

Kainic Acid-induced Neuronal Death is Attenuated by Aminoguanidine but Aggravated by L-NAME in Mouse Hippocampus

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Nitric oxide (NO) has both neuroprotective and neurotoxic effects depending on its concentration and the experimental model. We tested the effects of NG-nitro-L-arginine methyl ester (L-NAME), a nonselective nitric oxide synthase (NOS) inhibitor, and aminoguanidine, a selective inducible NOS (iNOS) inhibitor, on kainic acid (KA)-induced seizures and hippocampal CA3 neuronal death. L-NAME (50 mg/kg, i.p.) and/or aminoguanidine (200 mg/kg, i.p.) were administered 1 h prior to the intracerebroventricular (i.c.v.) injection of KA. Pretreatment with L-NAME significantly increased KA-induced CA3 neuronal death, iNOS expression, and activation of microglia. However, pretreatment with aminoguanidine significantly suppressed both the KA-induced and L-NAME-aggravated hippocampal CA3 neuronal death with concomitant decreases in iNOS expression and microglial activation. The protective effect of aminoguanidine was maintained for up to 2 weeks. Furthermore, iNOS knockout mice (iNOS^{-/-}) were resistant to KA-induced neuronal death. The present study demonstrates that aminoguanidine attenuates KA-induced neuronal death, whereas L-NAME aggravates neuronal death, in the CA3 region of the hippocampus, suggesting that NOS isoforms play different roles in KA-induced excitotoxicity.

Key Words: Kainic acid, Nitric oxide, L-NAME, Aminoguanidine, iNOS, Neuronal death

INTRODUCTION

Nitric oxide (NO), a diffusible messenger molecule, plays many important roles in the brain (Bredt and Snyder, 1992; Garthwaite and Boulton, 1995). However, the uncontrolled production of NO may be harmful and contributes to the development of several neuropathological states (Gross and Wolin, 1995). The synthesis of NO is mediated by three distinct isoforms of nitric oxide synthases (NOSs): neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) (Moncada et al., 1997). The fact that the activation of ionotropic glutamate receptors generates NO production by a calcium-dependent process led to the hypothesis that NO could be involved in excitotoxicity (Kiedrowski et al., 1992). Also, a role for NO in certain neurodegenerative processes (Southam and Garthwaite, 1993) and memory disorders (Du and Harvey, 1996; Du et al., 2000) has been proposed. NO production is increased in kainic acid (KA)-induced seizures (Mulsch et al., 1994) and inhibition of NOS activity with

7-nitroindazole, a selective nNOS inhibitor, and N-nitro-L-arginine (L-NAME), a NOS inhibitor, decreases NO production and hippocampal damage, and attenuates seizure activity (Takei et al., 1999; Takei et al., 2001). Aminoguanidine, a selective iNOS inhibitor, protects against ischemic damage in rats by suppressing the excessive production of NO (Danielisova et al., 2004). However, inhibition of NOS with L-NAME or N-omega-nitro-L-arginine (NNA) aggravates KA-induced neuronal damage and focal ischemic neuronal damage (Yamamoto et al., 1992; Ciani et al., 2001). Therefore, it is still controversial whether inhibition of NOS is beneficial to excitotoxic brain injuries (Ciani et al., 2001). Given that the inhibition of NOS can be either protective or neurotoxic depending on the experimental conditions (Buisson et al., 1993a; Buisson et al., 1993b; Rondouin et al., 1993; Schulz et al., 1995), it is necessary to determine the exact physiological consequences of NOS inhibition in instances of excitotoxic brain damage.

In the present study, we examined the different effects of two NOS inhibitors, L-NAME and aminoguanidine, on the hippocampal CA3 neuronal damage induced by the intracerebroventricular injection of KA.

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ABBREVIATIONS: KA, kainic acid; L-NAME, NG-nitro-L-arginine methyl ester; NO, nitric oxide; iNOS, inducible nitric oxide synthase; icv, intracerebroventricular.

METHODS

Animals and reagents

Male ICR mice weighing 23~25 g were obtained from Folas-International, Ltd. (Seoul, Korea). Inducible NOS knockout (iNOS^{-/-}) and littermate control mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). All of the animal experiments were conducted in accordance with the animal care guidelines of the National Institutes of Health (NIH) and Korean Academy of Medical Sciences (KAMS). Mice were housed five per cage in a room maintained at 22±2°C with an alternating 12/12 h light/dark cycle. Food and water were available *ad libitum*. KA, L-NAME, and aminoguanidine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). KA was prepared as a stock solution at 1 mg/ml in sterile 0.1 M phosphate-buffered saline (PBS, pH 7.4), and aliquots were stored at -20°C until use. L-NAME (50 mg/kg) or aminoguanidine (200 mg/kg) was administered intraperitoneally 1 h prior to KA injection.

