Nitric Oxide Is Involved in Acetylcholinesterase Inhibitor-Induced Myopathy in Rats[1]

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ABSTRACT

Excess activation of muscle nicotinic acetylcholine receptors due to genetic mutations, as seen in slow channel congenital myasthenic syndrome, or acetylcholinesterase (AChE) inhibition results in muscle cell degeneration. Our recent work showed that nitric oxide synthase (NOS) inhibitors prevent nicotine-induced muscle cell death in culture. In the present study, we examined the effects of NOS inhibition on nicotinic receptor-mediated myopathy in vivo. Rats injected with the AChE inhibitor paraoxon demonstrate a 90-fold increase in the number of dying muscle cells compared with control as evidenced histologically by centralized nuclei and the presence of degenerating profiles. Coadministration of the nonspecific NOS inhibitor nitro-L-arginine methyl ester or the neuronal NOS-specific inhibitor 7-nitroindazole dramatically reduced the presence of such degenerating profiles to ~20% of that seen with paraoxon alone. These results show that inhibition of NOS, as well as neuronal NOS, significantly reduces AChE inhibitor-induced muscle cell degeneration, suggesting that increased nitric oxide production mediates such myopathy.

Nicotinic acetylcholine receptors play a prominent role in synaptic transmission at the neuromuscular junction and have also been implicated in cellular differentiation, maturation, and degeneration. In humans, the excess activation of nicotinic acetylcholine receptors, observed in slow channel congenital myasthenic disorder, results in severe myopathy that is associated with muscle fatigue and eventual paralysis (Engel et al., 1982). Similarly, chronic fatigue is a symptom of Gulf War syndrome, a disorder proposed to result from exposure to acetylcholinesterase (AChE) inhibitors (Sapolsky, 1998). Although the degree of muscle viability in these patients has not been examined, it is well established that rodents exposed to AChE inhibitors demonstrate muscle fiber degeneration (Ariens et al., 1969; Fenichel et al., 1972; Leonard and Salpeter, 1979). For example, injection of paraoxon, the active metabolite of the insecticide parathion, results in marked muscle degeneration (Ariens et al., 1969), the severity of which is correlated with the duration of cholinesterase inhibition (Wecker et al., 1978).

Several experimental approaches indicate that administration of AChE inhibitors leads to muscle degeneration by enhancing the concentration of acetylcholine at the synaptic cleft. Prior nerve transection or administration of the nicotinic receptor antagonist *d*-tubocurarine both prevented AChE inhibitor-induced myopathy (Ariens et al., 1969). As well, AChE inhibitor-mediated degenerative effects were blocked by administration of the acetylcholine transport blocker hemicholinium, which depletes nerve terminal acetylcholine (Fenichel et al., 1972). Furthermore, agents that reactivate AchE activity, such as pyridine-2-aldoxime methochloride, attenuated myopathy (Wecker et al., 1978).

Although the majority of intracellular events that mediate nicotinic receptor-induced myopathy have not yet been identified, calcium may play a role. Evidence for this stems from studies that show that carbachol-induced muscle degeneration in culture is prevented by removing calcium from the bathing solution (Leonard and Salpeter, 1979, 1982). Moreover, generation of the free radical messenger nitric oxide (NO) has been shown to play an intermediary role in nicotinic receptor-mediated muscle cell death in culture (El-Dada and Quik, 1997). Similarly, NO has been implicated in cytotoxicity in the central nervous system (CNS) (Garthwaite and Boulton, 1995; Schulz et al., 1996; Brenman and Bredt, 1997). For example, NO mediates *N*-methyl-D-aspartate (NMDA) receptor-induced excitotoxicity (Dawson et al., 1991) where calcium influx resulting from receptor activation stimulates NO production and leads to neuronal death.

ABBREVIATIONS: AChE, acetylcholinesterase; NO, nitric oxide; CNS, central nervous system; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; L-NAME, nitro-L-arginine methyl ester; 7-NI, 7-nitroindazole.

NO is synthesized from L-arginine via the enzyme nitric oxide synthase (NOS). Three different NOS isoforms have been identified: two constitutive, calcium/calmodulin-dependent forms, endothelial (eNOS) and neuronal NOS (nNOS), and an inducible form. Both nNOS and eNOS have been localized to skeletal muscle (Kobzik et al., 1994, 1995; Silvagno et al., 1996) and have been demonstrated to affect muscle contractility (Kobzik et al., 1994). In addition, nNOS is concentrated at the synaptic junction of motor end plates of skeletal muscle, colocalizing with nicotinic receptors (Brenman et al., 1995, 1996).

