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NERVE GROWTH FACTOR PROTECTS CHOLINERGIC NEURONS AGAINST QUINOLINIC ACID-INDUCED EXCITOTOXICITY IN WISTAR RATS

VASILJEVIĆ IVANA*, JOVANOVIĆ MARINA*, ČOLIĆ M*, MIĆIĆ D**, NINKOVIĆ MILICA* and MALIČEVIĆ Ž**

**Military Medical Academy - Institute for Medical Research*, ***School of Medicine, Belgrade*

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The etiology of neuronal death in neurodegenerative diseases, including Huntington's disease (HD) is still unknown. There could be a complex interplay between altered energy metabolism, excitotoxicity and oxidative stress. Excitotoxic striatal lesions induced by quinolinic acid (QA), were used to test for the neuroprotective actions of nerve growth factor (NGF) on striatal cholinergic and GABAergic neurons. QA is an endogenous excitotoxin acting on N-methyl-D-aspartate (NMDA) receptors, that leads to neurotoxic damage resembling the alterations observed in HD. Unilateral administration of QA, in to the rat striatum in a single dose of 150 nM/L was used as the model of Huntington's disease. The second group was treated both with QA in the same dose and NGF in a dose of $7x10^{-9}$ g. NGF was applied immediately before the neurotoxin. The control group was treated with 0.154 mmol/L saline solution likewise. The activity of acetylcholinesterase (AChE) was increased in both the ipsi- and contralateral striatum, forebrain cortex, hippocampus and basal forebrain of QA-treated animals. This was prevented by NGF Some evidence suggests that interaction between neurotrophin receptors and glutamatergic activity may play an important role in plastic changes and synaptic reorganization of striatal circuits after excitotoxic injury. Considering that NGF mediates their activity across glutamatergic synapses, and, besides others, also has antioxidative effects, the obtained results indicate its protective role towards functional defects caused by QA.

Key words: acetylcholinesterase, Huntington disease, nerve growth factor, quinolinic acid.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder marked by selective striatal degeneration, motor dysfunction, and cognitive deterioration with learning impairment, memory loss and dementia (Hansson *et al.,* 2001). Neuropsychiatric problems, including personality changes, depression, and cognitive deficits, often precede the manifestation of chorea by as much as ten years. Cognitive decline consists of spatial learning

deficits, working memory and retrieval dysfunction, as well as obsessive behavior (Behan *et al.*, 1999).

The striatum, as a part of the basal ganglia and limbic system, has a very important role in motor and behavior regulation (Mink, 1999). Besides the striatum, the forebrain cortex, hippocampus and basal forebrain are also included in the selectively vulnerable brain structures. Massive afferents from all areas of the cortex and the thalamus are the most important source of excitatory amino acids, whereas the intrinsic circuits provide the striatum with acetylcholine, GABA, nitric oxide and adenosine (Popoli *et al.,* 2002). All these neurotransmitter systems interact with each other and with voltage-dependent conductances to regulate the efficacy of synaptic transmission within this nucleus. The integrative action exerted by striatal projection neurons on this converging information dictates the final output of the striatum to the other basal ganglia structures.

Neurotoxicity of excitatory amino acids (EAA) contributes to the pathogenesis of HD (Blundell *et al.*, 2000). The N-methyl-D-aspartate (NMDA) class of glutamate receptors is believed to play a prominent role in the pathogenesis of CNS excitotoxicity (Tkac *et al.*, 2001). 2,3 pyridine dicarboxylic acid (quinolinic acid-QA) has recently drawn considerable attention as an endogenous excitotoxic metabolite of l-tryptophan that mediates neuronal injury through NMDA receptor activation and subsequent elevation of intracellular calcium and oxidative stress (Harris *et al.*, 1998). Our studies have indicated that acute intrastriatal injections of the endogenous NMDA receptor agonist, QA, can mimic some of the neuroanatomical and behavioral deficits of HD. Intrastriatal injections of QA were used to test for neuroprotective actions of nerve growth factor (NGF) on cholinergic neurons (Moroni, 1999).

NGF is a member of the neurotrophin family of proteins that can regulate neuronal development, maintenance and recovery from injury (Jiang *et al.*, 2001). Additional effects of NGF stimulation include membrane ruffling, increased intracellular Ca^{2+} levels, increased transcription of c-fos and c-jun, together with increased phosphorylation of a number of intracellular proteins (Wu *et al.*, 2001).

Acetylcholine is a modulatory central nervous system neurotransmitter involved in diverse brain processes.

MATERIALS AND METHODS

The investigation was made on adult Wistar rats of both sexes and body weight sabout 250 g. The animals were divided into three groups and kept in macrolen cages (Erath, FRG) with free access to food and water. Average microclimate conditions were as follows: room temperature 23±2 ^{o}C, air humidity 55±10%, 10-50 air exchanges per hour, and the light regime was a cycle of 12 hours from 7-19 hours. The animals were anesthetized by pentobarbital sodium i.p. in a dose of 0.0405 g/kg b.w., and were placed in a stereotaxic frame.

