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Short communication

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

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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. © 1997 Elsevier Science B.V.

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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 ($n =$

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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 purchased 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/choloroform (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% paraformaldehyde solution and cryoprotected in a 20% sucrose solution.

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 previously [3].

Lesion volume was determined by multiplying the rostral/caudal extent of decreased cytochrome oxidase staining by the average area of decreased cytochrome oxidase staining through the striatum. Quantification of lesion volume was accomplished using computer assisted morphometry 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 lesion volume in striata exposed to the two highest concentrations of L-MIN co-administered with QUIN (mean lesion volume $mm^3 \pm$ 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).

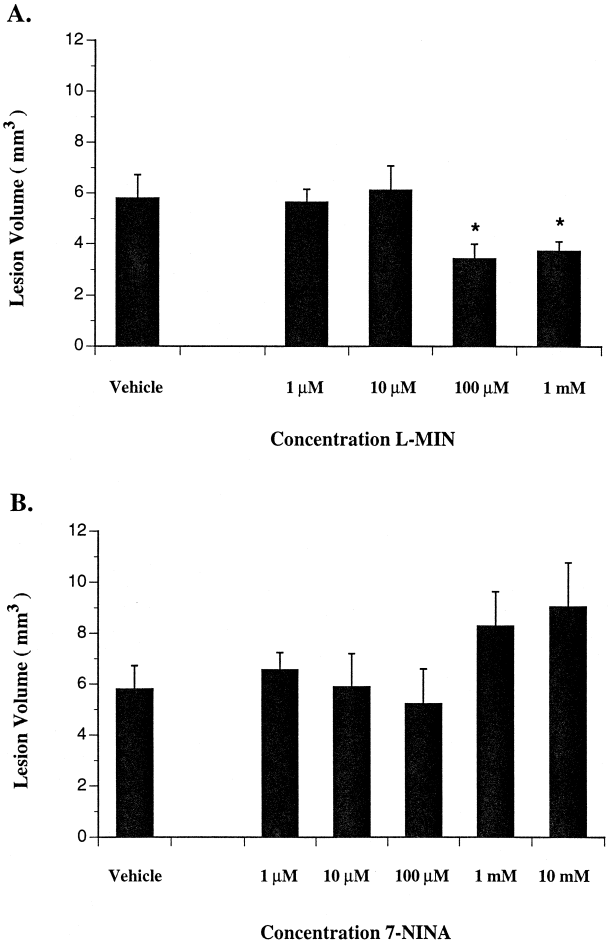

Fig. 1. A. There was a significant reduction in lesion volume produced by 15 mM QUIN co-administered with 100 μM L-MIN and 1 mM L-MIN compared to lesion volume produced by 15 mM QUIN alone ($P < 0.05$). There was no significant effect of 1 μM or 10 μM L-MIN co-administered with 15 mM QUIN. B. There was no significant effect of five separate doses of 7-NINA co-administered with 15 mM QUIN on striatal lesion volume compared to lesion volume produced by 15 mM QUIN alone. There was a trend toward an increase in lesion volume at the two highest doses.

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 concentrations 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-administered 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 intrastriatal admin-

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 excitotoxicty. 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_{50} 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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References