Chronic muscle stimulation increases nNOS protein expression and NOS activity (Reiser et al., 1997). Although enhanced NOS activity under physiological conditions may be essential for normal muscle function, it is plausible that excessive receptor stimulation increases NO production to pathological levels. Such a hypothesis is consistent with our earlier studies that implicate NO in receptor-mediated muscle cell death in culture (El-Dada and Quik, 1997). The purpose of the present study was to investigate whether NOS inhibitors prevent myopathy induced by the AChE inhibitor paraoxon in vivo.

Experimental Procedures

Materials. Paraoxon, nitro-L-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI), hematoxylin, eosin, leupeptin, aprotinin, pepstatin, calmodulin, EDTA, phenylmethylsulfonyl fluoride, NADPH, and β-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, MO), [^{3}H]arginine from New England Nuclear (Boston, MA), and Dowex 50W-X8 resin from Bio-Rad Laboratories (Mississauga, Ontario, Canada). All other chemicals were purchased from standard commercial sources.

Animals. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats weighing 180 to 200 g were used. Paraoxon or saline was injected i.p. once daily for 4 days, unless indicated otherwise, at doses of 0.25 or 0.4 mg/kg. The rats showed few adverse effects with the i.p. route of administration. These doses were selected based on the results of previous studies that 1) demonstrated the occurrence of myopathy with such treatments (Fenichel et al., 1972; Laskowski et al., 1975, 1977; Wecker et al., 1978). Furthermore, 2) they showed an almost complete decline in AChE activity immediately after the administration of paraoxon that returned to about 20 to 50% of control after 24 h, depending on the specific skeletal muscle group studied; these levels of inhibition persisted with repeated administration (Wecker and Dettbarn, 1976; Wecker et al., 1978).

L-NAME (5–20 mg/kg) or saline was injected i.p. 1 h before and 6 h after each paraoxon injection. This scheduling regimen was selected because previous studies had shown that two systemic injections of L-NAME given 8 h apart produced an inhibition that was rapid in onset and lasted for at least 24 h (Connop et al., 1995). 7-NI (5–20 mg/kg), dissolved in peanut oil, or vehicle was injected i.p. 1 h before and 4 h after each paraoxon injection. 7-NI was administered 4 h rather than 6 h after paraoxon administration because of the shorter duration of action of this NOS inhibitor compared with L-NAME. Previous reports had shown that maximal inhibition of NOS activity was obtained 30 to 60 min after injection of 7-NI, with a 30% inhibition remaining after 4 h (Connop et al., 1996; Kalisch et al., 1996; Przedborski et al., 1996). The concentration range for L-NAME and 7-NI used in these experiments was within that reported in the literature (Connop et al., 1995, 1996; Kalisch et al., 1996; Przedborski et al., 1996; Taraska and Finnegan, 1997).

Tissue Preparation. Twenty-four hours after the last injection, animals were euthanized with CO_2, and the diaphragm and leg muscles, including soleus, gastrocnemius, and plantaris, were dissected. Muscle tissue was washed in 0.9% saline, mounted in histoprep mounting medium, snap frozen in liquid N_2-cooled isopentane, and sectioned at 10 μm on a cryostat. Sections were fixed in 100% ethanol for 10 min, and washed in running tap water for 5 min before staining. Sections were then incubated in Harris' hematoxylin for 15 to 20 min, washed in tap water, incubated in acid alcohol, and rinsed in tap water, followed by incubation in ammonia water. Sections were rinsed again before being stained in eosin for 5 min, washed and dehydrated in graded series of ethanol, cleared in xylene, and coverslipped using Depex.

Cell Counts. A minimum of 10 grids from each tissue section was counted at a final magnification of 100×. A cell was considered degenerating if it had centralized nuclei, macrophage infiltration, or eosinophilia (Ariens et al., 1969). The number of degenerating profiles is expressed as the number of lesions per 1000 total cells.

NOS Assay. NOS activity was determined by monitoring the conversion of [^{3}H]arginine to [^{3}H]citrulline as previously described by Kobzik et al. (1995) with minor modifications. Rat skeletal muscle tissue was homogenized in 4 volumes of 50 mM HEPES (pH 7.5) containing 5 mM KCl, 120 mM NaCl, 10 mM glucose, 1 mM EDTA, 10 μg/ml pepstatin A, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride, and 5 mM β-mercaptoethanol. Homogenate was then incubated in 50 mM HEPES (pH 7.5) containing 5 mM KCl, 120 mM NaCl, 10 mM glucose, 20 μg/ml calmodulin, 2 mM NADPH, 2.5 mM $CaCl_2$, and 1 μCi/ml [^{3}H]arginine for 45 min at 37°C. The reaction was terminated by addition of 1 ml of 20 mM HEPES (pH 5.5) containing 2 mM EDTA. The samples were applied to 1-ml columns of Bio-Rad AG 50W-X8 resin and eluted with 2×1.0 ml of distilled water. [^{3}H]Citrulline was quantified by liquid scintillation spectroscopy of the 3×1 ml flow through.