Two groups of 8 animals received unilateral injection of QA (Aldrich Chemical Company, Inc.) in a single dose of 250.7 mg dissolved in H_2O (150 nM/L) using a stereotaxic instrument for small animals and coordinates for striatum (8.4; 2.4; 5.0 mm). The second group was also treated with NGF (Sigma,

Aldrich Chemie, Germany) immediately after the neurotoxin. The injected intracerebral volume was $10x10^{-6}$ ml. The control group received the same volume of 0,154 mmol/L saline solution and served as a sham-operated group.

All animals were sacrificed by decapitation 7 days after the treatment and the brains immediately removed. Ipsi- and contralateral striatum, forebrain cortex, hippocampus as well as basal forebrain from individual animals were quickly isolated and homogenized in ice-cold buffer containing 0.25 mol sucrose, 0.1 mmol EDTA, 50 mmol K-Na phosphate buffer, pH=7.2. Homogenates were centrifuged twice at 1580 g for 15 min at 4°C. The supernatant obtained by this procedure was then frozen and stored at -70°C (Gurd *et al.*, 1974).

The activity of acetylcholinesterase (AChE) was measured in the ipsi- and contralateral striatum, forebrain cortex, hippocampus and basal forebrain. The method is based the ability of AChE to degrade acetylthiocholine iodide into a product which binds DTNB (5,5-dithiobis-2-nitrobenzoic acid) forming a yellow compound. The extinction change is followed at a 3-5 minute interval at 412 nm (Mićić and Petronijević, 2000).

The content of protein in the rat brain homogenates (ipsi- and contralateral striatum, forebrain cortex, hippocampus and basal forebrain) was measured by the method of Lowry et al. using bovine serum albumin (Sigma) as the standard (Lowry *et al.,* 1974).

All experiments were done with n=8. Data are expressed as means±SD. Differences between groups were examined using Student's independent *t*-test. Statistical significance was accepted at $p<0.05$.

RESULTS

The results presented in Figure 1. show the AChE activity (μmol acetyl thiocholine/g prot.) in the ipsi- and contralateral striatum homogenates. QA injection was followed by a significant increase in AChE production in the ipsi- and contralateral striatum compared to the control animals (ipsilateral striatum = 6.6 ±1.6; contralateral striatum=5.6±1.5). There was no statistically significant difference in activity of acetylcholinesterase between each hemisphere, although the injection site was in the ipsilateral striatum in all experimental groups. NGF treatment before QA, very clearly maintained the lower levels of AChE in this brain structure compared to the QA-treated group (ipsilateral striatum = 28.3±4.0; contralateral striatum =134.1±19.5).

The effect of different intrastriatal drugs on the activity of AChE in forebrain cortex is shown in Figure 2. After QA, AChE activity in the ipsi- and contralateral forebrain cortex showed a significant increases compared to control animals (ipsilateral forebrain cortex=5.9±1.8; contralateral forebrain cortex =4.9±1.1). The AChE activity remained low in the ipsi- and contralateral forebrain cortex in NGF+QA treated animals compared with the QA-treated group (ipsilateral forebrain cortex = 56.0±10.1; contralateral forebrain cortex = 37.0±10.7). There was no statistically significant difference in the activity of AChE between each hemisphere.

The effect of different intrastriatal drugs on AChE activity in the hippocampus is shown in Figure 3. In the QA-treated group the activity of AChE was significantly increased compared to control animals (ipsilateral hippocampus = 8.2±1.1; contralateral hippocampus = 6.2 ± 1.8). NGF+QA showed significantly lower AChE activity both in the ipsi- and contralateral sides compared to QA-treated animals (ipsilateral hippocampus = 131.6±16.4; contralateral hippocampus=118.7±23.1). There was no statistically significant difference in the activity of AChE between each hemisphere.

The results obtained (Figure 4.) showed that QA induced a significant increase of AchE compared to the control group in the basal forebrain(ipsilateral basal forebrain = 6.5±1.8; contralateral basal forebrain=9.0±1.8). AChE activity was depleted in the ipsi- and contralateral basal forebrain of NGF+QA-treated animals, compared to the QA-treated group (ipsilateral basal forebrain = 92.1±20.3; contralateral basal forebrain = 132.5±29.4). There was no statistically significant difference in the activity of AChE between each hemisphere.

