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# Attenuation of Malonate-induced Degeneration of the Nigrostriatal Pathway by Inhibitors of Nitric Oxide Synthase

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**Summary**—Focal infusions of the succinate dehydrogenase inhibitor, malonate, into the substantia nigra pars compacta (SNc) of adult Sprague–Dawley rats resulted in a substantial depletion of ipsilateral striatal tyrosine hydroxylase (TH) activity. The percentage decrease in striatal TH activity following intranigral malonate (0.5 μmol/0.5 μl) infusion was similar at 4 (58%) and 7 days (62%) post-infusion. To assess the role of *N*-methyl-D-aspartate (NMDA) receptor activation in malonate neurotoxicity, animals were pretreated with the NMDA receptor antagonist MK-801 (2 × 5 mg/kg, i.p.). Four days post-infusion of malonate (0.5 μmol/0.5 μl) into the SNc, striatal TH activity was depleted by 58% in vehicle pretreated animals and 14% in the presence of MK-801 indicating a significant neuroprotective effect of MK-801 on malonate action. To determine the role of nitric oxide (NO) in malonate-induced nigral toxicity, the actions of malonate were evaluated in the presence of the nitric oxide synthase (NOS) inhibitors, 7-nitro indazole (7-NI) and $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME). Systemic injections of 7-NI (20, 30, 40, 50 and 75 mg/kg, i.p.) produced a dose-related inhibition of nigral NOS activity which was maximal at a dose of 40 mg/kg. Intranigral infusion of malonate with 20 and 50 mg/kg 7-NI pretreatment produced a 46 and 31% decrease in striatal TH activity, respectively. Thus, a significant protective effect at the higher but not lower dose of 7-NI was observed. Pretreatment with a L-NAME regimen (2 × 250 mg/kg; i.p.), previously shown to inhibit brain NOS activity by greater than 86%, also produced a significant neuroprotective effect against malonate-induced neurotoxicity (30% decrease). The results of this study suggest that malonate-induced toxicity to the dopaminergic neurons of the nigrostriatal pathway is mediated, at least in part, by NMDA receptor activation and the formation of NO. Copyright © 1996 Elsevier Science Ltd.

**Keywords**—Parkinson's disease, malonate, nitric oxide synthase (NOS), 7-nitro indazole (7-NI), $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME), excitotoxicity.

Deficits in energy metabolism have been implicated in the pathophysiology of various neurodegenerative disorders (Albin and Greenamyre, 1992; Beal, 1992, 1993). Considerable evidence suggests that there is decreased activity of complex 1 of the mitochondrial respiratory chain in Parkinson's disease (PD) (see Shapira, 1994) and in incidental Lewy Body disease, which may be considered asymptomatic PD (Dexter *et al.*, 1994). The neurotoxin MPTP which produces selective damage to nigral dopaminergic neurons mimics deficits seen in PD (Bloem *et al.*, 1990) by disrupting oxidative phosphorylation through inhibition of mitochondrial complex 1 activity at the rotenone binding site (Ramsay *et al.*, 1991; Saporito *et al.*, 1991; Gluck *et al.*, 1994). Thus, although the etiology of PD remains unknown, there is evidence to suggest that metabolic compromise of the dopaminergic nigrostriatal neurons may underlie its pathogenesis.

Metabolic compromise can indirectly lead to *N*-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity. Novelli *et al.* (1988) reported that decreased energy levels in primary cultures of rat cerebellar neurons enabled glutamate to become neurotoxic through activation of the NMDA receptor. Similarly, Zeevalk and Nicklas (1990) demonstrated that mild metabolic compromise of the chick retina led to toxicity which was predominately NMDA receptor-mediated. It has been suggested that decreased ATP levels due to mitochondrial dysfunction impairs the $Na^+/K^+$ ATPase which maintains resting membrane potential. Subsequent depo-

