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NITRIC OXIDE SYNTHASE INHIBITION PREVENTS ACUTE QA-INDUCED NEUROTOXICITY

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In the present study we employed 7-NI, reportedly a selective inhibitor of neuronal nitric oxide synthase (NOS) and the non-specific potent NOS inhibitor l-NAME, to investigate the possible involvement of nitric oxide (NO) in quinolinic acid (QA)-induced striatal toxicity in the rat. QA was administered unilaterally into the striatum of adult Wistar rats in the single dose of 150 nmol/L. The second and third group were treated with 7-NI and QA and l-NAME and QA. The control group was treated with 0.9% saline solution likewise. Nitrite levels were decreased in the ipsi- and contralateral striatum, forebrain cortex and hippocampus in the group treated with NOS inhibitors (7-NI, l-NAME) and QA compared to QA-treated animals. As 7-NI selectively inhibits the neuronal form of NOS, this study suggests that NO produced from a neuronal and not an epithelial source may contribute to neuronal damage in this model.

Key words: Huntington disease, nitric oxide, nitric oxide synthase inhibitors, quinolinic acid.

INTRODUCTION

Huntingtons disease (HD) is an autosomal dominant, fully penetrant disorder caused by an unstable CAG trinucleotide repeat on chromosome 4 that encodes an unknown protein (huntingtin) of approximately 340 kDa (Sathasivam *et al.,* 1999). Some evidence suggests that the abnormal gene product acts by a so-called gain of function effect in which the abnormal allele has gained a new and pathological effect, possibly unrelated to the function of the normal protein. HD is associated with a characteristic combination of pathological features: loss of striatal projection neurons with sparing of the patch-matrix compartmentation of the striatum and the NADPH-diaphorase-positive large aspiny neurons together with proliferative changes in spiny neuron dendrites (Hirsch *et al.,* 2000). The preferential loss of striatal output neurons observed in HD, and the resulting dyskinesia, are mimicked in a rat model in which an N-methyl-D-aspartate (NMDA) receptor agonist, quinolinic acid (QA), is injected into the striatum (Harris *et al.,* 1998). If an NMDA excitotoxic process does play a role in neuronal degeneration in HD, one would expect the neurons containing high densities of these receptors to be preferentially vulnerable, resulting in a depletion of NMDA receptors (Moroni, 1999).

Nitric oxide (NO) released from a new chemical class of donors enhances NMDA channel activity. NMDA receptor-mediated cell death provides firm evidence that NO modulates the NMDA channel in a manner consistent with both a physiological and pathophysiological role (Rodeberg *et al.*, 1995).

NO is produced in endothelial cells, neurons, glia and microglia/ macrophages by a family of calcium/calmodulin-linked enzymes known as NO synthases (NOS). The neuronal isoform of NOS (nNOS) is localized to postsynaptic regions in many neurons in the central nervous system and is activated by an influx of calcium (Perez-Severiano *et al.*, 1998). In the present study we employed 7-NI, reportedly a selective inhibitor of nNOS and the non-specific potent NOS inhibitor, l-NAME, to investigate the possible involvement of NO in QA-induced striatal toxicity in the rat.

MATERIAL AND METHODS

QA was administered unilaterally into the striatum of adult Wistar rats of both sexes in the single dose of 250.7μg (150 nmol/L) using a stereotaxic instrument for small animals. The second and third group were treated with 7-NI and QA (7-NI in the dose of $1x10^{-4}$g) and l-NAME and QA (l-NAME in the dose of $1x10^{-4}$g). 7-NI was immediately applied before the neurotoxin, in contrast to l-NAME which was given thirty minutes before QA. Control groups of animals were treated with 0.9% saline solution in the same manner.

Before the treatment animals were anesthetized by pentobarbital sodium i.p. in a dose of 0.0405g/kg b.w.

Animals were sacrificed by decapitation 7 days after the treatment.

A prepared crude mitochondrial fraction of striatum, forebrain cortex and hippocampus was used for the appropriate biochemical analysis (Gurd *et al.*, 1974).

NO is an important intra- and intercellular mediator, the half-life of which *in vivo* is only a few seconds. Most of the NO is oxidized to nitrite/nitrate, and the concentrations of these anions have been used as quantitative indices of NO production. The simplest and most widely used technique is spectrophotometric measurement of nitrite using the Griess reagent, which consists of naphthylethylenediamine dihydrochloride in water and sulphanilamide in phosphoric acid. The Griess reagent forms a purple azo dye with nitrite, which can be measured with a spectrophotometer (Viinikka, 1996).

