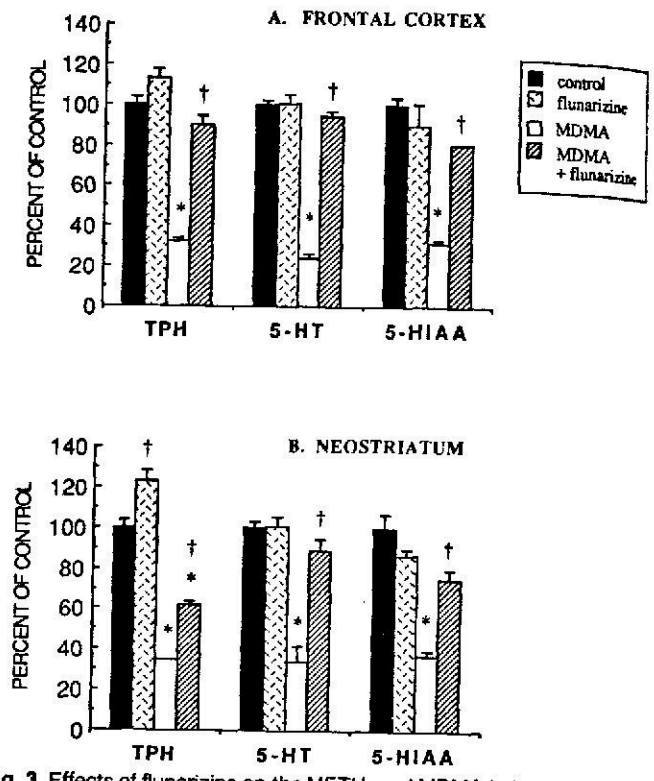

Fig. 3. Effects of flunarizine on the METH- and MDMA-induced decrease in TPH activity, in 5-HT and 5-HIAA concentrations measured in the frontal cortex (A) and in the neostriatum (B) after 1 week. The rats received five doses of MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-h intervals. Animals received flunarizine (30 mg/kg, i.p.) 15 min before the administration of MDMA or vehicle. The animals were sacrificed by decapitation 1 week later. The results are expressed as percent of their respective control (means ± S.E.; n = 3–6 per group). The control values for TPH activity in the frontal cortex (A) and in the neostriatum (B) were 49.2 ± 1.8 and 37.7 ± 1.5 nmol/h/g of tissue (n = 6), respectively. The control values for 5-HT concentrations in the frontal cortex (A) and in the neostriatum (B) were 0.63 ± 0.01 and 0.57 ± 0.02 μg/g of tissue (n = 6), respectively. The control values for 5-HIAA concentrations in the frontal cortex (A) and in the neostriatum (B) were 0.18 ± 0.01 and 0.42 ± 0.03 μg/g of tissue (n = 6), respectively. Statistical analyses were performed with a one-way analysis of variance followed by a Scheffe multiple comparison test. *P < .05 *vs.* control group; †P < .05 *vs.* respective MDMA-treated group.

measured 18 h after the last drug administration (table 1). METH and MDMA reduced neostriatal TPH activity to 36 and 21% of control, respectively, whereas cortical TPH activity was reduced to 13 and 19% of their respective controls. A 1 mg/kg dose of nimodipine was the highest dose tolerated by the METH-treated animals. A 5 mg/kg dose of nimodipine was given to MDMA-treated animals. Nimodipine failed to alter the decline in TPH activity measured 18 h after MDMA or METH. Although not significant, the METH-treated group receiving nimodipine appeared to have a lower TPH activity than the animals treated with METH alone. Cortical 5-HT and 5-HIAA concentrations were affected in a similar fashion as TPH activity by the various drug treatments.

Effects of diltiazem and TA 3090 on the METH- and MDMA-induced changes in neostriatal TPH activity. The administration of diltiazem 15 min before each of the four injections of METH or MDMA failed to alter the decline in neostriatal TPH activity measured 18 h after the last drug administration (fig. 4A). As with flunarizine and nimodipine, the administration of diltiazem with METH resulted in a greater decline in TPH activity than the administration of METH alone.