Intracerebroventricular (i.c.v.) injection of KA

The administration of KA (0.1 µg/5 µl) was performed

according to the procedure established by Laursen and Belknap (Laursen and Belknap, 1986). Briefly, KA was injected at the bregma with a 50-µl Hamilton microsyringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm.

In situ labeling of DNA fragmentation

DNA fragmentation analysis, which indicates apoptosis as previously described (Henshall et al., 2001), was performed according to the manufacturer's instructions using terminal deoxynucleotidyl transferase with peroxide-12-UTP nick-end labeling (TUNEL) (Roche Molecular Biochemicals, Indianapolis, IN, USA). The percentage of TUNEL-positive cells (bright fluorescent green) was assessed by analysis of digitized images from 5 or more microscopic fields of TUNEL-stained cells from TIFF files (Adobe Photoshop).

Immunohistochemistry

All mice were sacrificed at 6 h or 24 h after KA injection. The mice were transcardially perfused and post-fixed for 4 h in 4% paraformaldehyde. The brains were cryoprotected in 30% sucrose, sectioned coronally (40 µm) on a freezing microtome, and collected in cryoprotectant for storage at

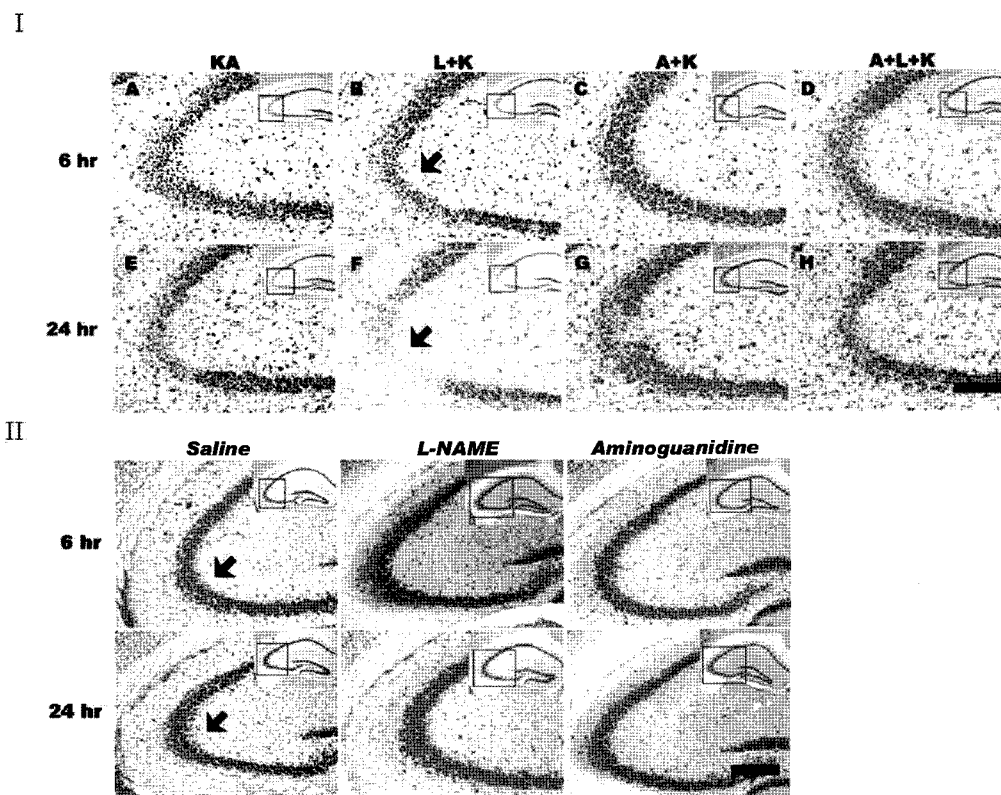


Fig. 1. Effects of NOS inhibitors on KA-induced neuronal cell death in the CA3 region of mouse hippocampus. Neuronal cell death was determined with cresyl violet staining. KA injection produced a marked loss of pyramidal neurons (IA, E) and L-NAME treatment prior to KA (IB, F) potentiated this KA-induced neuronal loss. Aminoguanidine pretreatment (IC, G) markedly attenuated KA-induced and L-NAME-aggravated (ID, H) neuronal death. Neuronal cell death was not observed in vehicle-, aminoguanidine-only-, or L-NAME-only-treated mice (II). Images in the upper right corner show the entire hippocampus and boxes in these images indicate the enlarged views of the CA3 region. Arrows indicate areas where most neuronal death appears. KA & K stand for kainic acid, L for L-NAME, and A for aminoguanidine. Scale bar: 100 µm.