RESULTS

Nitrite levels were decreased in the ipsi- and contralateral striatum, forebrain cortex and hippocampus in the group treated with NOS inhibitors and QA, as well as in the equivalent ipsi- and contralateral parts of the brain from the control group treated with 0.9% saline solution, compared to QA-treated animals (striatum (S) ipsi=2.13±0.52 and contra=1.43±0.39; forebrain cortex (Cx) ipsi=1.80±0.34 and contra=1.71±0.23; hippocampus (H) ipsi=2.41±0.50 and contralateral=2.22 ± 0.70 micromol nitrite/mg prot) (Figures 1, 2, 3).

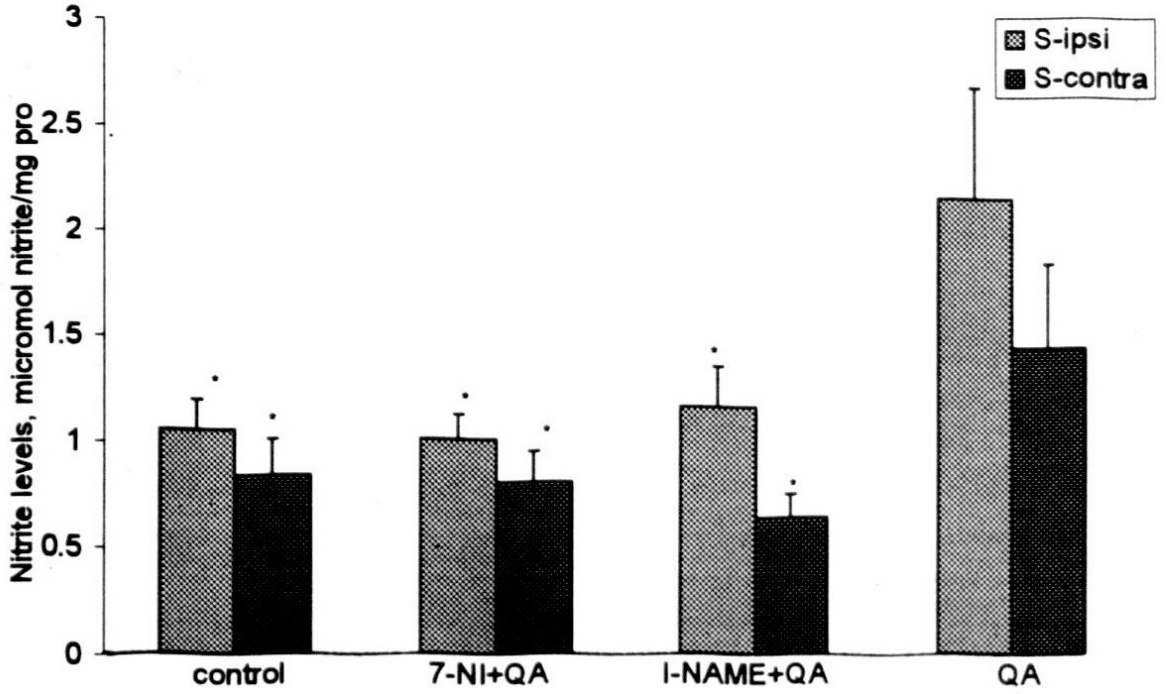

Fig. 1 Nitrite levels in the ipsi- and contralateral striatum of QA- and NOS inhibitor treated Wistar rats (Si, Sc = striatum ipsi-, contralateral). Values are given as micromol nitrite/mg prot. Mean ± S.D. *- Statistical significance of difference from corresponding values of QA-treated animals. (Students t-test, $p<0.05$).