Data Analysis. All values are expressed as the mean $\pm$ S.E. Results of cell counts were analyzed using a one-way ANOVA followed by a Fisher's protected least-significant difference test. Results of the NOS assay were analyzed using a Student's t test.

Results

Paraoxon Induces Muscle Cell Degeneration in a Time- and Dose-Dependent Manner. Adult rats were injected once daily with 0.25 or 0.4 mg/kg of the AChE inhibitor paraoxon for 2, 4, or 8 days. Such a treatment was selected because previous studies by Wecker and Dettbarn (1976) had shown that paraoxon at somewhat lower doses had resulted in an almost complete inhibition of AChE activity 30 min after administration with 20 to 50% declines 24 h after the initial injection. Degenerating muscle fibers were identified based on the presence of centralized nuclei, eosinophilia, and macrophage infiltration (Ariens et al., 1969). There was no significant increase in the number of degenerating muscle cells with either dose of paraoxon after 2 days of treatment in diaphragm or leg muscle (Fig. 1). However, the number of degenerating fibers was significantly enhanced over control after administration of 0.4 mg/kg paraoxon for 4 days in muscle sections from both diaphragm and leg (Figs. 1–3). Considerably less degeneration was observed in leg muscle compared with diaphragm as has been reported previously (Ariens et al., 1969; Wecker and Dettbarn, 1976). Similar to 4 days of treatment, injection of 0.4 mg/kg paraoxon for 8 days resulted in a significant increase in the number of degenerating profiles compared with control, although this was not significantly different from the 4-day time point (Fig. 1). We, therefore, used an injection regimen of 0.4 mg/kg

Diaphragm

Leg
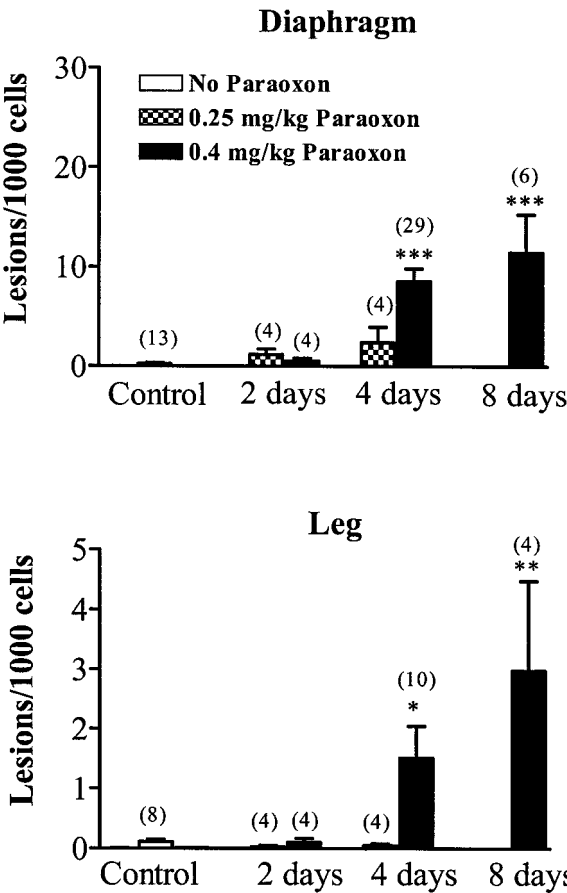
Fig. 1. Effect of varying dose and time of paraoxon treatment on diaphragm and leg muscle degeneration. Rats were injected with either vehicle, 0.25 or 0.4 mg/kg paraoxon for 2, 4, or 8 days. The number of degenerating fibers per 1000 cells was counted. The columns represent mean ± S.E. of the number of animals indicated in parentheses. Significance of difference from control: *$P < .05$, **$P < .01$, ***$P < .001$.