[1] T.J. Bazzett, J.B. Becker, R.L. Albin, A novel device for chronic intracranial drug delivery via microdialysis, J. Neurosci. Methods 40 (1991) 1–8.
[2] T.J. Bazzett, J.B. Becker, R.L. Albin, Construction and implantation of a chronic intracranial dialytic delivery system, Neurosci. Prot. 60 (1) (1994) 1–9.
[3] T.J. Bazzett, J.B. Becker, K.W. Kaatz, R.L. Albin, Chronic intrastriatal dialytic administration of quinolinic acid produces selective neural degeneration, Exp. Neurol. 120 (1993) 177–185.
[4] J. Church, S. Zeman, D. Lodge, The neuroprotective action of ketamine and MK-801 after transient cerebral ischemia in rats, Anesthesiology 69 (1988) 702–709.
[5] B.P. Connop, R.J. Boegman, K. Jhamandas, R.J. Beninger, Excitotoxic action of NMDA agonists on nigrostriatal dopaminergic neurons: Modulation by inhibition of nitric oxide synthase, Brain Res. 676 (1995) 124–132.
[6] A.R. Crossman, D. Peggs, S. Boyce, M.R. Luquin, M.A. Sambrook, Effect of the NMDA antagonist MK-801 on MPTP-induced parkinsonism in the monkey, Neuropharmacology 28 (1989) 1271–1273.
[7] V.L. Dawson, T.M. Dawson, Nitric oxide actions in neurochemistry, Neurochem. Int. 29 (1996) 97–110.
[8] V.L. Dawson, V.M. Kizushi, P.L. Huang, S.H. Snyder, T.M. Dawson, Resistance to neurotoxicity in cortical cultures from neuronal nitric oxide synthase deficient mice, J. Neurosci. 16 (1996) 2479–2487.
[9] G. Figueredo-Cardenas, Q. Chen, A. Reiner, Different survival pattern of striatal interneurons after slow vs. fast intrastriatal injections of quinolinic acid, Conf. Neurosci. Abstr. 73 (1992) 3.
[10] J.M. Galasso, T.J. Bazzett, J.B. Becker, R.L. Albin, Synergistic effect of intrastriatal co-administration of L-NAME and quinolinic acid, Neuroreport 6 (1995) 1505–1508.
[11] K.A. Haberny, S. Pou, C.U. Eccles, Potentiation of quinolinate-induced hippocampal lesions by inhibition of NO synthesis, Neurosci. Lett. 146 (1992) 187–190.
[12] I. Hanbauer, D. Wink, Y. Osawa, G.M. Edelman, J.A. Gally, Role of nitric oxide in NMDA-evoked release of [^{3}H]dopamine from striatal slices, Neuroreport 3 (1992) 409–412.
[13] H. Hara, P.L. Huang, N. Panahian, M.C. Fishman, M.A. Moskowitz, Reduced brain edema and infarction volume in mice lacking the neuronal isoform of nitric oxide synthase after transient MCA occlusion, J. Cereb. Blood Flow Metab. 16 (1997) 605–611.
[14] Z. Huang, P.L. Huang, J. Ma, W. Meng, C. Ayata, M.C. Fishman, M.A. Moskowitz, Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine, J. Cereb. Blood Flow Metab. 16 (1996) 981–987.
[15] Y. Izumi, A.M. Benz, D.B. Clifford, C.F. Zorumski, Nitric oxide inhibitors attenuate *N*-methyl-D-aspartate excitotoxicity in rat hippocampal slices, Neurosci. Lett. 135 (1992) 227–230.
[16] H. Kato, T. Araki, K. Kogure, Role of the excitotoxic mechanism in

the development of neural damage following repeated brief cerebral ischemia in the gerbil: protective effects of MK-801 and pentobarbital, Brain Res. 516 (1990) 175–179.

[17] G.M. Mackenzie, P. Jenner, C.D. Marsden, Effect of 7-nitro indazole on quinolinic acid-induced striatal toxicity in the rat, Neuroreport 6 (1995) 1789–1794.

[18] G.M. MacKenzie, P. Jenner, C.C. Marsden, The effect of nitric oxide synthase inhibition on quinolinic acid toxicity in the rat striatum, Neuroscience 1995 (1995) 357–371.

[19] T. Nagafuji, M. Sugiyama, A. Muto, T. Makino, T. Miyauchi, H. Nabata, The neuroprotective effect of a potent and selective inhibitor of the type I NOS (L-MIN) in a rat model of focal cerebral ischaemia, Neuroreport 6 (1995) 1541–1545.

[20] D.D. Rees, R.M.J. Palmer, S. Moncada, Role of endotheial-derived nitric oxide in the regulation of blood pressure, Proc. Natl. Acad. Sci. USA 86 (1989) 3375–3378.

[21] J.B. Schulz, R.T. Matthews, B.G. Jenkins, R.R. Ferrante, D. Siwek, D.R. Henshaw, P.B. Cipolloni, P. Mecocci, N.W. Kowall, B.R. Rosen, M.F. Beal, Blockade of neuronal nitric oxide synthase protects against excitotoxicity in vivo, J. Neurosci. 15 (1995) 8419–8429.

[22] M.T. Silva, S. Rose, J.G. Hindmarsh, G. Aislaitner, J.W. Gorrod, P.K. Moore, P. Jenner, C.D. Marsden, Increased striatal dopamine efflux in vivo following inhibition of cerebral nitric oxide synthase by the novel monosodium salt of 7-nitro indazole, Br. J. Pharm. 114 (1995) 257–258.

[23] A. Tabernero, J. Giraldo, E. Vila, Effect of N^G-nitro-L-arginine methylester (L-NAME) on functional and biochemical α_1-adrenoceptor-mediated responses in rat blood vessels, Br. J. Pharm. 117 (1996) 757–763.

[24] A. Vezzani, R. Serafini, M.A. Stasi, S. Caccia, I. Conti, R.V. Tridico, R. Samanin, Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity in rats, J. Pharm. Exp. Ther. 249 (1988) 278–283.

[25] M.A. Warner, K.H. Neill, J.V. Nadler, B.J. Crain, Regionally selective effects of NMDA receptor antagonists against ischemic brain damage in the gerbil, J. Cereb. Blood Flow 11 (1991) 600–610.

[26] G. Zaharchuk, H. Hara, P.L. Huang, M.C. Fishman, M.A. Moskowitz, B.G. Jenkins, B.R. Rosen, Neuronal nitric oxide synthase mutant mice show smaller infarcts and attenuated apparent diffusion coefficient changes in the peri-infarct zone during focal cerebral ischemia, Magn. Reson. Med. 37 (1997) 170–175.