–20°C. For cresyl violet staining, the sections were mounted in gelatin-coated slides and allowed to air-dry overnight. The mounted sections were submerged in 0.1% cresyl violet solution for 5 min. The sections were rinsed in 70% ethanol and dehydrated in graded series of ethanol, immersed in xylene, mounted in Permount (Fisher Scientific, NJ, USA), and cover slipped. The free-floating immunohistochemistry of the brain sections was processed as previously described (Baker and Farbman, 1993). Sections collected from cryoprotectant were washed with PBS, pre-incubated for 30 min in 0.1 M PBS with 1% bovine serum albumin and 0.2% Triton X-100, and incubated for 2 days at 4°C with the following primary antibodies: iNOS (1 : 1,000; BD Pharmingen, San Jose, CA, USA), OX-6 (1 : 1,000; BD Pharmingen), or NeuN (1 : 1,000; Santa Cruz, Santa Cruz, CA, USA). The antigens were then detected with 3,3'-diaminobenzidine tetrahydrochloride using an Elite ABC kit (Vector, Burlingame, CA, USA). The sections were mounted, air-dried, dehydrated through graded ethanol, cleared in histoclear, and cover slipped using Permount.

Statistical analysis

The data were analyzed with the Mann-Whitney U test

using SPSS software 12K (SPSS, Chicago, IL, USA) for independent samples to compare KA-only and treatment groups. A value of $p < 0.05$ was accepted as statistically significant. The results are expressed as mean \pm SEM values.

RESULTS

L-NAME aggravates KA-induced neuronal death and aminoguanidine attenuates both KA-induced and L-NAME-aggravated neuronal death

The i.c.v. injection of KA time-dependently killed hippocampal neurons in the CA3 region (Fig. 1IA, E); neuronal death was negligible in vehicle-injected mice (Fig. 1II). L-NAME treatment prior to the KA injection further increased the KA-induced neuronal death (Fig. 1IB, F), whereas pretreatment with aminoguanidine significantly attenuated the KA-induced neuronal loss (Fig. 1IC, G). Co-administration of aminoguanidine with L-NAME showed reduced neuronal death compared with L-NAME alone (Fig. 1ID, H). Similarly, L-NAME pretreatment increased the number of early TUNEL-positive neurons in the CA3 region compared with KA alone (Fig. 2), and aminoguanidine re-