Fig. 2. Photomicrographs of diaphragm muscle sections treated with vehicle, paraoxon (0.4 mg/kg), and paraoxon + L-NAME (10 mg/kg). Arrows indicate degenerating fibers. Scale bar, 100 μm.
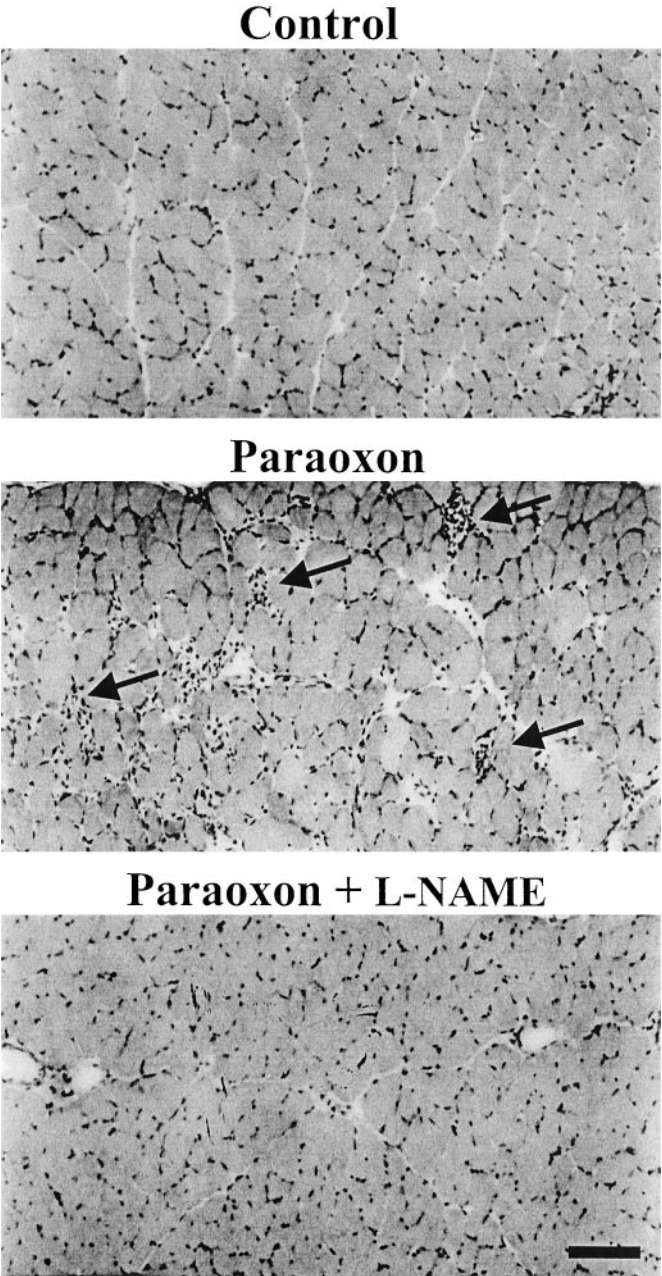
daily for 4 days for all subsequent experiments to produce a significant level of degeneration.

L-NAME Decreases Paraoxon-Induced Degeneration. To determine whether NO mediates paraoxon-induced myopathy, rats were treated with the reversible NOS inhibitor L-NAME 1 h before and 6 h after paraoxon treatment. L-NAME injections (20 mg/kg) alone produced no effect on muscle cell morphology compared with control (Table 1). However, L-NAME at a dose of 10 mg/kg significantly decreased paraoxon-induced muscle fiber degeneration in sections from both diaphragm (Figs. 2 and 4) and leg (Figs. 3 and

TABLE 1
Effect of NOS inhibitor administration on muscle cell morphology

Each animal received saline injections daily for 4 days and either vehicle or the indicated NOS inhibitor at the dose specified 1 h before and 6 h (L-NAME), or 4 h (7-NI) after saline injection. Animals were sacrificed 24 h after the last injection, and the tissue was sectioned, stained, and counted for the presence of degenerating profiles. Each value represents the mean ± S.E. of the number of animals (*n*) indicated in parentheses.

NOS Inhibitor	Dose	Degenerating Muscle Cells Lesions/1000 cells	
		Diaphragm	Leg
	mg/kg		
None	0	0.1 ± 0.1 (5)	0.09 ± 0.04 (9)
L-NAME	20	0 (4)	0.17 ± 0.11 (5)
7-NI	20	0 (5)	0 (4)