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larization of neurons results in the removal of the voltage-dependent $Mg^{2+}$ blockade of the NMDA receptor and will allow endogenous glutamate to produce excitotoxic damage. This process has been referred to as "weak" or "secondary" excitotoxicity (Albin and Greenamyre, 1992; Beal, 1992, 1993). In agreement with this hypothesis it has been found that various mitochondrial inhibitors can produce "excitotoxic" lesions both *in vitro* and *in vivo*. The succinate dehydrogenase (SDH) inhibitors, malonate and 3-nitropropionic acid (3-NPA) have been reported to produce striatal lesions which can be prevented by pretreatment with various NMDA receptor antagonists (Beal *et al.*, 1993a,b; Greene *et al.*, 1993; Greene and Greenamyre, 1995a). Similarly, the toxicity of MPTP and $MPP^+$ on nigral dopaminergic neurons has been reported by some investigators to be attenuated by MK-801 (Turski *et al.*, 1991; Storey *et al.*, 1992) although others failed to obtain significant neuroprotection with NMDA antagonists (Sonsalla *et al.*, 1992). NMDA receptors have been localized on the dopaminergic neurons of the substantia nigra pars compacta (SNc) (Albin *et al.*, 1992; Difazio *et al.*, 1992) and are thought to be predominantly responsible for both the excitatory (Christoffersen and Meltzer, 1995) and excitotoxic (Kikuchi and Kim, 1993) properties of glutamate on these neurons.

Activation of NMDA receptors on NOS containing neurons leads to nitric oxide (NO) formation (Garthwaite, 1991) which has been implicated in NMDA receptor-mediated toxicity (Dawson *et al.*, 1991). However, conflicting findings have also been reported (Demerle-Pallardy *et al.*, 1991). Previously, we have shown that systemic administration of the nitric oxide synthase (NOS) inhibitors, $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) and 7-nitro indazole (7-NI) potentiated the toxicity produced by focal infusions of quinolinic acid (QUIN) and NMDA into the SNc (Connop *et al.*, 1994, 1995). These findings suggest that inhibition of NOS may influence NMDA receptor-dependent neurotoxicity produced by metabolic impairment. To elucidate the role of NO in the toxicity associated with indirect activation of NMDA receptors, the present study examined the effect of focal infusions of malonate on the integrity of the nigrostriatal pathway in animals which have been pretreated with either 7-NI or L-NAME.

## MATERIALS AND METHODS

### *Animal treatments*

Animals were used in accordance with the *Canadian Council on Animal Care Guidelines* and the protocol was approved by the University Committee on Animal Care. Prior to determining the role of NOS inhibition on malonate toxicity, the ability of systemic administration of 7-NI to inhibit nigral NOS activity was investigated. To determine the relationship between 7-NI and NOS activity, male Sprague–Dawley rats (250–275 g) were given a single injection of the vehicle (arachis oil; i.p.) or 7-NI (20, 30, 40, 50, and 75 mg/kg; i.p.). 7-NI was heated and sonicated in arachis oil. Thirty minutes post-injection, animals were sacrificed and the substantia nigra (SN) removed and assayed for NOS activity. To test the short duration of 7-NI-induced inhibition of brain NOS activity reported by MacKenzie *et al.* (1994), 7-NI (20 and 50 mg/kg, i.p.) was also injected 4.5 hr prior to sacrifice. The SN from each hemisphere was dissected from a 3 mm thick coronal section which was removed 4.5 mm posterior to the optic chiasm. The SN from both hemispheres was pooled for each nigral sample. The respective tissue samples were homogenized with a Teflon® pestle in a solution containing 1 mM dithiothreitol, 10 μg/ml leupeptin, 1 mM EDTA and 50 mM HEPES buffer at pH 7.4. Each pooled nigral sample was homogenized in 175 μl. The homogenates were centrifuged at 20,000 *g* for 30 min at 4°C and the supernatant retained for the NOS assay.