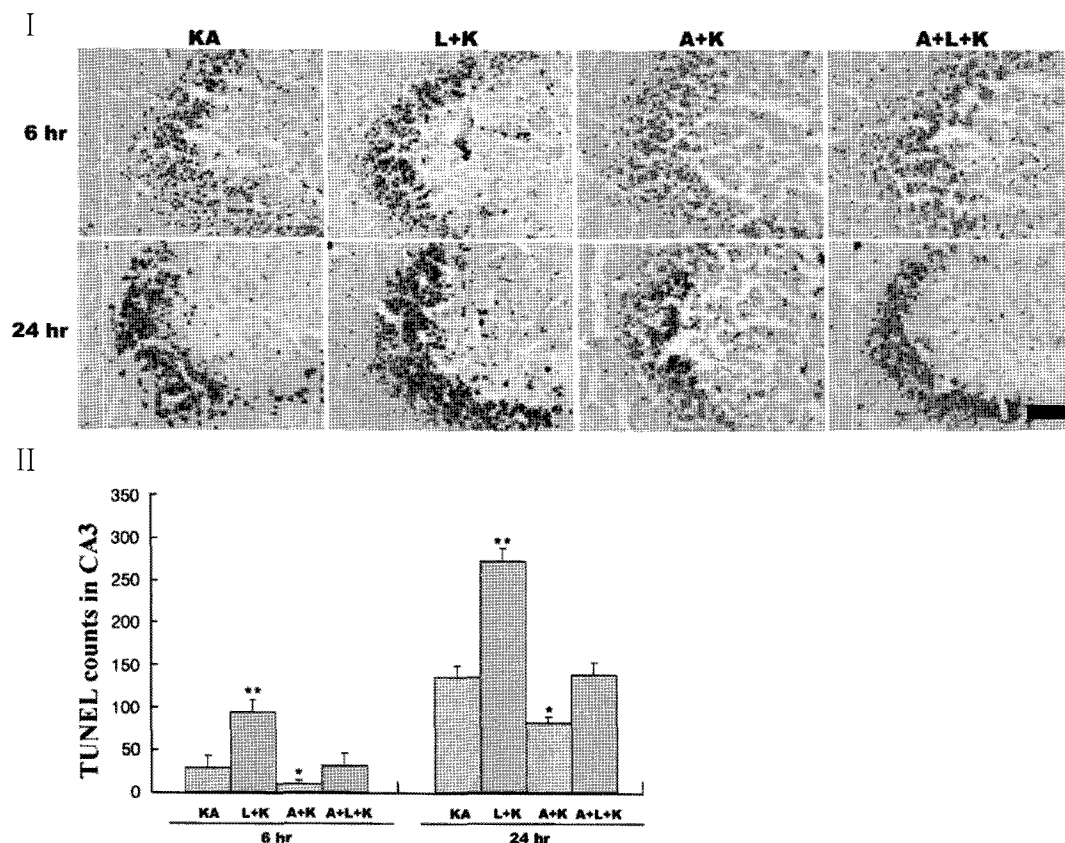


Fig. 2. Representative (I) and quantitative (II) neuronal death measured with Terminal deoxynucleotidyl transferase-mediated dUTP-nick end labeling (TUNEL) assay. KA produces TUNEL-positive neurons within 24 h, and L-NAME (L+KA) treatment potentiates this induction. Aminoguanidine (A+KA) reduces the numbers of TUNEL-positive cells compared with KA-only (KA) and L-NAME-aggravated (A+L+KA) groups. L-NAME and aminoguanidine alone did not affect cell survival (data not shown). Quantitative data represent three independent experiments and are expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ versus the KA-only treated group. KA & K stand for kainic acid, L for L-NAME, and A for aminoguanidine.

duced both KA-induced and L-NAME-aggravated TUNEL staining. Vehicle-treated mice showed no TUNEL staining (i.p. administration of saline as pretreatment and i.c.v. injection of PBS instead of KA). In addition, no TUNEL staining was observed with L-NAME or aminoguanidine alone, respectively (data not shown).

Aminoguanidine produces prolonged survival of hippocampal CA3 neurons

To test the time-dependency of the neuroprotective effects of aminoguanidine, we measured levels of viable neurons in the CA3 region with cresyl violet staining and NeuN im-

munoreactivity for up to 2 weeks after the KA injection. Neuron number did not change dramatically between 24 h and 2 weeks after KA and aminoguanidine treatment, suggesting that the neuroprotective effects of aminoguanidine were permanent (Fig. 3).

L-NAME potentiates KA-induced iNOS expression in activated microglia

KA induced microglial activation (Fig. 4E) as determined by immunostaining of the microglial marker, OX-6, and the subsequent induction of inducible iNOS (Fig. 4A) in the region of neuronal death. iNOS expression was localized to

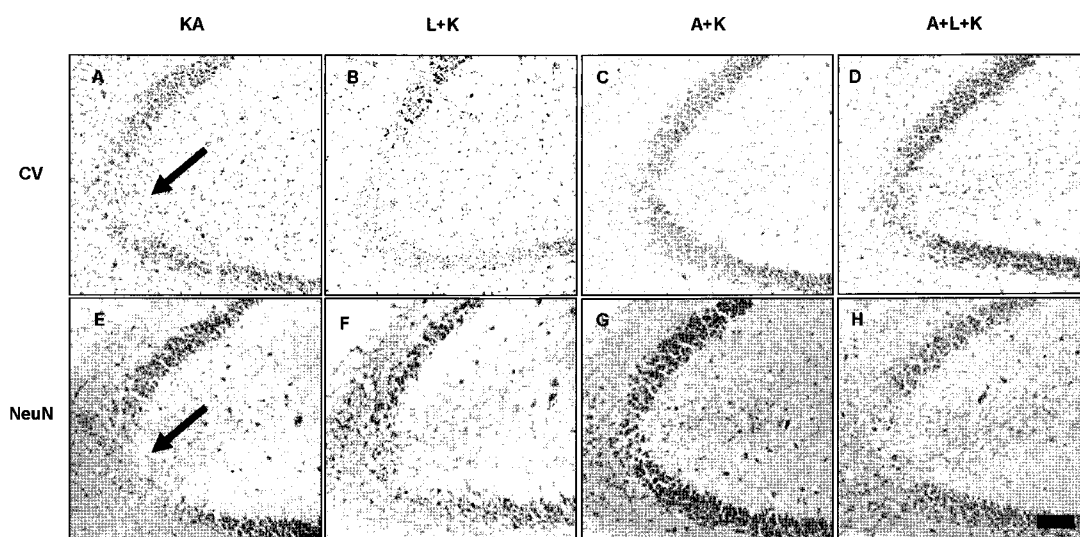


Fig. 3. Aminoguanidine produces persistent neuronal survival against KA and L-NAME injury. Pyramidal neurons were measured with cresyl violet staining (Top panels) and NeuN immunoreactivity (Bottom panels) at 2 weeks after KA injection. Arrows indicate areas with highest neuronal death. KA & K stand for kainic acid, L for L-NAME, A for aminoguanidine.