4). Higher concentrations of L-NAME did not further reduce paraoxon-induced damage. In fact, there appears to be somewhat less of a protective effect at 20 mg/kg L-NAME.
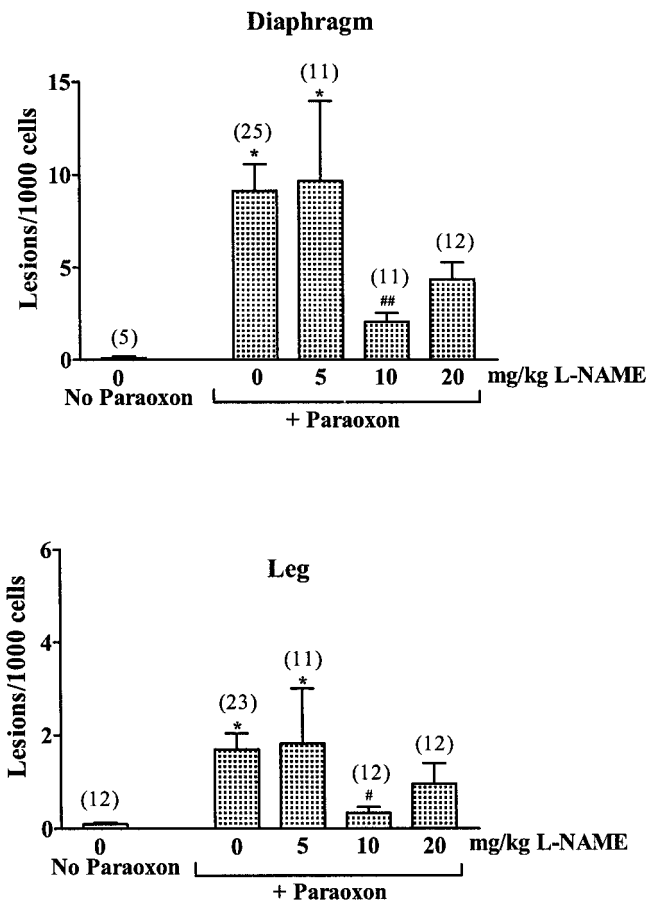
Neuronal NOS Mediates AChE Inhibitor-Induced Myopathy. The results with L-NAME suggest that NO mediates AChE inhibitor-induced myopathy, at least in part. Because the neuronal isoform has been shown to colocalize with nicotinic receptors (Brenman et al., 1995, 1996), we sought to determine whether activation of nNOS may mediate paraoxon-induced muscle degeneration. Rats were treated with 7-NI, an NOS inhibitor specific for the neuronal isoform, 1 h before and 4 h after paraoxon treatment every day for 4 days. As seen with L-NAME, 7-NI administration (20 mg/kg) alone had no effect on muscle tissue degeneration