In order to rule out the possibility that diltiazem failed to block the METH- or MDMA-induced changes due to its poor ability to cross the blood-brain barrier, we repeated the above experiment by replacing diltiazem with its 8-chloro analog, TA-3090. In this experiment, the concentration of METH was reduced to 10 mg/kg in order to avoid animal mortality. Under these conditions, METH and MDMA reduced striatal TPH activity to 68 and 27% of control, respectively (fig. 4B). As described for diltiazem, TA-3090 failed to prevent these alterations. Moreover, the combined treatment with METH and TA-3090 significantly potentiated the METH-induced decline in enzyme activity, whereas TPH activity measured in the animals treated with MDMA and TA-3090 remained similar to the activity measured in MDMA-treated animals.

Role of DA D-2 receptors in mediating the flunarizine effect. Because flunarizine blocks DA D-2 receptors (Fadda *et al.*, 1989; Govoni *et al.*, 1988), we considered the possibility that flunarizine (30 mg/kg) could be preventing the MDMA-induced changes by its action on these receptors.

The antagonist properties of flunarizine on the D-2 autoreceptor is supported by the ability of a single dose of flunarizine (1 to 60 mg/kg) to increase neostriatal DOPAC (117–275% of control) and HVA (115–300% of control) concentrations 3 h after administration (fig. 5). DA concentrations remained unaltered except for a small increase observed at a 1 mg/kg dose of flunarizine.

If flunarizine attenuated the MDMA-induced changes by blocking D-2 receptors, other D-2 antagonists should have a similar effect. This possibility was tested by determining the

TABLE 1

Effects of nimodipine on the MDMA- and METH-induced decrease in cortical and neostriatal TPH activity and on cortical 5-HT and 5-HIAA concentrations

The animals were administered four doses of MDMA or METH at 6-h intervals and sacrificed 18 h after the last dose. Nimodipine was administered 15 min before MDMA or METH. The percent of the respective controls is shown in parentheses and the number of animals per group is shown in the treatment column.

Treatments	Doses	Neostriatum	Frontal Cortex		
		TPH	TPH	5-HT	5-HIAA
	mg/kg/dose	nmol/h/g tissue	nmol/h/g tissue	μg/g tissue	μg/g tissue
Control (n = 6)		33.0 ± 2.6	59.6 ± 2.5	0.66 ± 0.03	0.24 ± 0.01
		(100 ± 7.9)	(100 ± 4.2)	(100 ± 4.6)	(100 ± 4.2)
Nimodipine (n = 6)	5	41.9 ± 2.7	57.9 ± 2.5	0.72 ± 0.02	0.27 ± 0.02
		(127.0 ± 8.2)	(97.2 ± 4.2)	(109.1 ± 3.0)	(112.5 ± 8.3)
MDMA (n = 6)	10	6.9 ± 0.9*	11.3 ± 1.2*	0.14 ± 0.01*	0.08 ± 0.01*
		(20.9 ± 2.7)	(19.0 ± 2.0)	(21.2 ± 1.5)	(33.3 ± 4.2)
MDMA + nimodipine (n = 6)	10 + 5	11.2 ± 3.2*	8.2 ± 1.2*	0.10 ± 0.01*	0.06 ± 0.01*
		(33.9 ± 9.7)	(13.8 ± 2.0)	(15.2 ± 1.5)	(25.0 ± 4.2)
METH (n = 8)	15	11.9 ± 4.2*	7.6 ± 2.1*	0.13 ± 0.03*	0.08 ± 0.01*
		(36.1 ± 12.7)	(12.8 ± 3.5)	(19.7 ± 4.6)	(33.3 ± 4.2)
METH + nimodipine (n = 3)	15 + 1	0.5 ± 0.3*	1.3 ± 0.6*	0.02 ± 0.01*	0.02 ± 0.01*
		(1.5 ± 0.9)	(2.2 ± 1.0)	(3.0 ± 1.5)	(8.3 ± 4.2)

*$P < .05$ vs. control.

