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Role of glutamate in neurodegeneration of dopamine neurons in several animal models of parkinsonism

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Summary. Although controversial, studies with methamphetamine and MPTP suggest a link between glutamate-mediated excitotoxicity and degeneration of dopamine cells. Both compounds are thonght to create a metabolic stress. To further explore glutamate actions in DA degeneration, we investigated the effects of other metabolic inhibitors. In mesencephalic cultures, DA cell loss produced by 3-NPA or malonate was potentiated by NMDA and prevented by MK-801. In vivo, striatal DA loss produced by intranigral infusions of malonate was also potentiated by intranigral NMDA and prevented by systemic MK-801. In contrast, systemic MK-801 did not prevent DA loss produced by intrastriatal malonate. Intrastriatal MK-801 or CGS 19755 did attenuate DA loss in METH-treated mice, but was confounded by the findings that METH-induced hyperthermia, an important component in toxicity, was also attenuated. Taken together, the data support the hypothesis of NMDA receptor involvement in degeneration of DA neurons. Furthermore, the data also suggest that this interaction is likely to occur in the substantia nigra rather than in the striatum.

Keywords: Glutamate – NMDA receptors – Dopamine – Malonate – Methamphetamine – MPTP – MK-801 – Mesencephalic cultures – Mice – Basal ganglia

A prominent feature of Parkinson's disease (PD) is the loss of nigrostriatal dopamine (DA) neurons. The hypothesis that an excitotoxic action of glutamate might contribute to the degeneration of these DA neurons was first proposed by Sonsalla and colleagues (1989, 1991) who observed that N-methyl-D-aspartate (NMDA) antagonists prevent the loss of striatal DA nerve terminals produced by systemic administration of methamphetamine (METH). However, NMDA antagonists also block the hyperthermia produced by METH, a physiological effect important in mediating its neurotoxicity (Bowyer et al., 1994; Albers et al., 1995). Thus, it is unclear whether the neuroprotective effects of the NMDA antagonists are due to inhibition of a direct glutamate-mediated action on DA neurons or to a secondary, indirect effect mediated by temperature alterations.

In another animal model of PD, created by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its metabolite, MPP^+, the efficacy of NMDA antagonists as neuroprotectants also remains controversial (reviewed in Chan et al., 1997). Although several factors may contribute to the dichotomous findings (age, weight, species or strain of animal), it is also possible that protection with NMDA antagonists may depend on the severity of the MPP^+ insult and the mechanism by which the cell degenerates. For example, Pang and Geddes (1997) found that MK-801 prevented necrotic, but not apoptotic, cell death produced by 3-nitroproprionate (3-NPA) in hippocampal cultures. Thus, protection against MPTP/MPP^+ by NMDA antagonists may occur only when the predominant form of cell loss is necrosis. However, if the insult produces apoptosis, then NMDA antagonists may be less protective.

Emerging evidence suggests that metabolic stress may be an important component of toxicity produced by MPTP or METH. Blockade of complex I activity by MPP^+ inhibits energy production (Sonsalla and Nicklas, 1992) whereas in METH toxicity it is proposed that energy demands may exceed energy stores within DA terminals (Chan et al., 1994; Albers et al., 1996). It has been proposed that decreased energy metabolism may produce a secondary excitotoxicity mediated by NMDA receptors (Novelli et al., 1988; Zeevalk and Nicklas, 1991). Furthermore, the discovery of mitochondrial defects in PD patients indicate that metabolic deficiencies may contribute to DA neurodegeneration in this neurological disorder. These observations have led us to further explore glutamate actions and metabolic stress in DA degeneration *in vitro* and *in vivo*.