### *Nitric oxide synthase assay*

NOS activity was assayed using a modification of the method described by Bredt and Snyder (1990) which measures the amount of $^{14}C$-labelled arginine converted to $^{14}C$-labelled citrulline. Briefly, tissue samples (25 μl) were incubated for 1 hr at 37°C with 25 μl of 100 μM $^{14}C$-arginine (0.319 Ci/mmol, 50 μ Ci/ml) and 100 μl of reaction buffer. The reaction buffer consisted of 1 mM dithiothreitol, 1.25 mM $CaCl_2$, 1 mM valine, 2 mM NADPH, 1 mM EDTA and 50 mM HEPES buffer pH 7.4. The reaction was terminated by the addition of 2 ml ice cold stop buffer which contained 2 mM EDTA and 20 mM HEPES buffer at pH 5.5. Each sample was passed through a 1 ml AG5OW-X8 cation exchange column ($Na^+$ form) which was washed with 2 ml of distilled water and the eluate collected and counted using a Beckman scintillation counter. Protein was determined by the method of Bradford (1976) and the enzyme activity was expressed as nmol citrulline formed/mg protein/hr.

### *Intranigral infusion of malonate*

Male Sprague–Dawley rats (250–275 g) were anesthetized with 4% halothane and placed in a Narashige® stereotaxic apparatus. Saline (0.9%; pH 7.4) or malonate (0.25 and 0.5 μmol) was unilaterally infused through a cannula into the SNc according to the following coordinates: 5.3 mm posterior to bregma, 2.2 mm lateral to the midline and 7.7 mm ventral to bregma (Paxinos and Watson, 1982). Malonate was dissolved in 0.9% saline and titrated to pH 7.4 with NaOH. Each solution was infused for 75 sec through a cannula (30 guage) connected to a Hamilton® syringe to deliver a total volume of 0.5 μl. The cannula was left in place for an additional 2 min and then withdrawn, bone wax applied and the incision closed. Four or seven days post-infusion, animals were decapitated and the brain rapidly removed

and placed in ice cold saline. Each of the ipsilateral and contralateral striata were dissected and homogenized separately with a glass hand-homogenizer in 175 μl of buffer consisting of 0.3 M sucrose in 0.01 M Tris–HCl at pH 7.3. The samples were subsequently centrifuged at 12,000 *g* for 6 min at 4°C and the supernatant retained for the assay of tyrosine hydroxylase (TH) activity.

*Pretreatment protocols*

In the 7-NI experiments, arachis oil was used as the vehicle control, and in experiments using MK-801 and L-NAME saline was used as the vehicle control. In the MK-801 pretreatment experiments, animals were injected with this agent (5 mg/kg, i.p.) 30 min prior to stereotaxic infusion of malonate (0.5 μmol) into the SNc and again 4 hr after the initial injection of MK-801. In the 7-NI pretreatment experiments, animals were injected with 7-NI (20 or 50 mg/kg, i.p.) 30 min prior to stereotaxic infusion of malonate (0.5 μmol) into the SNc. Due to its short duration of action (MacKenzie *et al*, 1994), animals were given subsequent injections of the same dose of 7-NI 4 and 8 hr following stereotaxic infusion of malonate. In the L-NAME pretreatment experiments, animals were injected twice (8 hr apart) with 250 mg/kg L-NAME with the second injection occurring 14 hr prior to stereotaxic infusion of malonate (0.5 μmol) into the SNc.

*Tyrosine hydroxylase assay*

TH activity was assayed using a modification of the method described by Yamauchi and Fujisawa (1978). The modifications included a 1.5 hr incubation period at 37°C as well as centrifugation at 2000 *g* for 10 min following each vortex after addition of alumina. The amount of L-dopa formed from L-tyrosine was determined by the trihydroxyindole fluorometric method (Yamauchi and Fujisawa, 1978). Protein was determined by the method of Bradford (1976) and the enzyme activity was expressed as nmol L-dopa formed/mg protein/hr. For each animal, the results were expressed as the per cent decrease from the contralateral (uninjected) side.

*Drugs and chemicals*

7-NI was obtained from the Lancaster Chemical Co. (Windham, NH) and radiolabelled [$^{14}$C]arginine (0.319 Ci/mmol, 50 μCi/ml) from Amersham (Toronto, Ont.). All other chemicals were obtained from the Sigma Chemical Co. (St Louis, MO).