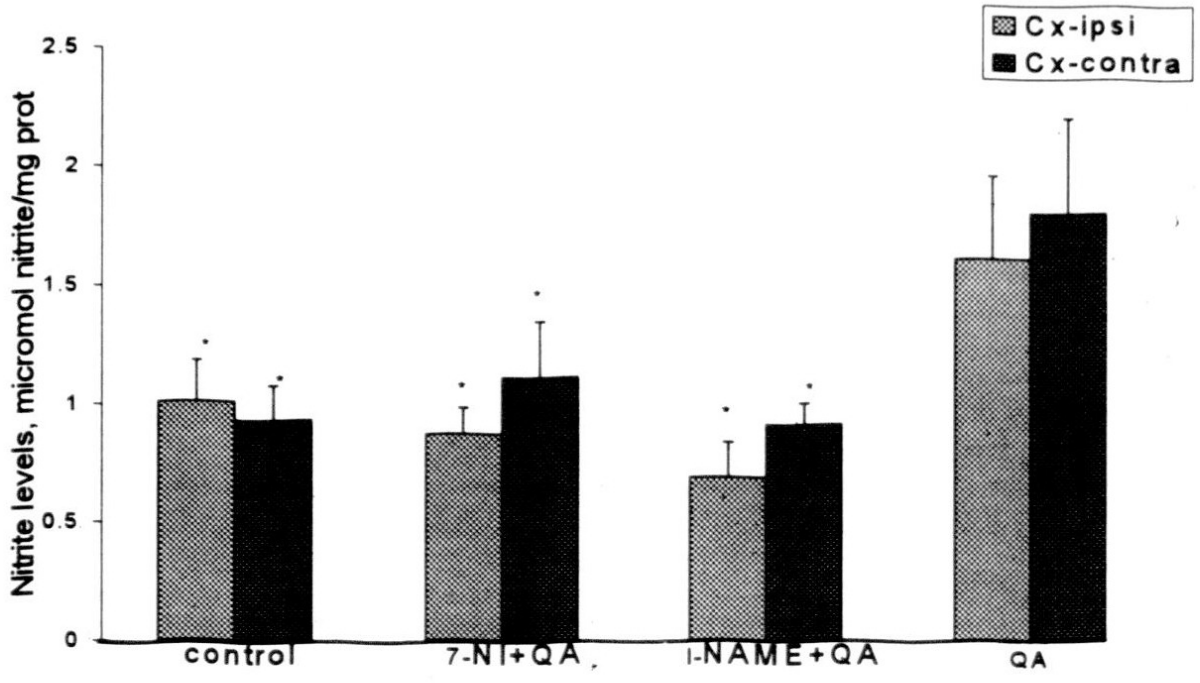

Fig. 2 Nitrite levels in the ipsi- and contralateral forebrain cortex of QA- and NOS inhibitor-treated Wistar rats (Cxi, Cxc = forebrain cortex ipsi-, contralateral). Values are given as micromol nitrite/mg prot. Mean ± S.D. *- Statistical significance of difference from corresponding values of QA-treated animals. (Students t-test, $p<0.05$).