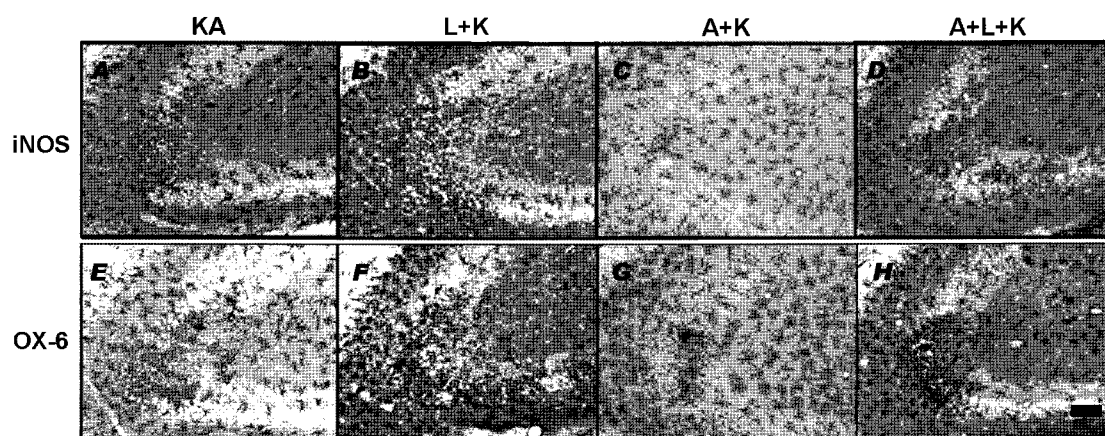


Fig. 4. Effects of NOS inhibitors on KA-induced microglial activation and subsequent iNOS expression. Expression of iNOS (top panels) and OX-6, a microglial marker, (bottom panels) within the CA3 was determined with immunohistochemical analysis at 24 h after treatment. KA increased iNOS expression (A) and microglial activation (E), and these phenomena were attenuated with aminoguanidine pretreatment (C, G). L-NAME pretreatment (B, F) potentiated the KA effect, but aminoguanidine reduced this potentiation (D, H). L-NAME and aminoguanidine alone did not affect microglial activation and iNOS expression (data not shown). KA & K stand for kainic acid, L for L-NAME, and A for aminoguanidine.

activated microglia. L-NAME treatment prior to KA produced earlier (data not shown) and increased (Fig. 4B) expression of iNOS in activated microglia. However, pre-treatment with aminoguanidine markedly attenuated the KA-induced microglial activation and iNOS expression (Fig. 4C, G). Aminoguanidine also attenuated the L-NAME-potentiated increases in microglial activation and iNOS expression to the level of the KA-only group (Fig. 4D, H).

iNOS knockout mice (iNOS^{-/-}) are resistant to KA-induced neuronal death

To further delineate the role of iNOS in KA-induced neuronal death, we next tested KA-induced neuronal death in iNOS knockout mice (iNOS^{-/-}). KA injection was less toxic in iNOS^{-/-} mice than in littermates with the iNOS gene (Fig. 5I), but L-NAME still potentiated this toxicity (Fig. 5I, II). Similarly, KA treatment produced fewer TUNEL-positive neurons in iNOS^{-/-} mice than wild-type littermates (Fig. 5II, III)

DISCUSSION

In the present study, we demonstrated that inhibiting NOS activity with L-NAME aggravated KA-induced hippocampal neuronal loss in the CA3 region, but that the selective inhibition of iNOS with aminoguanidine significantly attenuated this KA-induced death.

Glutamate and other excitatory amino acids induce neuronal cell death by excitotoxicity, which may contribute to the neuronal cell loss caused by acute insults and chronic degeneration in the central nervous system (Choi, 1988; Coyle and Puttfarcken, 1993; Lee et al., 1999; McNamara, 1999). However, the role of NO in brain excitotoxicity is controversial.

L-NAME has a 20-fold selectivity for the constitutive isoforms of NOS (n- and e-NOS) (Boer et al., 2000) and aminoguanidine has a 5.5 (iNOS/nNOS)- or 11 (iNOS/eNOS)-fold selectivity (Alderton et al., 2001). Therefore, NO originating from iNOS could cause neuronal death, whereas NO from eNOS and nNOS are protective.