*Statistical analysis*

Statistical analysis was performed by one-way ANOVA followed by Newman–Keuls, *post hoc* testing to determine statistical significance at $p < 0.05$. From four to six animals were used to determine each point and values were expressed as mean ± SEM.

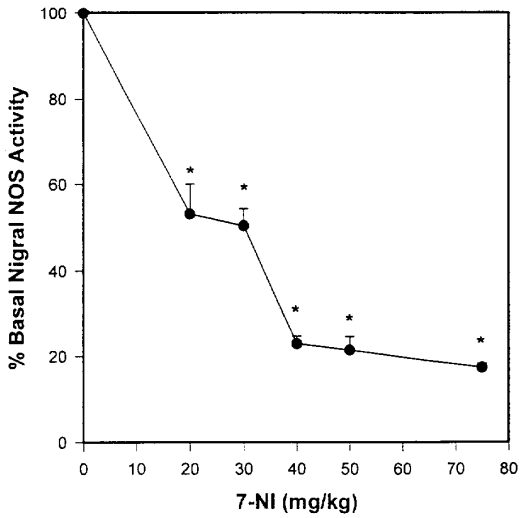


Fig. 1. Nigral NOS activity–dose–response curve following a single i.p. injection of varying doses of vehicle or 7-NI with dissection 30 min post-injection. Values are expressed as mean ± SEM with $n = 4$ or 5 for each point. *$p < 0.05$.

## RESULTS

*Inhibition of nigral NOS by 7-NI*

Prior to examining the effect of systemic 7-NI on the neurotoxicity of malonate on nigral dopaminergic neurons, inhibition of nigral NOS activity was assessed to determine an optimal dosing regimen that would provide maximal and sustained enzyme inhibition. Basal nigral NOS activity in vehicle treated animals was found to be 3.5 ± 0.2 nmol/mg/hr. Figure 1 represents the dose–response relationship between systemic 7-NI and nigral NOS activity expressed as the per cent basal enzyme activity observed 30 min following injection of 7-NI (20, 30, 40, 50 and 75 mg/kg; i.p.). Maximal nigral NOS inhibition was obtained following a dose of 40 mg/kg 7-NI (22.9 ± 1.8% basal). Based on this dose–response relationship, both a submaximal (20 mg/kg) and maximal (50 mg/kg) dose of 7-NI were used in subsequent malonate neurotoxicity experiments. Due to the short duration of action of 7-NI (MacKenzie *et al.*, 1994), nigral NOS activity was also measured 4.5 hr post-injection of 7-NI (20 and 50 mg/kg) to determine the degree of enzyme inhibition at the end of this time period. Doses of 20 and 50 mg/kg resulted in 86.9 ± 5.4% and 69.9 ± 3.8% of basal NOS activity, respectively, indicating partial recovery of enzyme activity 4.5 hr post-injection. In view of these results and those of Mackenzie *et al.* (1994), 7-NI (20 or 50 mg/kg i.p.) was given 30 min prior to nigral malonate infusion with two subsequent injections 4 hr apart. Thus, three injections of the same dose of 7-NI were given to each animal in this treatment group.

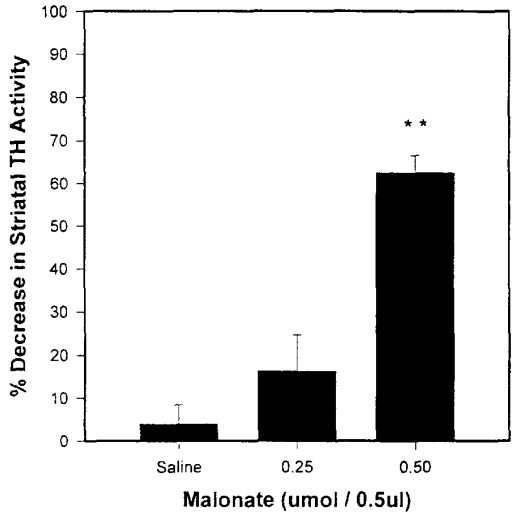


Fig. 2. Per cent decrease in striatal TH activity measured seven days following intranigral infusions of saline or varying doses of malonate. Values are expressed as per cent decrease in striatal TH activity from the contralateral (unlesioned) side. Data are mean ± SEM with $n = 4$ or 5 for each point. $^{**}p < 0.01$.

