(*15*) and their presence in the pituitary, adrenal, bone marrow, kidney, spinal cord, and brain as well as ovary, testis, and placenta (*16*), it seems likely that these factors also play an important role in a number of nonreproductive functions. The discovery of a protein homologous to this gene family in *Drosophila* (*17*) emphasizes the potential importance and widespread occurrence of these factors. Production of inhibin and activin by two associated cell types may then be one of a number of ways in which these factors regulate the growth and differentiated function of organs.

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Role for Excitatory Amino Acids in Methamphetamine-Induced Nigrostriatal Dopaminergic Toxicity

PATRICIA K. SONSALLA, WILLIAM J. NICKLAS, RICHARD E. HEIKKILA

The systemic administration of either methamphetamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to experimental animals produces degenerative changes in nigrostriatal dopaminergic neurons or their axon terminals. This study was conducted to determine if excitatory amino acids, which appear to be involved in various neurodegenerative disorders, might also contribute to the dopaminergic neurotoxicity produced in mice by either methamphetamine or MPTP. MK-801, phencyclidine, and ketamine, noncompetitive antagonists of one subtype of excitatory amino acid receptor, the *N*-methyl-D-aspartate receptor, provided substantial protection against neurotoxicity produced by methamphetamine but not that produced by MPTP. These findings indicate that excitatory amino acids play an important role in the nigrostriatal dopaminergic damage induced by methamphetamine.

OVERACTIVITY OF EXCITATORY AMINO acid (EAA) neurotransmission may be associated with the pathophysiology of a number of neurodegenerative disorders, including epilepsy (*1*), olivopontocerebellar atrophy (*2*), stroke or hypoxia-induced brain damage (*3*), hypoglycemia-induced brain damage (*4*), spinal cord injury (*5*), and possibly even Huntington's disease (*6*) and Alzheimer's disease (*7*). The infusion of EAA agonists such as kainate, ibotenate, or *N*-methyl-D-aspartate (NMDA) directly into a particular brain region such as the cortex (*8*), hippocampus (*9*), or striatum (*6*) of experimental animals causes neurodegeneration within that brain region. Excitotoxin-induced cell death appears to be mediated by excessive stimulation of EAA receptors, of which there are at least three subtypes: NMDA, kainate, and quisqualate (*10*).

The excitotoxins cause a prolonged neuronal depolarization (*11*), an influx of Ca^{2+} (*12*), and a depletion of adenosine triphosphate with a concomitant increase in purine catabolites (*13*). Each of these effects, alone or in combination, could contribute to cell death. Cultured cerebellar neurons can be protected from excitotoxin-induced death by compounds that reduce levels of superoxide or hydroxyl radicals (*14*). These latter findings are consistent with the premise that excitotoxin-induced neuronal degeneration may be mediated by oxidative stress associated with the production of superoxide and hydroxyl radicals.

Parkinson's disease (PD) is a neurodegenerative disorder of unknown etiology that is characterized by a loss of nigrostriatal dopaminergic neurons. One theory of neurodegeneration in PD is that oxidation products derived from dopamine (DA), such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals, are neurotoxic (*15*). There are several experimental models of PD, one of which is the amphetamine- or methamphetamine (METH)–treated rodent or nonhuman primate. The amphetamines cause damage to nigrostriatal dopaminergic neurons as evidenced by marked decrements in the neostriatal content of DA and its metabolites, the number of high-affinity DA uptake sites, and the activity of tyrosine hydroxylase (TH), as well as histochemical indications of nerve terminal degeneration within the neostriatum (*16*). The amphetamines cause a release of newly synthesized

Table 1. Effect of (+)MK-801 on METH-induced decreases in TH activity and DA content in the mouse neostriatum. Mice received four intraperitoneal injections of METH at the doses indicated at 2-hour intervals. Other groups were injected intraperitoneally with (+)MK-801 at the doses indicated 15 min before and 3 hours after the first injection of METH. Results are the means ± SD of three to five mice per group killed 3 days after treatment.

(+)MK-801 (mg/kg)	METH (mg/kg)	DA (μg/g)	TH activity (nmol/g per hour)
		14.9 ± 1.8	465 ± 54
	1.25	13.3 ± 0.4	367 ± 16
	2.5	5.3 ± 4.2*	197 ± 111*
	5	3.3 ± 0.8*	154 ± 44*
	10	1.1 ± 0.6*	64 ± 26*
0.5	5	10.5 ± 3.4*†	240 ± 82*
1.0	5	14.3 ± 1.0†	369 ± 80†
2.5	5	12.6 ± 2.6†	358 ± 77*†
2.5	10	15.4 ± 0.8†	420 ± 50†

*Statistically different ($P < 0.05$) from naive group (analysis of variance with Duncan's multiple range test). †Statistically different ($P < 0.05$) from METH-only group.

Department of Neurology, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ 08854.