NO produced from iNOS can cause neuronal damage,

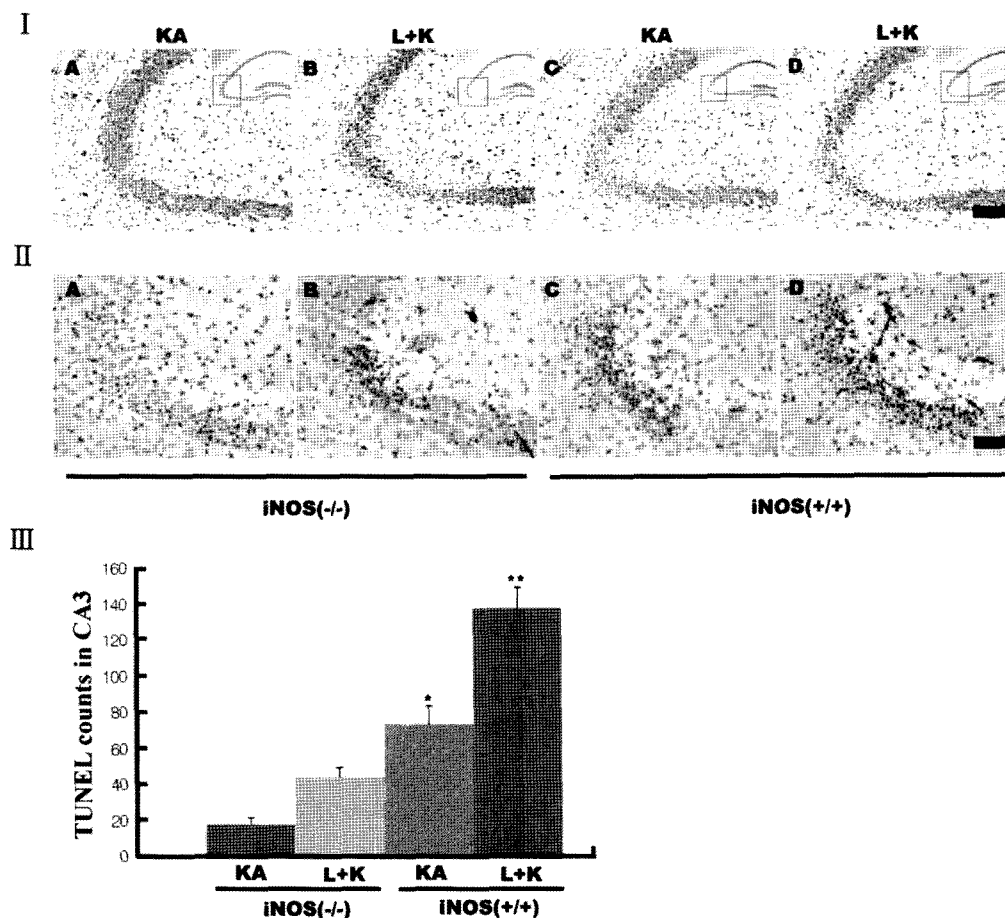


Fig. 5. Decreased neuronal death after KA in iNOS knockout (iNOS^{-/-}) mice. Neuronal death was measured with cresyl violet (I) and TUNEL staining (II&III). KA produced less neuronal death in iNOS^{-/-} mice (I&II-As) than littermates (I&II-Cs). L-NAME pretreatment potentiated neuronal death in both littermates (I&II-Ds) and iNOS^{-/-} mice (I&II-Bs) compared to KA alone, but iNOS^{-/-} mice showed less potentiation (III). Quantitative data represent three independent experiments and are expressed as mean±SEM. **p<0.01 versus KA-only treated iNOS^{-/-} mice. Images in the upper right corner show the entire hippocampus and boxes in these images indicate the enlarged views of the CA3 region. KA & K stand for kainic acid, L for L-NAME, and A for aminoguanidine. Scale bars: I, 100 μm; II, 50 μm.

such as neurological disorders (Koprowski et al., 1993; Sugimoto and Iadecola, 2002) and apoptotic cell death (Kim et al., 1999). Inhibiting iNOS activity decreases glutamate release and improves stroke outcomes after experimental ischemia (Perez-Asensio et al., 2005). Similarly, we found that blockade of NO production from iNOS either with a specific inhibitor, aminoguanidine, or in an iNOS gene knockout significantly attenuated KA-induced neuronal death. However, the role of eNOS inhibition is still controversial. L-NAME protects hippocampal pyramidal neurons against kainite-induced excitotoxicity in rats (Jones et al., 1998) and in newborn rabbits (Takei et al., 2001), but eNOS inhibition can also aggravate seizures in animal models (Haberny et al., 1992; Starr and Starr, 1993; Penix et al., 1994; Tsuda et al., 1997). For example, L-NAME treatment facilitated pilocarpine-induced seizures (Starr and Starr, 1993) and seizures induced by DMCN (methyl-6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate), an inverse agonist for the GABA_A receptor benzodiazepine binding site, in mice (Tsuda et al., 1997). Thus, endogenous NO originating from eNOS may protect against epileptogenesis by excitotoxins. Chronic L-NAME treatment aggravates animal mortality and neuronal damage in KA-induced excitotoxic brain injury (Ciani et al., 2001).