In mesencephalic cultures, [3H]-DA uptake, a measure of DA cell damage/loss, was substantially reduced by NMDA as well as by 3-NPA and malonate, inhibitors of succinate dehydrogenase (Table 1). Loss of DA uptake produced by these metabolic inhibitors was attenuated or prevented by coincubation of the cultures with MK-801. Furthermore, combined treatment of cells with a non-toxic concentration of malonate (10 mM) and NMDA poten-

Table 1. In vitro studies with NMDAor metabolic inhibitors

Treatment	MK-801 (1 μM)	^{3}H-DA Uptake (%Control)
NMDA (100 μM)[c]	–	1 ± 1[a]
NMDA (100 μM)[c]	+	56 ± 12[a,b]
3-NPA (0.25 mM)[d]	–	41 ± 10[a]
3-NPA (0.25 mM)[d]	+	99 ± 4[b]
Malonate (50 mM)[d]	–	31 ± 11[a]
Malonate (50 mM)[d]	+	92 ± 15[b]

Mesencephalic cultures were treated with the compounds for 24 h and allowed to recover for 48 h. Results are mean ± SEM for ^{3}H-DA uptake of 3–5 experiments performed in duplicate. [a]$p < 0.05$ from controls; [b]$p < 0.05$ from respective group incubated in absence of MK-801; [c]data from Zeevalk et al., 1995a, reprinted with permission; [d]data from Zeevalk et al., 1995b, reprinted with permission.

tiated damage (data not shown). These *in vitro* findings demonstrate that NMDA receptor activation can produce DA degeneration and also implicate NMDA receptor involvement in DA degeneration produced by metabolic stress.

In vivo, our initial studies focussed on striatal terminals because they are more sensitive to neurodegeneration than are the cell bodies after treatment with MPTP or METH and thus appear to be a primary target for the neurotoxic actions of these substances. A large bolus of NMDA (250 nmol) infused into the striatum of mice produced a 42% loss of striatal DA (Table 2). A lower non-toxic concentration (100 nmol) administered with a non-toxic dose of METH produced a small but significant 33% reduction of striatal DA. These findings suggest a contributory role for NMDA receptor activation in the striatum in METH-induced toxicity. In an attempt to eliminate the temperature-blocking effects of NMDA antagonists in METH-treated animals, but yet allow for investigation of direct NMDA receptor involvement in DA terminal toxicity, MK-801 or CGS 19755 was infused directly into the striatum prior to and 3 h after METH treatment. Protection was observed but only at concentrations that also prevented the METH-induced hyperthermia (Table 3). Taken together, these data suggest that striatal NMDA receptors may contribute to METH-induced toxicity. However, the inability of the antagonists, when infused directly into the striatum, to protect at concentrations which do not block temperature elevations does not provide compelling evidence that striatal NMDA receptors are important mediators of METH-induced toxicity in this brain region.

In other studies, the contribution of metabolic stress to DA nerve terminal damage was investigated. Intrastriatal malonate produced a dose-dependent loss of striatal DA (Table 4). This DA loss was enhanced by low doses of systemic MPTP or METH. However, in contrast to the *in vitro* data in which MK-801 protected against malonate damage, the DA loss produced by intrastriatal malonate in mice or rats was not prevented by either systemic or intrastriatal MK-801 (Table 4). Taken together, these data suggest that meta-

Table 2. Effect of intrastriatal NMDA and systemic METH in mice

NMDA (nmol)	Other	Striatal DA (% Control)
100	–	92 ± 30
250	–	58 ± 10[a]
–	METH	96 ± 12
100	METH	67 ± 14[a,b]

Saline or METH (30 mg/kg, sc) was injected into mice that had received a unilateral intrastriatal infusion of NMDA (1 μl). Results are the mean ± SEM DA content in % of control obtained from striata of 3–6 mice 10 days after treatment. [a]$p < 0.05$ vs control; [b]$p < 0.05$ vs 100 nmol NMDA or METH alone. Data from Sonsalla et al., 1991; reprinted with permission.