DA from a cytosolic pool (*17*). The administration to experimental animals of α-methyl-*p*-tyrosine, which inhibits TH activity and therefore prevents DA synthesis, attenuates METH-induced neurotoxicity (*18*). Oxidation products of DA may play a role in this METH-induced toxicity (*19*), and it is possible that there may be important similarities in the mechanisms of cell death in idiopathic PD and the dopaminergic cell damage induced by METH administration.

The neostriatum receives a large input of glutamatergic neurons from the cortex (*20*). NMDA causes DA to be released from neostriatal slices, a part of which, at least, may be mediated by NMDA receptors on dopaminergic terminals (*21*). Moreover, a population of these glutamatergic neurons have presynaptic DA receptors which, when activated, may decrease the high-affinity uptake of glutamate (*22*). The competitive blockade of NMDA receptors or the blockade of the NMDA receptor–associated ion channel prevents EAA-mediated damage in in vitro experimental systems (*23*) and in experimental animals retards seizure activity produced by electroshock and convulsants (*24*), reduces or prevents neurodegeneration induced by hypoglycemia (*4*) or reperfusion ischemia (*3*), and improves neurological outcome after spinal cord injury (*5*). On the basis of these observations, we have evaluated the effect of noncompetitive NMDA receptor antagonists that readily penetrate into the brain on METH-induced dopaminergic toxicity. In addition, we tested for the efficacy of one of these agents, namely (+)MK-801, in preventing dopaminergic neurotoxicity induced by another dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (*25*). The mechanism of cell death by MPTP is not well understood. Although oxidative stress may be a component of MPTP-induced dopaminergic toxicity (*26*), other mechanisms involving cellular respiration may be more important (*27*).

The intraperitoneal administration of METH (*28*) produced a dose-dependent decrease in TH activity and in DA content in the mouse neostriatum (Table 1). Three days after treatment with METH (four injections of 5 or 10 mg per kilogram of body weight every 2 hours), there was a marked loss of neostriatal DA (78% and 93%, respectively) and TH activity (67% and 86%, respectively). The compound (+)MK-801 (0.5 to 2.5 mg/kg) protected against these large decrements (Tables 1 and 2).

No protection against the METH-induced dopaminergic deficits was observed in mice treated with the less active isomer (−)MK-801 (2.5 mg/kg) (Table 2). However, (−)MK-801 does display a moderate affinity for the ion channel binding site but is only one-seventh as potent as (+)MK-801 (*29*). A larger dose of (−)MK-801 (10 mg/kg) provided substantial, although not complete, protection against METH-induced toxicity (*30*).

In contrast to its protective effect against METH-induced toxicity, (+)MK-801 failed to provide substantial protection against MPTP-induced decrements in DA content and TH activity (Table 2). Although DA content was significantly higher in mice that received (+)MK-801 and MPTP than in those that received MPTP alone, no statistical differences in TH activity were seen between the two groups (Table 2). Moreover, the effects of (−)MK-801 on MPTP-induced decreases in DA and TH were similar to those of (+)MK-801. In two other experiments, the effects of (+)MK-801 were variable: in one experiment there was no significant protection, whereas in the other experiment there was a small but significant attenuation by (+)MK-801 of the MPTP-induced decrement in DA content. Although a more complete investigation is needed to determine if there is any protective effect of (+)MK-801 against MPTP, it is clear that (+)MK-801 does not provide the same degree of protection against MPTP as against METH.

Both phencyclidine (PCP) and ketamine antagonize NMDA responses in a noncompetitive fashion, presumably by blocking the NMDA receptor–associated ion channel in a manner analogous to that for MK-801. Both PCP and ketamine are much less potent than either (+)MK-801 or (−)MK-801 in their binding affinity for the ion channel (*29*) and in their related pharmacological effects (*24*). Neither PCP (10 mg/kg) nor ketamine (10 mg/kg) given intraperitoneally 15 min before and 3 hours after the first METH injection protected against METH-induced toxicity. However, when the doses were increased (PCP, 20 mg/kg; ketamine, 100 mg/kg) and the compounds were administered more frequently (four times, 15 min before each METH injection), each provided extensive protection (Table 3).

Although both METH and MPTP pro-

Table 2. Effect of (+) or (−)MK-801 on METH- or MPTP-induced decreases in TH activity and DA content in the mouse neostriatum. Mice were injected with (+) or (−)MK-801 (2.5 mg/kg, intraperitoneally) 15 min before and 3 hours after the first injection of METH or MPTP. Four intraperitoneal injections of METH (10 mg/kg) or MPTP (20 mg/kg) were given at 2-hour intervals. Results are the means ± SD of three to five mice per group killed 3 days after treatment.