Relatively low doses of L-NAME, up to 20 mg/kg, were beneficial against excitotoxic neuronal damage (Jones et al., 1998; Takei et al., 2001), whereas higher doses, ranging from 50 mg/kg to 125 mg/kg, were detrimental (Starr and Starr, 1993; Tsuda et al., 1997). We therefore used a relatively high dose of L-NAME, 50 mg/kg, which aggravated KA-induced death of pyramidal neurons in the CA3 region. NARG (N-nitro-L-arginine), an irreversible NOS inhibitor, exhibits biphasic dosing effects during transient forebrain ischemia in gerbils (Shapira et al., 1994). Low levels of NO from eNOS and/or nNOS, which may not be completely blocked by low doses of L-NAME, could protect brain tissue. However, higher doses of L-NAME, which may completely block these enzymes, produces neuronal death.

In conclusion, the present data demonstrate that NOS isoforms play a different role in KA-induced excitotoxicity. Pathological levels of NO originating from iNOS play a detrimental role, whereas NO from eNOS and/or nNOS can be beneficial depending on the magnitude of inhibition. Careful consideration should be applied in selecting NOS inhibitors for pharmacological approaches to treating and preventing diseases associated with excitotoxic insults such as epilepsy, ischemia, and traumatic brain injury.

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REFERENCES

- Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 357: 593–615, 2001.
- Baker H, Farbman AI. Olfactory afferent regulation of the dopamine phenotype in the fetal rat olfactory system. *Neuroscience* 52: 115–134, 1993.
- Boer R UMPW, Klein T, Mirau B, Haas S, Baur I. The inhibitory potency and selectivity of arginine substrate site nitric-oxide synthase inhibitors is solely determined by their affinity toward the different isoenzymes. *Mol Pharmacol* 58: 1026–1034, 2000.
- Bredt DS, Snyder SH. Nitric oxide, a novel neuronal messenger. *Neuron* 8: 3–11, 1992.
- Buisson A, Lakhmeche N, Verrecchia C, Plotkine M, Boulu RG. Nitric oxide: an endogenous anticonvulsant substance. *Neuroreport* 4: 444–446, 1993a.
- Buisson A, Margail I, Callebert J, Plotkine M, Boulu RG. Mechanisms involved in the neuroprotective activity of a nitric oxide synthase inhibitor during focal cerebral ischemia. *J Neurochem* 61: 690–696, 1993b.
- Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1: 623–634, 1988.
- Ciani E, Baldinotti I, Contestabile A. Sustained, long-lasting inhibition of nitric oxide synthase aggravates the neural damage in some models of excitotoxic brain injury. *Brain Res Bull* 56: 29–35, 2001.
- Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262: 689–695, 1993.
- Danielisova V, Nemethova M, Burda J. The protective effect of aminoguanidine on cerebral ischemic damage in the rat brain. *Physiol Res* 53: 533–540, 2004.
- Du W, Harvey JA. The nitric oxide synthesis inhibitor L-NAME facilitates associative learning. *Prog Neuropsychopharmacol Biol Psychiatry* 20: 1183–1195, 1996.
- Du W, Weiss H, Harvey JA. Associative learning is enhanced by selective neuronal nitric oxide synthase inhibitors and retarded by a nitric oxide donor in the rabbit. *Psychopharmacology (Berl)* 150: 264–271, 2000.
- Garthwaite J, Boulton CL. Nitric oxide signaling in the central nervous system. *Annu Rev Physiol* 57: 683–706, 1995.
- Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. *Annu Rev Physiol* 57: 737–769, 1995.
- Haberny KA, Pou S, Eccles CU. Potentiation of quinolinate-induced hippocampal lesions by inhibition of NO synthesis. *Neurosci Lett* 146: 187–190, 1992.
- Henshall DC, Bonislawski DP, Skradski SL, Araki T, Lan JQ, Schindler CK, Meller R, Simon RP. Formation of the Apaf-1/cytochrome c complex precedes activation of caspase-9 during seizure-induced neuronal death. *Cell Death Differ* 8: 1169–1181, 2001.
- Jones PA, Smith RA, Stone TW. Nitric oxide synthase inhibitors L-NAME and 7-nitroindazole protect rat hippocampus against kainate-induced excitotoxicity. *Neurosci Lett* 249: 75–78, 1998.
- Kiedrowski L, Costa E, Wroblewski JT. Glutamate receptor agonists stimulate nitric oxide synthase in primary cultures of cerebellar granule cells. *J Neurochem* 58: 335–341, 1992.
- Kim YM, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. *Circ Res* 84: 253–256, 1999.
- Koprowski H, Zheng YM, Heber-Katz E, Fraser N, Rorke L, Fu ZF, Hanlon C, Dietzschold B. In vivo expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. *Proc Natl Acad Sci USA* 90: 3024–3027, 1993.
- Laursen SE, Belknap JK. Intracerebroventricular injections in mice. Some methodological refinements. *J Pharmacol Methods* 16: 355–357, 1986.
- Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. *Nature* 399: A7–14, 1999.
- McNamara JO. Emerging insights into the genesis of epilepsy. *Nature* 399: A15–22, 1999.
- Moncada S, Higgs A, Furchgott R. International Union of Pharmacology Nomenclature in Nitric Oxide Research. *Pharmacol Rev* 49: 137–142, 1997.
- Mulsch A, Busse R, Mordvintsev PI, Vanin AF, Nielsen EO, Scheel-Kruger J, Olesen SP. Nitric oxide promotes seizure activity in kainate-treated rats. *Neuroreport* 5: 2325–2328, 1994.
- Penix LP, Davis W, Subramaniam S. Inhibition of NO synthase increases the severity of kainic acid-induced seizures in rodents. *Epilepsy Res* 18: 177–184, 1994.
- Perez-Asensio FJ, Hurtado O, Burguete MC, Moro MA, Salom JB,