*Intranigral infusions of malonate*

Basal TH activity in the contralateral (unlesioned) striatum was found to be 614 ± 30 pmol/mg/hr, a value which was not significantly different from that in naive animals (Connop *et al.*, 1995). These values are in agreement with those reported by Vrana *et al.* (1992) who used an HPLC assay to measure TH activity. No difference in contralateral striatal TH activity was observed between any of the animal treatment groups. Figure 2 represents the per cent decrease in ipsilateral striatal TH activity measured 7 days following intranigral infusions of malonate (0.25 and 0.5 μmol). The reductions in striatal TH activity measured 4 and 7 days post-infusion of malonate (0.5 μmol) were not significantly different (58.1 ± 5.2% and 62.5 ± 3.9%, respectively). Thus, in subsequent malonate infusion studies, striatal TH activity was assessed four days post-infusion.

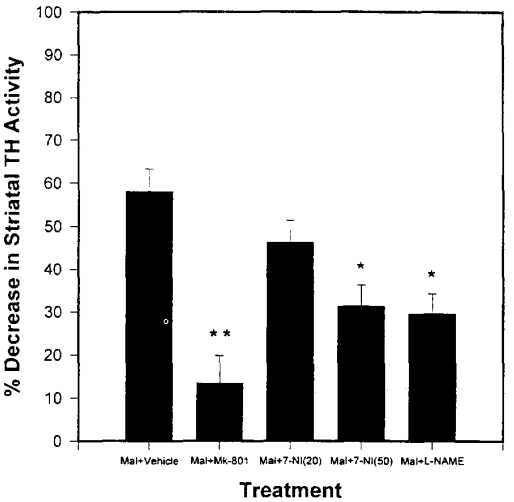


Fig. 3. Per cent decrease in striatal TH activity measured 4 days following intranigral infusions of malonate (0.5 μmol/0.5 μl) in the presence of vehicle, MK-801 (5 mg/kg), 7-NI (20 and 50 mg/kg) and L-NAME (250 mg/kg) pretreatment. Values are expressed as per cent decrease in striatal TH activity from the contralateral (unlesioned) side. Data are mean ± SEM with $n = 4$ to 6 for each point. $^{*}p < 0.05$, $^{**}p < 0.01$.

*Pretreatment studies on the effect of intranigral malonate*

In the present study, an MK-801 pretreatment regimen (2 × 5 mg/kg, i.p.) which was found to be neuroprotective against striatal malonate infusions (Greene *et al.*, 1993) was used to evaluate NMDA receptor involvement in nigral malonate toxicity. The decrease in striatal TH activity following an infusion of 0.5 μmol malonate into the SNc of vehicle pretreated animals (58.1 ± 5.2%) was significantly reduced by MK-801 pretreatment (13.5 ± 6.4%) indicating a neuroprotective effect (Fig. 3). To determine the role of NO in malonate-induced nigral toxicity, animals were pretreated with two doses of 7-NI (3 × 20 or 3 × 50 mg/kg, i.p.) based on the dosing regimen described previously. Pretreatment with 20 mg/kg 7-NI resulted in a 46.3 ± 5.1% decrease in striatal TH activity following intranigral malonate (0.5 μmol) infusion. This was not significantly different from values obtained with malonate (0.5 μmol) infusion into vehicle pretreated animals (Fig. 3). Pretreatment with a higher dose of 7-NI (50 mg/kg, i.p.) resulted in a 31.4 ± 4.9% decrease in striatal TH activity following intranigral infusion of malonate. Thus, a significant neuroprotective effect was observed at the higher but not lower dose of 7-NI. Pretreatment with L-NAME (250 mg/kg) which we have previously shown to inhibit brain NOS activity by more than 86% for at least 24 hr (Connop *et al.*, 1995) was used to examine the effect of a non-selective NOS inhibitor on nigral malonate toxicity. This L-NAME pretreatment resulted in a 29.6 ± 4.7% decrease in striatal TH activity following an infusion of malonate (0.5 μmol). This value was significantly less than the values obtained following malonate (0.5 μmol) infusion into vehicle pretreated animals (58.1 ± 5.2%) (Fig. 3). Thus, the toxicity of focal infusions of malonate into the SNc, as assessed by per cent decrease in striatal TH activity, was attenuated by MK-801, a high dose of 7-NI (50 mg/kg) and L-NAME.