Treatment	Neurotoxin	DA (μg/g)	TH activity (nmol/g per hour)
		13.6 ± 1.7	458 ± 81
(+)MK-801		13.8 ± 1.8	497 ± 62
(−)MK-801		13.2 ± 1.2	482 ± 50
	METH	3.7 ± 2.2*	202 ± 88*
(+)MK-801	METH	9.9 ± 2.2*†	369 ± 68*†
(−)MK-801	METH	1.5 ± 0.2*	97 ± 28*†
	MPTP	4.4 ± 1.5*	242 ± 59*
(+)MK-801	MPTP	6.9 ± 1.0*‡	296 ± 39*
(−)MK-801	MPTP	6.9 ± 1.8*‡	292 ± 58*

*Statistically different ($P < 0.05$) from naive or corresponding control (*) group (analysis of variance with Duncan's multiple range test). †Statistically different ($P < 0.05$) from METH-only group. ‡Statistically different ($P < 0.05$) from MPTP-only group.

Table 3. Effect of ketamine or PCP on METH-induced decreases in neostriatal TH activity and DA content. Ketamine (100 mg/kg) or PCP (20 mg/kg) was administered intraperitoneally 15 min before each injection of METH. METH was administered as described in Table 2. Results are the means ± SD of four to nine mice per group killed 7 days after treatment.

Treatment	METH	DA (μg/g)	TH activity (nmol/g per hour)
	−	15.4 ± 1.0	545 ± 72
Ketamine	−	15.0 ± 2.1	546 ± 87
	+	2.2 ± 1.0*	134 ± 65*
Ketamine	+	10.4 ± 2.0*†	448 ± 42†
	−	13.2 ± 1.2	657 ± 66
PCP	−	14.4 ± 1.8	615 ± 64
	+	1.8 ± 0.6*	140 ± 36*
PCP	+	13.4 ± 1.3†	644 ± 81†

*Statistically different ($P < 0.05$) from naive or corresponding control group (analysis of variance with Duncan's multiple range test). †Statistically different ($P < 0.05$) from METH-only group.

duce nigrostriatal dopaminergic neurotoxicity, different mechanisms appear to be involved. The lack of any substantial protective effect of (+)MK-801 on MPTP-induced neurotoxicity suggests that EAA receptors, at least of the NMDA type, are not involved in MPTP-induced neurotoxicity. In contrast, our data are consistent with a role for the actions of EAAs, mediated via NMDA receptors, in the dopaminergic toxicity induced by METH. Not only did several different noncompetitive antagonists of the NMDA receptors provide protection against METH toxicity, but the rank order for the potency of these compounds in protecting against METH-induced toxicity [(+)MK-801 > (−)MK-801 > PCP > ketamine] is the same as the order observed in binding experiments (*29*) and in other in vivo experiments (*24*).

Our findings implicate EAAs in yet another model of neurodegeneration. These findings and those of others may lead to an understanding of the etiology of and the development of effective preventive measures for PD and other neurodegenerative disorders. Exactly how the EAAs are involved in METH-induced and other forms of neurotoxicity is not known. Although the noncompetitive antagonists might inhibit the direct actions of EAAs on neostriatal DA terminals, there is no evidence that the infusion of NMDA or other excitotoxins into the neostriatum damages neostriatal dopaminergic neurons. Other indirect mechanisms involving the EAAs may be important. The fact that the EAAs have been implicated in many neurodegenerative disorders of many brain areas may indicate that overactivity of EAAs may be a common denominator in neurodegeneration.

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28. Male Swiss Webster mice (25 to 30 g, Taconic Farms) were injected intraperitoneally with MPTP hydrochloride (Research Biochemicals) (20 mg of free base per kilogram per injection) or METH hydrochloride (Sigma) (1.25 to 10 mg of free base per kilogram per injection) four times at 2-hour intervals. Some of the mice were also injected intraperitoneally with various doses of (+)MK-801 maleate (Merck or Research Biochemicals) or (−)MK-801 maleate (Research Biochemicals) 15 min before and 3 hours after the first injection of MPTP or METH. Other mice received intraperitoneal injections of PCP hydrochloride (Sigma) (20 mg of free base per kilogram per injection) or ketamine hydrochloride (Research Biochemicals) (100 mg of free base per kilogram per injection) before each injection of METH. Three or 7 days later the mice were killed and the neostriata were removed. TH activity and DA content were measured as described by J. F. Reinhard, Jr., *et al.* [*Life Sci.* **39**, 2185 (1986)] and P. K. Sonsalla *et al.* [*J. Pharmacol. Exp. Ther.* **242**, 850 (1987)], respectively.
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30. Mice (four to eight per group) were injected intraperitoneally with 10 mg of (−)MK-801 or METH, or both, per kilogram as described in (*28*), and were killed 7 days later. TH activities (in nanomoles per gram of tissue per hour) ± SD were as follows: naives, 657 ± 66; (−)MK-801, 574 ± 31; METH, 140 ± 36*, and (−)MK-801 plus METH, 433 ± 111*†. DA levels (in micrograms per gram of tissue) ± SD were as follows: naives, 13.2 ± 1.2; (−)MK-801, 12.7 ± 1.5; METH, 1.8 ± 0.6*; and (−)MK-801 plus METH, 8.3 ± 2.2*†. $P < 0.05$ versus naives or corresponding controls (*) or METH-only group (†).

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