- Lizasoain I, Torregrosa G, Leza JC, Alborch E, Castillo J, Knowles RG, Lorenzo P.** Inhibition of iNOS activity by 1400W decreases glutamate release and ameliorates stroke outcome after experimental ischemia. *Neurobiol Dis* 18: 375–384, 2005.
- Rondouin G, Bockaert J, Lerner-Natoli M.** L-nitroarginine, an inhibitor of NO synthase, dramatically worsens limbic epilepsy in rats. *Neuroreport* 4: 1187–1190, 1993.
- Schulz JB, Matthews RT, Jenkins BG, Ferrante RJ, Siwek D, Henshaw DR, Cipolloni PB, Mecucci P, Kowall NW, Rosen BR.** Blockade of neuronal nitric oxide synthase protects against excitotoxicity *in vivo*. *J Neurosci* 15: 8419–8429, 1995.
- Shapira S, Kadar T, Weissman BA.** Dose-dependent effect of nitric oxide synthase inhibition following transient forebrain ischemia in gerbils. *Brain Res* 668: 80–84, 1994.
- Southam E, Garthwaite J.** The nitric oxide-cyclic GMP signalling pathway in rat brain. *Neuropharmacology* 32: 1267–1277, 1993.
- Starr MS, Starr BS.** Paradoxical facilitation of pilocarpine-induced seizures in the mouse by MK-801 and the nitric oxide synthesis inhibitor L-NAME. *Pharmacol Biochem Behav* 45: 321–325, 1993.
- Sugimoto K, Iadecola C.** Effects of aminoguanidine on cerebral ischemia in mice: comparison between mice with and without inducible nitric oxide synthase gene. *Neurosci Lett* 331: 25–28, 2002.
- Takei Y, Takashima S, Ohyu J, Matsuura K, Katoh N, Takami T, Miyajima T, Hoshika A.** Different effects between 7-nitroindazole and L-NAME on cerebral hemodynamics and hippocampal lesions during kainic acid-induced seizures in newborn rabbits. *Brain Dev* 23: 406–413, 2001.
- Takei Y, Takashima S, Ohyu J, Takami T, Miyajima T, Hoshika A.** Effects of nitric oxide synthase inhibition on the cerebral circulation and brain damage during kainic acid-induced seizures in newborn rabbits. *Brain Dev* 21: 253–259, 1999.
- Tsuda M, Suzuki T, Misawa M.** Aggravation of DMCM-induced seizure by nitric oxide synthase inhibitors in mice. *Life Sci* 60: 339–343, 1997.
- Yamamoto S, Golanov EV, Berger SB, Reis DJ.** Inhibition of nitric oxide synthesis increases focal ischemic infarction in rat. *J Cereb Blood Flow Metab* 12: 717–726, 1992.