## DISCUSSION

In the present study, we report that unilateral focal infusions of the competitive SDH inhibitor malonate (0.5 μmol/0.5 μl; 1 M) into the SNc of the rat produces substantial neurotoxicity as assessed by depletion of ipsilateral striatal TH activity. Previously, we have demonstrated that intranigral infusions of NMDA

receptor agonists resulted in depletions of striatal TH activity which correlated with the loss of TH immunoreactivity in the SNc, thus confirming an association between loss of striatal TH activity and degeneration of the nigrostriatal pathway (Connop *et al.*, 1995). A lower dose of malonate (0.5 M) was not found to be neurotoxic, an observation which is in agreement with the finding of Greene and Greenamyre (1995a) who showed that a dose of less than 0.5 M (0.6 $\mu$mol/2 $\mu$l) produced little or no toxicity when injected into the striatum. It was suggested that this may be due to metabolism of malonate within the brain and/or the accumulation of the endogenous substrate, succinate, which may successfully compete with lower doses of malonate (Greene and Greenamyre, 1995a).

To determine the role of NMDA receptor activation in malonate toxicity to dopaminergic neurons *in vivo*, the effect of MK-801 was tested. In this study, the MK-801 pretreatment regimen was based on a protocol which had been found to protect striatal neurons against infusions of malonate independent of the hypothermic effects induced by MK-801 (Greene *et al.*, 1993; Greene and Greenamyre, 1995a). Pretreatment with MK-801 protected nigral neurons against malonate toxicity and implies that, as in the striatum (Beal *et al.*, 1993b; Greene *et al.*, 1993; Greene and Greenamyre, 1995a; Henshaw *et al.*, 1994), malonate is toxic to dopaminergic neurons *in vivo* through an indirect or "secondary" activation of the NMDA receptor. Similarly, Zeevalk *et al.* (1995) have demonstrated that the irreversible SDH inhibitor, 3-NPA, produced a dose-dependent toxicity on cultured mesencephalic dopamine neurons, an effect which was mediated through the NMDA receptor.