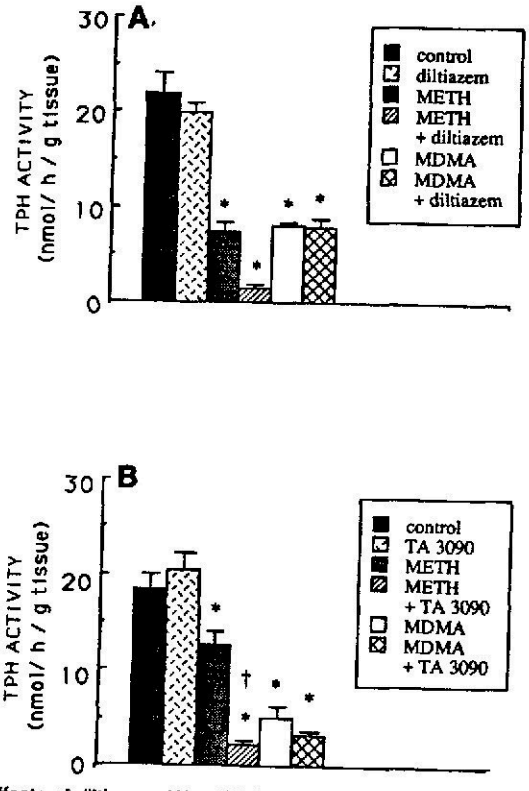

Fig. 4. Effects of diltiazem (A) or TA 3090 (B) on the METH- and MDMA-induced decrease in neostriatal TPH activity. In figure 4A, the rats received four doses of METH (15 mg/kg, s.c.), MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-h intervals. Diltiazem (50 mg/kg, i.p.) was injected 15 min before the administration of METH, MDMA or vehicle. In figure 4B, the rats were treated as described above, except that a 10 mg/kg dose of METH was used. TA 3090 (20 mg/kg, i.p.) was injected 15 min before the administration of METH, MDMA or vehicle. The animals were sacrificed by decapitation 18 to 20 h after the last drug administration. Means ± S.E. of TPH activity are expressed as nmol/h/g of tissue (n = 6 per group). Statistical analyses were performed with a one-way analysis of variance followed by a Scheffe multiple comparison test. *$P < .05$ vs. control group; †$P < .05$ vs. METH group.

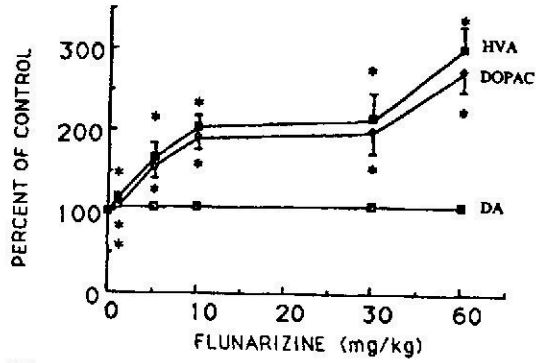

Fig. 5. Effects of different doses of flunarizine (1–60 mg/kg, i.p.) on neostriatal DA, DOPAC and HVA concentrations. The rats received a single administration of flunarizine and were sacrificed 3 h later. The results are expressed as percent of their respective control (means ± S.E.; n = 5–6 per group). The control values expressed in μg/g tissue were: DA, 10.5 ± 0.3; DOPAC, 0.75 ± 0.06; HVA, 0.74 ± 0.03. Statistical analyses were performed with a Student's t test. *$P < .05$ vs. control group.

effects of haloperidol (2 mg/kg, i.p.) on the decrease in central TPH induced by multiple administrations of MDMA (fig. 6). Cortical and neostriatal TPH activity were reduced to 15 and 18% of control, respectively, 18 h after the last MDMA administration. Haloperidol treatment failed to alter significantly these decreases. This suggests that D_2 receptors are not involved in the protective effects of flunarizine.