Control

Paraoxon
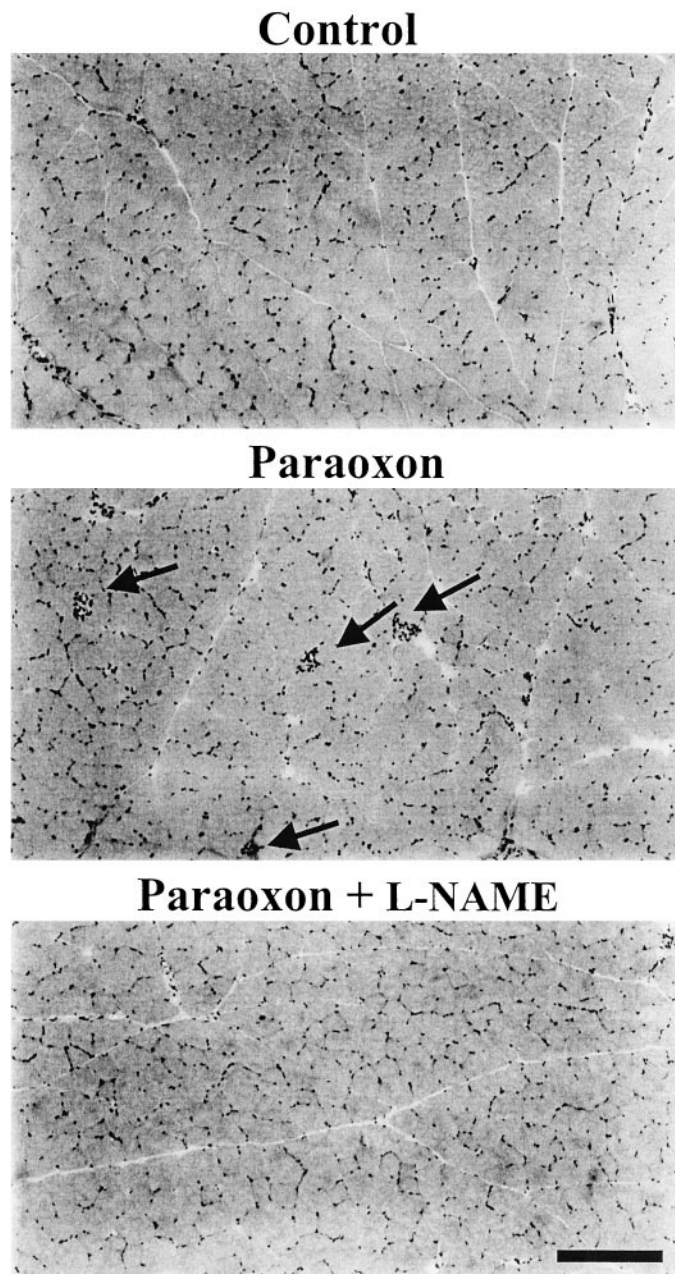
Paraoxon + L-NAME

Fig. 3. Photomicrographs of leg muscle sections treated with vehicle, paraoxon (0.4 mg/kg), and paraoxon + L-NAME (10 mg/kg). Arrows indicate degenerating fibers. Scale bar, 200 μm.
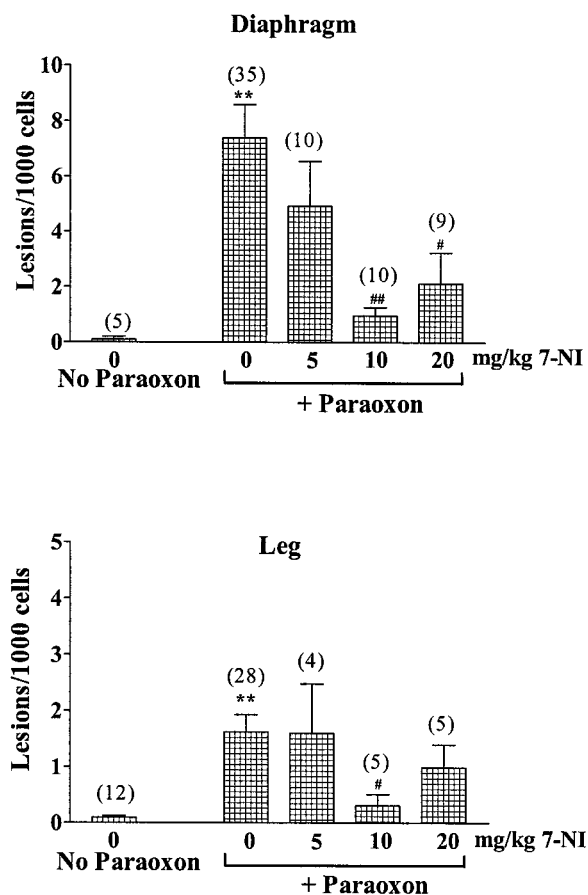

Fig. 4. Effect of L-NAME on paraoxon-induced muscle degeneration. Rats were injected with L-NAME (doses in mg/kg listed below each column) 1 h before and 6 h after paraoxon treatment (0.4 mg/kg). Note that 10 mg/kg L-NAME significantly reduced paraoxon-induced myopathy in both diaphragm and leg. The columns represent mean $\pm$ S.E. of the number of animals indicated in parentheses. Significance of difference from control: *$P < .05$. Significance of difference from paraoxon in the absence of L-NAME: #$P < .05$, ##$P < .01$.

(Table 1). The ability of 7-NI to attenuate paraoxon-induced myopathy was similar to that observed with L-NAME. At concentrations of 7-NI lower than 10 mg/kg, there was no significant reversal of paraoxon-induced myopathy. However, 7-NI at a dose of 10 mg/kg significantly reduced paraoxon-induced muscle cell death in both diaphragm and leg sections (Fig. 5). A dose of 20 mg/kg 7-NI also significantly reduced the incidence of degenerating profiles in diaphragm compared with paraoxon treatment alone, although to a lesser degree than the 10-mg/kg dose. This, however, was not the case for leg muscle sections, where the extent of muscle degeneration observed after treatment with 20 mg/kg 7-NI was not significantly different from paraoxon treatment alone, although it was also not significantly different from

control. The maximal protective effect of 7-NI is comparable with that produced by L-NAME, suggesting that paraoxon-induced muscle cell degeneration is due to the activation of nNOS.

NOS Activity in Control and Paraoxon-Treated Muscle Tissue. To determine whether paraoxon treatment resulted in an increase in muscle NOS activity, we assessed the calcium-dependent NOS-mediated conversion of [³H]arginine to [³H]citrulline in homogenized muscle tissue from control and paraoxon-treated animals. Rats were injected i.p. once daily with paraoxon (0.4 mg/kg) for 4 days, a similar regimen in which myopathy was observed (Fig. 1). There was a small (13%) although not statistically significant increase in muscle NOS activity in paraoxon-treated rats (1.96 $\pm$ 0.1 fmol/mg of tissue, $n = 5$) compared with control (1.74 $\pm$ 0.2 fmol/mg of tissue, $n = 4$).