Although some studies employing neuronal culture, brain slices and models of ischemia have provided evidence that NO may play an intermediary role in NMDA receptor-mediated toxicity (Dawson *et al.*, 1991), other reports have suggested that NO is not involved (Demerle-Pallardy *et al.*, 1991). In the present study the role of NO formation in malonate-induced toxicity to nigral dopaminergic neurons was examined following pretreatment with both the selective neuronal NOS inhibitor, 7-NI, and the non-selective NOS inhibitor, L-NAME. Based on the finding that an intrastriatal infusion of 1 $\mu$mol malonate caused a decrease in ATP levels 3 hr post-infusion with a return to basal levels within 12 hr (Beal *et al.*, 1993b), NOS inhibitor pretreatment regimens that would maintain decreased nigral NOS activity for approximately 12 hr were used. A dose of 20 mg/kg 7-NI which resulted in 47% inhibition of nigral NOS activity did not alter malonate toxicity, however, a dose of 50 mg/kg 7-NI which inhibited nigral NOS by 79% was found to be neuroprotective. A higher dose of 7-NI could not be examined due to the high rate of delayed mortality following surgery. Pretreatment with L-NAME which was previously found to inhibit brain NOS activity by greater than 86% (Connop *et al.*, 1995) was also neuroprotective against the toxicity associated with nigral infusions of malonate. This is in agreement with reports that $N^{\omega}$-nitro-L-arginine attenuates striatal malonate lesions in rats (Maragos and Silverstein, 1995) and that 7-NI protects against MPTP-induced neurotoxicity in mice (Schulz *et al.*, 1995). The fact that both malonate and MPTP neurotoxicity can be attenuated by the selective neuronal NOS inhibitor, 7-NI, implies that NO formation by the neuronal isoform of NOS may be neurotoxic. Immunohistochemical mapping of NOS containing neurons in the rat brain have shown that NOS does not co-localize with nigral dopaminergic neurons and that there is a sparse network of NOS positive fibers in the SNc with essentially no cell body staining (Vincent and Kimura, 1992; Johnson and Ma, 1993). In addition, Iadecola *et al.* (1993) have reported that NOS containing neurons possess a large number of processes that appear to form an intricate network with cerebral blood vessels. This suggests that NO formation in the SNc may originate from dendritic and axonal processes of relatively distant neurons either under basal conditions or in response to local malonate-induced depolarization. This formation of NO from NOS containing fibers may affect the viability of dopaminergic neurons exposed to malonate. Potential mechanisms by which NO would be neurotoxic in this paradigm may involve formation of peroxynitrite (Beckman *et al.*, 1990), ADP ribosylation of cellular proteins (Brune and Lapetina, 1989) or inhibition of mitochondrial function (Bolaños *et al.*, 1994).

In contrast to the present study, we have previously reported that the excitotoxic action of NMDA and QUIN on the dopaminergic neurons of the nigrostriatal pathway is potentiated by systemic L-NAME (Connop *et al.*, 1995) and similarly, that the action of NMDA on this pathway is augmented by a low dose of 7-NI (Connop *et al.*, 1994). The fact that both L-NAME and 7-NI potentiated NMDA and QUIN toxicity yet attenuated malonate toxicity is of interest since the neurotoxic properties of these compounds all appear to be NMDA receptor-mediated. However, a crucial difference between the toxic actions of direct acting NMDA receptor agonists and the indirect acting mitochondrial inhibitors is the absence of observable neuronal excitation with the latter. In the present study, a dose of malonate which resulted in a 58% decrease in striatal TH activity was not accompanied by circling or barrel rolling behaviour which was previously observed with doses of NMDA and QUIN that produced similar decreases in striatal TH activity (Connop *et al.*, 1995). In the brain, neuronal excitation has been found to produce activity dependent vasodilation in order to compensate for increased neuronal energy requirements (see Iadecola, 1993). In situations where extensive neuronal excitation is present, such as infusion of NMDA or QUIN, blockade of activity-dependent vasodilation through inhibition of NOS activity (Dirnagl *et al.*, 1993; Rigaud *et al.*, 1993; Faraci and Breese, 1994) may be

detrimental by producing metabolic impairment that would augment excitotoxicity. This is supported by the observation that mild metabolic compromise markedly potentiates direct glutamate receptor-mediated toxicity (Greene and Greenamyre, 1995b). Thus, in our previous studies, the potential neuroprotective action of a NOS inhibitor on NMDA receptor-mediated toxicity may have been overcome by the loss of accompanying activity-dependent vasodilation which would result in compounding metabolic impairment. Thus, an attractive hypothesis is that NOS inhibitors will protect against indirect (i.e. malonate) but not direct NMDA receptor-mediated toxicity. Further studies on the effect of mitochondrial inhibitors vs NMDA agonists on activity-dependent vasodilation and resultant metabolic impairment may provide insight on differential effects of NOS inhibition on the toxicity of these compounds.

In summary, malonate-induced degeneration of the nigrostriatal pathway appears to be mediated, at least in part, through the NMDA receptor and nitric oxide formation. Further studies on mechanisms of neuroprotection against metabolic inhibitor-mediated toxicity on the nigrostriatal pathway may be useful if deficits in mitochondrial function are involved in the etiology of Parkinson's disease.

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