Table 3. Effect of intrastriatal NMDA antagonists vs systemic METH

NMDA antag (nmol)	METH	Striatal DA (% control)
–	+	17 ± 12[a]
MK-801 (3)	+	37 ± 28[a]
MK-801 (30)	+	68 ± 27[a,b,c]
CGS 19755 (3)	+	83 ± 13[b,c]

NMDA antagonists (2 μl) were infused into the left striatum 0.5 h before before METH treatment (2 × 10 mg/kg ip; 2 h apart) and again 3 h later. Results are for DA content (mean ± SD) in % of control obtained from striata of 3–6 mice 5–7 days after treatment. [a] $p < 0.05$ vs controls; [b] $p < 0.05$ vs METH only group; [c] rectal temperatures were significantly lower than in METH only group. DA content did not differ significantly in mice infused with antagonist alone.

Table 4. Effect of intrastriatal malonate and concurrent treatments on DA depletion

	Malonate (μmol)	Other	Striatal DA (% control)
Intrastriatal infusions – mice[d]	3	–	70 ± 23[a]
	–	MPTP ip	101 ± 13
	3	MPTP ip	43 ± 30[a,b,c]
	–	METH ip	71 ± 13[a]
	3	METH ip	29 ± 11[a,b,c]
	4	–	34 ± 20[a]
	4	MK-801 ip	56 ± 32[a]
Intrastriatal infusions – rats	2	–	32 ± 2[a]
	2	MK-801 is	40 ± 8[a]

Malonate was infused in the left striatum of animals immediately prior to treatment with MPTP or METH; see Albers et al. (1996) for details of methods. MK-801 (2.5 mg/kg ip or 80 nmol intrastriatally) was administered prior to malonate and again 3 h later. Results are for DA content (mean ± SD) in % of control from 4–8 animals killed 5–7 days after treatment. [a] $p < 0.05$ vs controls; [b] $p < 0.05$ vs MPTP or METH alone; [c] $p < 0.05$ vs malonate alone; [d] some of mouse data is from Albers et al. (1996) reprinted with permission; *ip* intraperitoneal, *is* intrastriatal.

bolic compromise is a component of the neurotoxicity produced by METH or MPTP. But the inability of MK-801 to block DA toxicity produced by an intrastriatal infusion of malonate suggests that NMDA receptor activation in the striatum does not contribute substantially to DA terminal loss produced by metabolic compromise.

Because observations of the *in vitro* and *in vivo* striatal malonate/MK-801 studies appeared incongruous, we explored the possibility that NMDA recep-

Table 5. Effect of NMDA receptor activation or blockade on malonate toxicity in substantia nigra of rats

NMDA (nmol)	MAL (umol)	Other	Striatal DA (% control)
10	–	–	66 ± 12[a]
–	0.25	–	81 ± 15
10	0.25	–	41 ± 28[a,b]
50	–	–	46 ± 4[a]
50	–	MK-801	86 ± 19[b]
–	0.5	–	71 ± 15[a]
–	0.5	MK-801	92 ± 9[b]

Rats received an intranigral infusion of MAL and or NMDA. Some of the rats also received MK-801 (2.5 mg/kg ip) just prior to intranigral infusions and again 3 h later. Results are for DA content (mean ± SD) in % of control from 4–6 animals killed 7 days after treatment. [a] $p < 0.05$ vs controls; [b] $p < 0.05$ from group treated with only malonate or NMDA.

tors located on the somata and/or dendrites of the DA neurons in the substantia nigra of animals might be more pertinent in mediating any excitotoxic insult as a result of metabolic inhibition. Intranigral NMDA infusions in rats produced a dose-dependent decrease in striatal DA which was enhanced by co-infusion of malonate (Table 5). Furthermore, DA loss produced by intranigral NMDA or malonate was prevented by blockade of the NMDA receptors with MK-801 treatment. These data suggest that the damage produced by metabolic compromise at the level of the DA cell bodies/dendrites is mediated, at least in part, by NMDA receptor activation.

In summary, our data support the hypothesis that glutamate actions mediated by NMDA receptors, possibly via a secondary excitotoxicity, can produce DA neurodegeneration. Additionally, it appears that glutamate receptor involvement in the neurodegeneration of DA neurons is more likely to be at the level of the substantia nigra than the striatum.

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