Discussion

We recently reported that the noncompetitive NMDA receptor antagonist, MK-801, attenuates the decline in neostriatal TPH activity induced by METH. The NMDA receptor is associated with a calcium channel which allows calcium influx into the neuronal cells after receptor stimulation, thus indicating that calcium plays a role in the METH-induced changes in the serotonergic system. In this study, the effects of non-NMDA calcium channel blockers on the METH- and MDMA-induced changes in the serotonergic system were examined. The calcium channel blocker, flunarizine, attenuated or blocked the changes in the central serotonergic system induced by MDMA, but did not influence the response to METH. This differential effect of flunarizine on the MDMA and METH

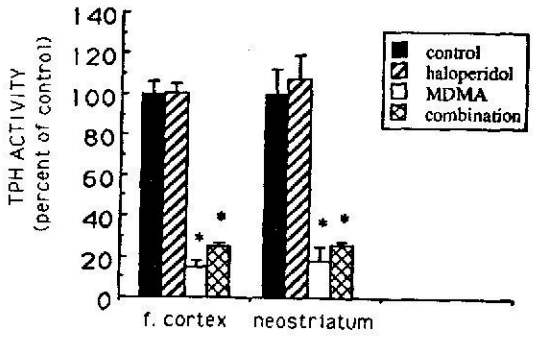

Fig. 6. Effects of haloperidol on the MDMA-induced decrease in TPH activity measured in the frontal cortex and in the neostriatum. The rats received five doses of MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-h intervals. Haloperidol (2 mg/kg, i.p.) was injected 15 min before the administration of MDMA or vehicle. The animals were sacrificed by decapitation 18 h after the last drug administration. The results are expressed as means ± S.E. of their respective control (n = 3–8 per group). The control values for TPH activity in the frontal cortex and in the neostriatum were 68.1 ± 4.1 and 39.9 ± 5.1 nmol/h/g of tissue (n = 6), respectively. Statistical analyses were performed with a one-way analysis of variance followed by a Scheffe multiple comparison test. *P < .05 *vs.* control group.

responses suggests that the decrease in TPH activity and in 5-HT concentrations induced by these drugs occurs by different mechanisms. The effect of flunarizine was selective, as other calcium channel blockers such as nimodipine, diltiazem and TA-3090, failed to block the changes induced by either METH or MDMA in serotonergic systems.

There is significant evidence that the loss of TPH activity measured 18 h after multiple administrations of MDMA reflects the destruction of serotonergic nerve terminals; this is in contrast to the rapid and reversible inactivation of the enzyme occurring shortly after administration of a single dose of MDMA. Reducing conditions reverse the decrease in enzymatic activity observed 3 h after a single dose of MDMA, whereas TPH activity measured 18 h after the last of multiple administrations of MDMA remains unaltered (Stone *et al.*, 1989). Moreover, flunarizine failed to prevent the decline measured 3 h after a single administration of MDMA (unpublished results), but blocked the decrease in activity measured 18 h and 1 week after multiple administrations of MDMA (figs. 1 and 3). This indicates that the changes observed 18 h after multiple administrations of MDMA reflect neurotoxic changes.

The precise mechanism by which flunarizine prevents the neuronal damage resulting from ischemia or from MDMA-induced changes remains unknown. Flunarizine fails to alter calcium accumulation into striatal slices under resting conditions, but inhibits the calcium influx during depolarization (Battaini *et al.*, 1986). This inhibition of stimulated calcium influx prevents the "calcium overload" resulting from an overstimulation of the neuron, leading to its damage (Van Zwieten, 1986). This inhibition of calcium uptake probably takes place at the membrane level, as flunarizine can prevent the lowering of extracellular calcium concentration measured *in vitro* after depolarization of an arterial smooth muscle preparation (Borgers *et al.*, 1980). Flunarizine antagonizes the L- and T-type voltage-operated calcium channels (Tytgat *et al.*, 1988), but apparently lacks affinity for the NMDA-linked calcium channel (Pauwels *et al.*, 1989). In our study, it is unlikely that L-type channels mediate the MDMA-induced decline in TPH, as the L-type channel inhibitor, nimodipine, failed to mimic the flunarizine response. The lack of effect of diltiazem on the METH- and MDMA-induced changes could be explained by its poor ability to cross the blood-brain barrier, although the potentiation of the METH-induced decrease may indicate that diltiazem penetrated the brain. TA 3090, a diltiazem analog, failed to block the changes induced by amphetamines, indicating that the diltiazem binding site may not be involved in the MDMA-induced toxicity. Thus, we were unable to identify a specific type of calcium channel linked to the MDMA-induced toxicity. However, the protective action of flunarizine still suggests that calcium is involved in the mechanism by which MDMA alters the serotonergic system.