DISCUSSION

Long-lasting changes in the efficacy of excitatory transmission have been proposed to represent the cellular basis of some forms of motor learning and are altered in animal models of HD (Davies *et al.*, 1999). During pathological conditions, striatal synaptic transmission is altered depending on presynaptic inhibition of transmitter release and the opposite membrane potential changes occur in projection neurones and in cholinergic interneurones. These ionic mechanisms might partially explain the selective neuronal vulnerability in the striatum during HD (Nakamura *et al.*, 1999). In the ipsi- and contralateral striatum of NGF-treated animals, we demonstrated low activity of AChE (Fig. 1).

AChE, a biochemical marker for cholinergic neurons, is secreted from various brain regions and may have alternative, non-cholinergic functions, one of which could be in development. Endogenous acetylcholine (ACh) exerts complex modulation of striatal synaptic transmission, which produces both short-term and long-term effects (Martinez *et al.*, 1998). ACh-mediated mechanisms might be of crucial importance in processing the cortical inputs to the striatum (Guzowski *et al.*, 1997).

One of the most prominent cholinergic deficits after application of NGF is the reduced number of nicotinic ACh receptors in the cortex and hippocampus. This deficit results in reduced nicotinic cholinergic excitation which may not only impair postsynaptic depolarization but also presynaptic neurotransmitter release and Ca^{2+}-dependent intracellular signaling, including transcriptional activity (Canals *et al.*, 1999). Our results indicate low AChE activity of the ipsi- and contralateral forebrain cortex and hippocampus in NGF-treated rats (Fig. 2, 3). Although NGF mRNA is widely distributed throughout the brain, levels are highest in the hippocampus and neocortex, target areas of basal forebrain cholinergic neurons. Unilateral striatal injections were found to lead to increases in the AChE activity of the ipsi- and contralateral basal forebrain (Fig. 4). It is postulated that both the trkA and p75 receptors are transported anterogradely to the target regions of the basal

forebrain and upon binding to the high-affinity receptor, NGF is retrogradely transported as a ligand/receptor complex to perikarya of BFCN, where NGF provides trophic support. It has been hypothesised that a NGF-trkA receptor complex (or NGF-trkA-p75 receptor complex) is transported to the neuronal cell body and that the activated trkA receptor initiates signal transduction mechanisms that results in the induction of trophic functions (Lee *et al.*, 1998).

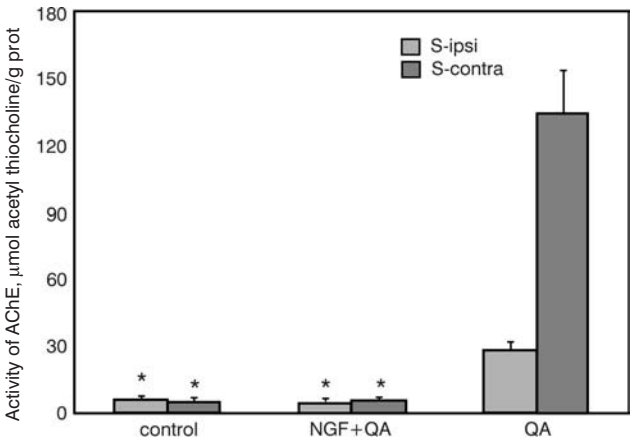

Figure 1. Activity of AChE in the ipsi- and contralateral striatum of QA- and NGF-treated Wistar rats. (Si, Sc = striatum ipsi-, contralateral). Values are given as micromol acetyl tiocholine/g prot. Mean ± S.D. * - Significance to corresponding values of QA-treated group. (Student's t-test, $p<0.05$).

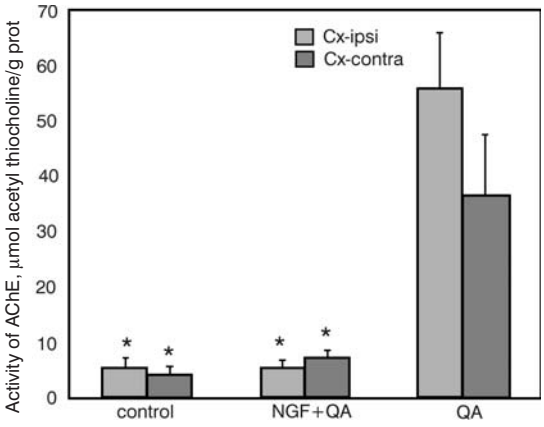

Figure 2. Activity of AChE in the ipsi- and contralateral forebrain cortex of QA- and NGF-treated Wistar rats. (Cxi, Cxc = forebrain cortex ipsi-, contralateral). Values are given as micromol acetyl tiocholine/g prot. Mean ± S.D. * - Significance to corresponding values of QA-treated group. (Student's t-test, $p<0.05$).

