Short communication

The neuronal NOS inhibitor L-MIN, but not 7-NINA, reduces neurotoxic effects of chronic intrastriatal administration of quinolinic acid

Terence Bazzett a,*, Adam Geiger b, Brian Coppola c, Roger Albin b,d

a Department of Psychology, SUNY Geneseo, 1 College Circle, Geneseo, NY 14454, USA
b Department of Neurology, University of Michigan, Ann Arbor, MI 48104, USA
c Department of Chemistry, University of Michigan, Ann Arbor, MI 48104, USA
d Geriatrics Research, Education and Clinical Center, Ann Arbor VAMC, Ann Arbor, MI 48104, USA

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Abstract

Rat striata were exposed to 15 mM quinolinic acid (QUIN), or QUIN plus the nitric oxide synthase inhibitors S-methyl-L-thiocitrulline dihydrochloride (L-MIN) or 7-nitroindazole monosodium salt (7-NINA) for 21 days. Co-administration of 100 µM or 1 mM L-MIN with QUIN significantly reduced lesion volume compared to QUIN alone. Co-administration of 1 µM or 10 µM L-MIN with QUIN had no significant effect. There was no significant effect of 7-NINA co-administered with QUIN compared to QUIN alone. L-MIN reduction of lesion volume supports the contention that neuronal nitric oxide synthase is a mediator of excitotoxic injury.

Keywords: NMDA receptor; Huntington’s disease; Excitotoxicity; Neuroprotection; Basal ganglia; Hydroxyl radical; Nitric oxide; Indazole

Under nonpathological conditions, nitric oxide (NO) acts as a calcium dependent neuromodulator and retrograde messenger following stimulation of the NMDA receptor complex [7,12]. However, excessive NO is speculated to interact with superoxide anions resulting ultimately in the production of neurotoxic hydroxyl radicals and nitrogen dioxide [7,18].

Inhibiting nitric oxide synthase (NOS), an essential enzyme for production of NO, as a means of reducing NMDA-induced neurotoxicity has produced conflicting results. Some researchers have found neuroprotection with NOS inhibitors [15,19,21] while others report no neuroprotection [17,18] or exacerbation of NMDA-induced toxicity [5,10,11]. These discrepancies may stem from at least two methodological problems. First, some compounds may affect endothelial NOS (eNOS), as well as neuronal (nNOS). An NOS antagonist induced reduction in endothelial NO may in turn reduce blood flow [20,23] introducing a potential variable to assessment of lesions attributed to NMDA receptor agonists. Second, variability in the neurotoxic effects of acute quinolinic acid (QUIN) injection, commonly used to induce NMDA receptor mediated lesions, may reduce reliability in assessing potentially neuroprotective compounds [9].

In the present study, we examined the neuroprotective potential of two separate NOS inhibitors. S-Methyl-L-thiocitrulline dihydrochloride (L-MIN) is known to be relatively specific for nNOS, whereas 7-nitroindazole monosodium salt (7-NINA) affects both nNOS and eNOS [19]. These compounds were co-administered with QUIN directly to the striatum using a chronic in-vivo microdialysis delivery system.

Male Sprague–Dawley rats (225–250 g) were anesthetized with ketamine/xylazine, and a stainless steel guide cannulae (8 mm, 21 ga) was stereotaxically implanted through the skull aimed at the right striatum (stereotaxic co-ordinates +0.5 mm anterior, +2.6 mm lateral from bregma, and 1 mm ventral from skull surface). Cannulae were held in place with dental acrylic anchored by four screws secured through the skull.

Six days after implantation of guide cannulae, Alzet osmotic pumps (model 2002) were filled with their respective solutions: 15 mM QUIN (n = 9), or 15 mM QUIN combined with one of the following: 1 µM 7-NINA (n = 4), 10 µM 7-NINA (n = 5), 100 µM 7-NINA (n =
6), 1 mM 7-NINA (n = 9), 10 mM 7-NINA (n = 5), 1 
µM L-MIN (n = 5), 10 µM L-MIN (n = 5), 100 µM
L-MIN (n = 9), 1 mM L-MIN (n = 9). Pumps were then
connected to chronic in vivo microdialysis probes. The
chronically implanted in-vivo microdialysis probe delivers
drug to the central region of the striatum via a 4 mm
length of dialysis fiber (approximately −3.5 to −7.5 mm
ventral from the skull surface). Design, construction and
implantation of the chronic dialysis probe apparatus are
described in detail elsewhere [1,2].

L-MIN and 7-nitroindazole (7-NI) were purchased from
Precision Biochemicals (Colton, CA). QUIN was pur-
chased from Sigma (St. Louis, MO). Conversion of 7-NI to
7-NINA was achieved using the methods of Silva and
colleagues [22]. Briefly, 7-NI was dissolved in
methanol/chloroform (3:1, v/v) + 14.5% 0.48 M sodium
hydroxide. The solution was then evaporated to dried
powder form in a rotary film evaporator at 35°C under
reduced pressure. 7-NINA was made fresh, 24–48 h prior
to use. All drugs were dissolved in phosphate-buffered
saline (PBS).

Twenty one days after implantation of the dialysis
probe apparatus, all rats were deeply anesthetized and
perfused through the heart with 200 ml PBS, followed by
300 ml 4% paraformaldehyde in phosphate buffer. Brains
were removed and post fixed for 24 h in a 4% paraformal-
dehyde solution and cryoprotected in a 20% sucrose solu-
tion.

Coronal tissue sections (40 µm) were collected from
brains frozen in dry ice and sliced on a sliding microtome.
Sections were collected through the entire rostral/caudal
extent of the striatum. Free-floating sections were then
stained for cytochrome oxidase activity as described previ-
ously [3].