Discussion

The present study demonstrates that paraoxon-induced muscle cell degeneration is decreased by exposure to the nonspecific NOS inhibitor L-NAME. Moreover, the nNOS-specific inhibitor 7-NI produces an equivalent level of protection as L-NAME against AChE inhibitor-induced myopathy,

Fig. 5. Effect of 7-NI on paraoxon-induced muscle degeneration. Rats were injected with 7-NI (doses in mg/kg listed below each column) 1 h before and 4 h after paraoxon treatment (0.4 mg/kg). Note that 10 mg/kg 7-NI significantly reduces paraoxon-induced myopathy in both diaphragm and leg, and 20 mg/kg 7-NI decreases myopathy in diaphragm. The columns represent mean ± S.E. of the number of animals indicated in parentheses. Significance of difference from control: $**P < .01$. Significance of difference from paraoxon in the absence of 7-NI: $^{\#}P < .05$, $^{\#\#}P < .01$.

suggesting that activation of nNOS is involved. These experiments extend our previous results with muscle cells in culture (El-Dada and Quik, 1997) to the in vivo situation, and may suggest that excess stimulation of nicotinic receptors after AChE inhibitor administration results in increased nNOS activity to induce muscle degeneration.

As an approach to directly test this possibility, we measured NOS activity in muscle tissue from control and paraoxon-treated animals. There was a small increase in enzyme activity after paraoxon treatment, although it was not significantly different from control. Our data with the nNOS-specific inhibitor 7-NI suggest that nNOS is the primary NOS isoform involved in AChE-induced myopathy. Because muscle tissue contains both the eNOS and nNOS isoforms (Kobzik et al., 1995), the present results may suggest that an increase in muscle nNOS activity in paraoxon-treated tissue is masked by high basal levels of eNOS, as well as nNOS. Experiments with nNOS knockout mice may prove useful in evaluating the involvement of nNOS in AchE-induced myopathy, although there is the possibility of compensatory changes by other NOS isoforms.

Indirect evidence from other in vivo studies has also implicated NO in both cardiac and skeletal muscle injury. One

major pathway through which NO induces cellular injury is through its reaction with superoxide anion and the formation of peroxynitrite, a highly reactive free radical species that is known to mediate oxidative injury. Consistent with this role of NO, peroxynitrite levels are elevated in cardiac muscle after ischemia-reperfusion injury (Wang and Zweier, 1996). Moreover, exposure to the NOS inhibitor L-NAME prevents such reperfusion-induced injury to cardiac myocytes (Wang and Zweier, 1996). Pretreatment with L-NAME also increases cell viability in gastrocnemius muscle after limb ischemia-reperfusion injury (Knight et al., 1997). NO has been implicated in other models of muscle injury as well. For example, L-NAME has been shown to rescue muscle cells from mechanical crush injury (Rubinstein et al., 1998). Similarly, in a mouse model of cachexia, a condition characterized by weight loss primarily due to muscle wasting, administration of the NOS inhibitor nitro-arginine prevented myopathy (Buck and Chojkier, 1996).

In addition to a destructive role in the periphery, NO has been implicated in many neurodegenerative processes in the CNS such as those involved in Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and cerebral ischemia (Dawson et al., 1991; Lipton and Rosenberg, 1994; Schulz et al., 1996). There is significant support for the idea that NMDA receptor-mediated neurotoxicity, which is thought to contribute to ischemic damage, occurs via NO. For example, NOS inhibitors prevent glutamate neurotoxicity in primary cortical cultures (Dawson et al., 1991). Moreover, administration of NOS inhibitors decreased the severity of NMDA-mediated excitotoxic lesions in mouse striatum (Ayata et al., 1997). Our data demonstrating that excess nicotinic receptor stimulation is attenuated by NOS inhibitors are consistent with the results from these models of CNS excitotoxicity.

AChE inhibitors such as paraoxon have been reported to have several actions in relation to nicotinic acetylcholine receptors. First, cholinesterase inhibitors enhance muscle responses to nerve stimulation due to the prolonged action of acetylcholine within the synaptic cleft (Clark and Hobbiger, 1983). In addition, such compounds act as weak noncompetitive agonists on acetylcholine receptors at low concentrations (Rao et al., 1987; Storch et al., 1995) unrelated to their actions as cholinesterase inhibitors. Although AChE inhibitors have low efficacy at activating the receptor, they do potentiate receptor responses to acetylcholine (Storch et al., 1995). Therefore, in addition, to the excess presence of acetylcholine in the synaptic cleft, the cholinesterase inhibitor itself may further increase the response of the receptor, resulting in excessive stimulation and thereby leading to calcium entry and ultimately muscle cell degeneration.