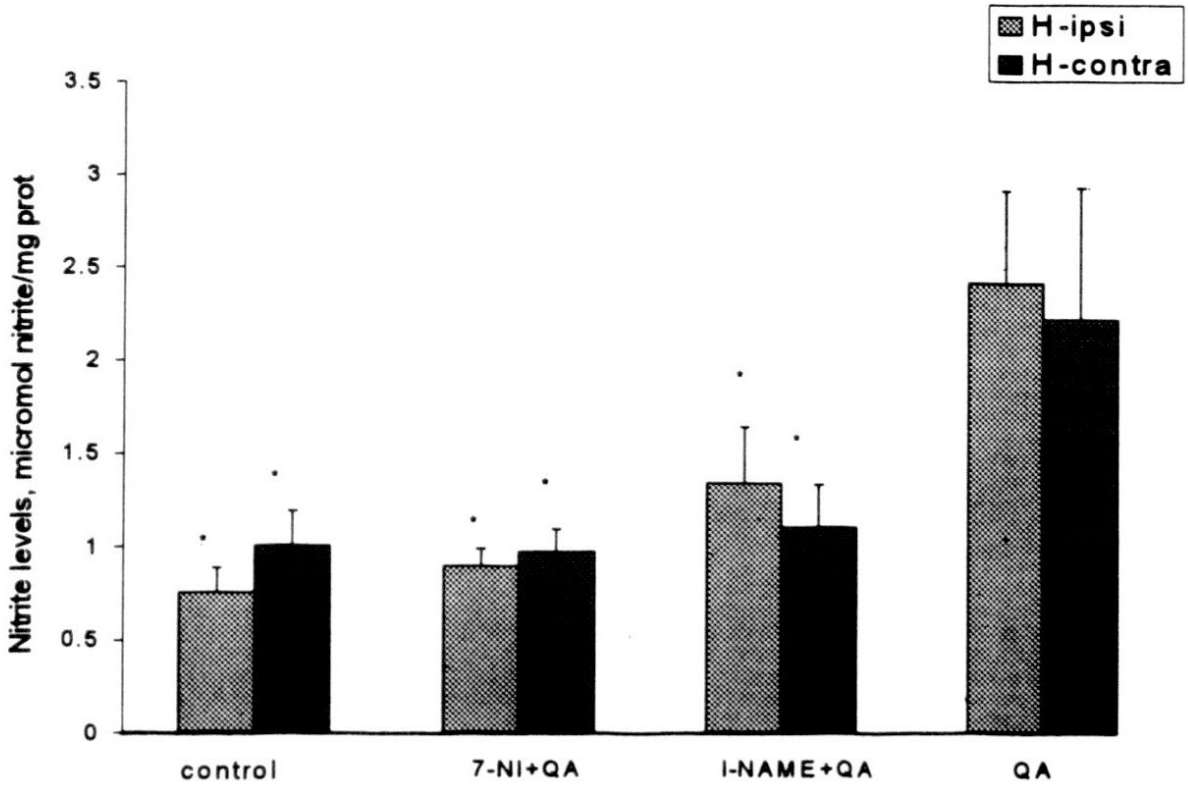

Fig. 3 Nitrite levels in the ipsi- and contralateral hippocampus of QA- and NOS inhibitor-treated Wistar rats (Hi, Hc = hippocampus ipsi-, contralateral). Values are given as micromol nitrite/mg prot. Mean S.D. *- Statistical significance of difference from corresponding values of QA-treated animals. (Students t-test, $p<0.05$).

DISCUSSION

Bioenergetic defects and oxidative stress could be critical links in excitotoxic mechanisms of neuronal death. Imbalance between production and removal of free radicals would be abrasive for a neuron. Increase in the intracellular levels of reactive oxygen species (ROS), frequently referred to as oxidative stress, represents a potentially toxic insult, which if not counteracted, will lead to membrane dysfunction, DNA damage and inactivation of proteins (Jenner, 1994).

Inhibiting NOS, an essential enzyme for production of nitric oxide (NO), as a means of reducing NMDA-induced neurotoxicity has produced conflicting results (Zhang and Snyder, 1995). Some investigators have found neuroprotection with NOS inhibitors, while others report no neuroprotection or exacerbation of NMDA-induced toxicity (Dawson *et al.*, 1992). These discrepancies may stem from at least two methodological problems. Firstly, some compounds may affect endothelial NOS (eNOS), as well as neuronal (n) NOS. Secondly, variability in the neurotoxic effects of acute QA injection, commonly used to induce NMDA receptor mediated lesions, may reduce reliability in assessing potentially neuroprotective compounds (Reddy *et al.*, 1999).

The relative effectiveness of NOS antagonists as neuroprotectants may depend in part on their relative specificity for the neuronal form of NOS (Chabrier *et al.*, 1999). Both the selective nNOS inhibitor, 7-NI, and the nonselective NOS

inhibitor l-NAME suppressed nitrite accumulation and attenuated neuronal damage induced by QA in the striatum, forebrain cortex and hippocampus (Fig. 1, 2, 3). As 7-NI selectively inhibits the neuronal form of NOS, this study suggests that NO produced from a neuronal and not an epithelial source may contribute to neuronal damage in this model.

The age-related onset and progressive course of HD may be due to a cycling process between impaired energy metabolism and oxidative stress (Schmidt *et al.*, 1995). The deleterious networks in the brain of HD are formed on the basis of the intimate interactions among the key pathogenic factors, including oxidative damage, aberrant calcium homeostasis and metabolic compromise (Milstien *et al.*, 1994).

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Vasiljević Ivana *et al.* Nitric oxide synthase inhibition prevents acute QA-induced neurotoxicity

INHIBICIJA AZOT OKSID SINTAZE SPREČAVA AKUTNU NEUROTOKSIČNOST IZAZVANU HINOLINSKOM KISELINOM

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SADRŽAJ

U ovoj studiji je ispitivan selektivni inhibitor neuronske azot oksid sintaze (NOS), 7-NI, kao i nespecifični NOS inhibitor, l-NAME, zbog moguće uključenosti azot oksida (NO) u toksičnost strijatuma izazvanu hinolinskom kiselinom (HK) kod pacova. HK je aplikovana unilateralno u strijatum adultnih Wistar pacova u pojedinačnoj dozi od 150 nmol/L. Druga i treća grupa životinja tretirane su sa 7-NI i HK i l-NAME i HK. Kontrolna grupa životinja primila je 0.9% fiziološki rastvor na isti način. Koncentracija nitrita snižena je u ipsi- i kontralateralnom strijatumu, kori prednjeg mozga i hipokampusu eksperimentalnih grupa životinja tretiranih NOS inhibitorima (7-NI, l-NAME) i HK u poređenju sa životinjama koje su dobile samo neurotoksin. S obzirom da 7-NI selektivno inhibira neuronsku izoformu NO sintaze, ova studija pokazuje da u oštećenju neurona u ovom eksperimentalnom modelu učestvuje NO produkovan iz neuronskog, a ne endotelnog izvora.