It is possible that flunarizine exerted its protective effect at a site other than a calcium channel. Flunarizine inhibits calmodulin (Lugnier *et al.*, 1984) and has dopaminergic (Fadda *et al.*, 1989; Govoni *et al.*, 1988), adrenergic (Descombes and Stoclet, 1985) and histaminic (Holmes *et al.*, 1984) antagonist properties. In this study, we explored the possibility that flunarizine exerted its protective action by blocking the D-2 receptors. The increase of neostriatal DOPAC and HVA shown in figure 5 is a typical response to the blockade of D-2 receptors. However, it is unlikely that the protective effect of flunarizine can be explained by the blockade of this receptor because the D-2 antagonist, haloperidol, failed to block the MDMA-induced changes. Also, it is unlikely that flunarizine protected the serotonergic system by blocking adrenergic receptors, as *alpha*-1 and *beta* adrenergic blockers fail to prevent the MDMA-induced decline in 5-HT concentrations (Schmidt *et al.*, 1990). Thus, further studies remain to be performed to understand fully the mechanism by which flunarizine prevents the MDMA-induced changes.

The ability of flunarizine to block the MDMA-induced changes, but not those induced by METH, suggests that the mechanism of action of these drugs differs. This conclusion is based on the results obtained when flunarizine was administered at a dose of 30 mg/kg. A dose of 1 mg/kg failed to alter the METH- or MDMA-induced changes (unpublished results). The use of a higher dose of flunarizine was not pursued due to the high rate of mortality observed in the METH-treated animals. The conclusion that METH and MDMA alter the serotonergic system by two distinct mechanisms is also supported by the observation that MK-801 and haloperidol attenuate or block the METH-induced decline in TPH activity, but do not prevent the MDMA-induced changes (Hotchkiss *et al.*, 1980; Johnson *et al.*, 1989; fig. 4). However, DA released by these two amphetamine analogs appears to be central to the response of the serotonergic system (Hotchkiss *et al.*, 1980; Stone *et al.*, 1988; Schmidt *et al.*, 1985, 1991). DA alone does not decrease TPH activity when its synaptic concentration is increased by a DA uptake blocker, such as amfonelic acid or GBR 12909, or by supplementation with L-dopa, the precursor of DA (Schmidt *et al.*, 1985). This suggests that other factors in combination with the increase in DA release are necessary in order to destroy the serotonergic nerve terminal leading to a long-lasting reduction in TPH activity. One of these factors occurring during METH treatment may be the increase in calcium influx into the serotonergic nerve terminal mediated by NMDA receptors, whereas the calcium influx during the MDMA treatment is mediated through a flunarizine-sensitive mechanism. Further evidence is required to clarify the role of calcium.

It is interesting to note that calcium channel blockers poten-

tiated the METH-induced changes. This effect is most apparent in figure 4B, where the METH-induced decrease in TPH activity was less due to the lower dosage. A possible explanation is that this effect may be due to the ability of several calcium channel blockers to inhibit liver drug metabolism (Hunt *et al.*, 1989). This could decrease METH metabolism and thus increase its bioavailability to the brain. This would explain how diltiazem, which crosses the blood-brain barrier poorly, appears to potentiate the action of METH. This suggests that calcium channel blockers could make low doses of METH toxic to the serotonergic system. However, preliminary experiments using nimodipine and two different nontoxic doses of METH failed to alter TPH activity (unpublished results). Thus, the potentiating effect of calcium channel blockers may occur only at doses of METH which have an initial effect on the serotonergic system.

In summary, this study demonstrated that flunarizine attenuates or blocks the MDMA-induced decline in central TPH activity and 5-HT concentration. Because flunarizine failed to alter the METH-induced changes, this study suggests that the mechanisms by which these two amphetamine analogs cause their effects on the serotonergic systems may be different.

Acknowledgments

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Send reprint requests to: Dr. James W. Gibb, Department of Pharmacolo and Toxicology, University of Utah, Salt Lake City, UT 84112.