In contrast to the above-mentioned effects, there have also been reports that AChE inhibitors block nicotinic receptor-mediated activity, particularly at high concentrations. Katz et al. (1997) showed that paraoxon bound to and desensitized muscle-type nicotinic receptors in membrane preparations with an IC_{50} of 300 μM, whereas Van Den Beukel et al. (1998) showed that paraoxon reduced nicotinic receptor-mediated currents in neuronal cell cultures with an IC_{50} value of 100 μM. If we assume a uniform distribution of paraoxon after injection of our standard dose of 0.4 mg/kg i.p., we estimate a tissue concentration of approximately 1 to 2 μM.

Thus, a direct inhibitory action of paraoxon at the level of the receptor appears unlikely in the present experiments.

Previous studies have demonstrated that nicotinic acetylcholine receptor activation results in a calcium influx, which may activate numerous intracellular calcium-dependent processes. Although the maximum calcium entry through nicotinic receptors only accounts for 2% of the total current, receptor stimulation may also activate voltage-gated calcium channels, resulting in a greater calcium influx (Decker and Dani, 1990). It is well known that calcium homeostasis is critical for maintaining cellular viability. Either lowering or raising intracellular calcium concentrations can result in cellular degeneration (McBurney and Neering, 1987). Therefore, excess activation of these receptors, as is seen after exposure to AChE inhibitors in vivo, may raise the intracellular calcium concentration to pathological levels. Such an interpretation is supported by the work of Leonard and Salpeter (1979, 1982) who showed that removal of calcium prevented nicotinic receptor-induced myopathy in vitro. The present results, as well as others, may now suggest that alterations in intracellular calcium subsequently trigger the activation of NOS.

The results show that a lower dose (10 mg/kg) of L-NAME reversed the toxic effects of paraoxon on muscle, whereas a higher dose (20 mg/kg) did not significantly reduce paraoxon-induced myopathy, although an approximate 50% decline in muscle degeneration was still observed. With 7-NI, on the other hand, there was still a significant decline in myopathy at the higher concentration, at least in diaphragm, albeit of a slightly smaller magnitude. NOS inhibitors on their own did not result in myopathy at any dose tested. These observations may suggest that the higher doses of the NOS inhibitors decrease NO production to levels below that necessary for optimal function only when the system is compromised as in the presence of an AChE inhibitor. Such an interpretation is consistent with studies that show that NO is involved in diverse biological functions ranging from development and homeostasis to degenerative effects (Garthwaite and Boulton, 1995; Brenman and Bredt, 1997). Another or alternate possibility may relate to the fact that L-NAME inhibits both nNOS and eNOS, whereas 7-NI preferentially inhibits nNOS. A reduction in nNOS activity may prevent myopathy; however, inhibition of eNOS may block vasodilation to enhance neurodegenerative effects (Brenman and Bredt, 1997).

A question that arises is the time requirements for AChE-induced degenerative effects. This is important because it may provide insight into the molecular mechanisms that mediate myopathy. In the CNS, it has been shown that an initial brief insult can generate a series of events to result in a delayed toxicity. For instance, a 5-min exposure to glutamate results in marked neurodegenerative effects 24 h later (Dawson et al., 1991). A similar mechanism may also apply in the current studies, especially in view of an extensive literature that shows that nicotinic receptor activation results in an initial excitatory response followed by a rapid desensitization, which may persist in the presence of continued AChE inhibition (Edmonds et al., 1995). As the effect of the AChE inhibitor diminishes over time, there may be a resensitization of receptor-mediated responses. Experiments with different treatment regimens will be necessary to determine whether exposure to short pulses of acetylcholine or chronic exposure to high levels of acetylcholine produce a more severe myopathy. However, because 1) paraoxon-induced myopathy is more robust than that which occurs in the presence of the more reversible AChE inhibitors such as neostigmine and physostigmine (Wecker et al., 1978), and 2) prolonged exposure to carbachol induces more severe degenerative effects in muscle cells in culture than a brief exposure period (Leonard and Salpeter, 1979), the extent of myopathy may represent a complex interplay between receptor stimulation, desensitization, and resensitization.

In summary, these studies show that NOS inhibition decreases paraoxon-induced muscle cell degeneration in rodents and support a role for nNOS in mediating this effect. These findings are the first to suggest that increased NO levels downstream of nicotinic receptor activation mediate agonist-induced myopathy in vivo. The current work may suggest that NOS inhibitors have the potential to provide therapeutic benefit in slow channel congenital myasthenic disorder.

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