Lesion volume was determined by multiplying the ro-
stral/caudal extent of decreased cytochrome oxidase stain-
ing by the average area of decreased cytochrome oxidase
staining through the striatum. Quantification of lesion vol-
ume was accomplished using computer assisted morphom-
etry on an MCID imaging system (Imaging Research, St.
Catherines, ONT).

Analysis of variance showed a significant effect of
L-MIN (F = 3.36; 4.32; P < 0.05) but not of 7-NINA
(F = 1.35; 5.32) on QUIN-induced lesion volume. Fisher’s
post-hoc comparison revealed a significantly reduced le-
sion volume in striata exposed to the two highest concen-
trations of L-MIN co-administered with QUIN (mean
lesion volume mm³ ± S.E.M.: QUIN + 100 µM L-MIN,
3.43 ± 0.57, P < 0.05; QUIN + 1 mM L-MIN, 3.74 ±
0.37, P < 0.05) compared to striata exposed to QUIN
alone (15 mM QUIN, 5.80 ± 0.92; Fig. 1A). There was no
significant difference in lesion volume in striata exposed to
the two lowest concentrations of L-MIN co-administered
with QUIN (QUIN + 1 µM L-MIN, 5.65 ± 0.51; QUIN +
10 µM L-MIN: 6.12 ± 0.97) compared to striata exposed
to QUIN alone (Fig. 1A).

In contrast to the effects of L-MIN on QUIN-induced
lesions, co-administration of 7-NINA had no significant
effect on lesion volume. There was, however, a trend
toward increased lesion volume at the two highest concen-
trations of 7-NINA when co-administered with QUIN
QUIN + 1 µM 7-NINA, 6.57 ± 0.68; QUIN + 10 µM
7-NINA, 5.90 ± 1.31; QUIN + 100 µM 7-NINA, 5.23 ±
1.38; QUIN + 1 mM 7-NINA, 8.30 ± 1.34; QUIN + 10
mM 7-NINA, 9.06 ± 1.73; (Fig. 1B).

In the present study we report that the selective nNOS
inhibitor L-MIN reduced the neurotoxic effects of QUIN
when the two compounds were chronically co-adminis-
tered to the rat striatum. Further, the less selective nNOS
inhibitor 7-NINA did not exhibit neuroprotective qualities
when administered using the same protocol.

Numerous laboratories now employ intrastratial admin-
istration of nNOS inhibitors to reduce QUIN-induced
lesions. In the present study we report a significant
decrease in lesion volume produced by 10 mM 7-NINA
co-administered with L-MIN. This suggests a possible
approach for reducing QUIN-induced damage in the
central nervous system.
istration of QUIN as an animal model of Huntington’s disease. Although this model has provided useful information regarding morphological changes associated with excitotoxicity, there has been less success in development of neuroprotective protocols. Several laboratories have shown significant reduction in excitotoxicity using the potent NMDA receptor antagonist MK-801 [4,16,24]. However, exceeding the therapeutically useful dose of MK-801 significantly increases morbidity rate of subjects [25]. In addition, when administered chronically, NMDA receptor antagonists may exacerbate abnormal motor behaviors [6]. These factors have raised concerns about the therapeutic potential of NMDA receptor blockade as a treatment for neurodegenerative disorders.

Excessive NOS dependent NO release during high levels of NMDA receptor stimulation results in production of toxic hydroxyl radicals and nitrogen dioxide [7,18]. Recently, NMDA-induced toxicity has been evaluated in mice deficient in NOS gene expression (NOS knockout). Cultured cortical cells from nNOS knockout mice show a high level of resistance to NMDA-induced excitotoxicity [8]. Similar results have been found in vivo where nNOS knockout mice exhibit decreased infarct volume following focal cerebral ischemia [13,26]. These reports suggest an essential role for nNOS in NMDA receptor mediated excitotoxicity. Decreasing nNOS thus offers a potential alternative to NMDA receptor blockade for attenuating this form of neurotoxicity.

In the present report L-MIN was effective in reducing QUIN-induced lesions while 7-NINA was ineffective. Furthermore, at high concentrations, 7-NINA produced a trend toward an increase in QUIN-induced lesion. One possible explanation for these results is the relative specificity of these two compounds for nNOS. Nagafuji and colleagues [19] reported the IC value of L-MIN to be more than 800-times lower than that of 7-NI for nNOS, and 10-times higher for eNOS. While the neuroprotective properties of L-MIN likely represent antagonism of nNOS, the trend toward increased lesion volume with 7-NINA may have resulted from nonspecific antagonism of eNOS. This hypothesis is supported by findings of Huang and colleagues who showed increased susceptibility to NMDA-induced lesions in eNOS knockout mice [14].

Our results contrast with previous results showing systemic administration of 7-NI afforded protection against chronic systemic administration of the mitochondrial inhibitor 3-NP [21]. It is possible that neuroprotection reported in this previous study resulted from 7-NI-induced alteration in peripheral metabolism of 3-NP, effectively reducing the neurotoxic potential of 3-NP prior to entry into the brain. Alternatively, the discrepancy may be a result of inherent differences in the neural mechanisms involved in 3-NP- and QUIN-induced neurodegeneration.

Transgenic models have definitively established a role for nNOS in NMDA-induced neurotoxicity. The present results support those earlier findings and further suggest therapeutic potential for nNOS antagonists. Finally, the relative effectiveness of NOS antagonists as neuroprotectants may depend in part on their relative specificity for the neuronal form of NOS.

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