Basal forebrain atrophy in HD may be due to a deficit in the NGF responsiveness of magnocellular cholinergic neurons that project to the cortex and hippocampus. A marked reduction in the density of NGF receptors was observed in the caudate nucleus, putamen, ventral striatum, nucleus basalis and tegmental nucleus in HD patients. NGF-receptor gene expression is selectively reduced within degenerating basal forebrain neuronal populations in HD (Francis *et al.*, 2000).

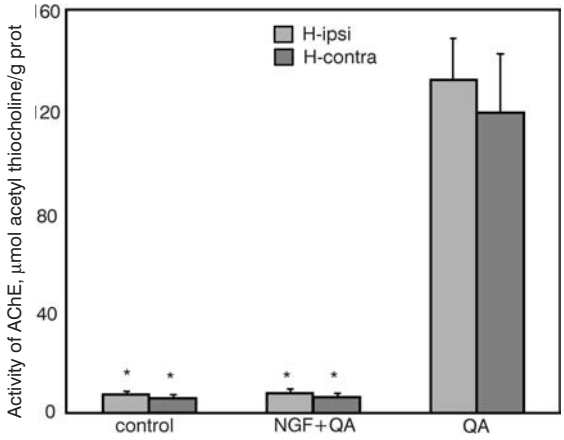

Figure 3. Activity of AChE in the ipsi- and contralateral hippocampus of QA- and NGF-treated Wistar rats. (Hi, Hc = hippocampus ipsi-, contralateral). Values are given as micromol acetyl tiocholine/g prot. Mean ± S.D. * - Significance to corresponding values of QA-treated group. (Student's t-test, $p<0.05$).

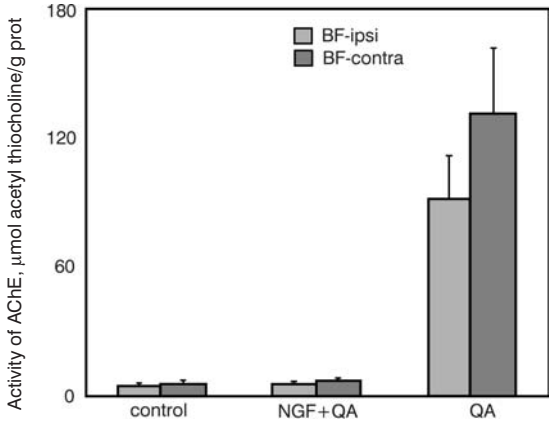

Figure 4. Activity of AChE in the ipsi- and contralateral basal forebrain of QA- and NGF-treated Wistar rats. (BFi, BFc = basal forebrain ipsi-, contralateral). Values are given as micromol acetyl tiocholine/g prot. Mean ± S.D. * - Significance to corresponding values of QA-treated group. (Student's t-test, $p<0.05$).

CONCLUSIONS

Immature neurons have mechanisms in place that ordinarily lead to cell death unless suppressed by NGF. A possible interpretation of these experiments is that neuronal death in response to deprivation of trophic factor is an active process carried out by a "suicide-program" within the cell. Interaction between neurotrophin receptors and glutamatergic activity may play an important role for plastic changes and synaptic reorganization of striatal circuits after excitotoxic injury (Jain, 2000). Considering that NGF mediates their activity across glutamatergic synapses and also has antioxidative effects, the obtained results indicate a protective role towards functional defects caused by QA.

Address for correspondence:
Vasiljević Ivana
Military Medical Academy,
Institute for Medical Research
Crnotravska 17, 11000 Belgrade
Serbia & Montenegro
e-mail: ivanav13@yahoo.ca

REFERENCES

1. *Behan WMH, McDonald M, Darlington LG, Stone TW,* 1999, Oxidative stress as a mechanism for quinolinic acid- induced hippocampal damage: protection by melatonin and deprenyl. *British J Pharmacol,* 128, 1754-60.
2. *Blundell TL, Burke DF, Chirgadze D, Dhanaraj V, Hyvonen M, Innis CA, Parisini E, Pellegrini L, Sayed M, Sibanda BL,* 2000, Protein-protein interactions in receptor activation and intracellular signalling. *Biol Chem*, 381(9-10):955-9.
3. *Canals JM, Checa N, Marco S, Michels A, Perez-Navarro E, Alberch J,* 1999, The neurotrophin receptors trkA, trkB and trkC are differentially regulated after excitotoxic lesion in rat striatum. *Mol Brain Res*, 69, 242-8.
4. *Davies SW, Turmaine M, Cozens BA, Raza AS, Mahal A, Mangiarini L, Bates GP,* 1999, From neuronal inclusions to neurodegeneration: neuropathological investigation of transgenic mouse model of Huntington's disease. *Philos Trans R Soc Lond B Biol Sci*, 354(1386), 981-9.
5. *Francis L, Cruz R, Antunez I, Rosillo JC,* 2000, Behavior characterization of a model of Huntington's disease in rats, induced by quinolinic acid. *Rev Neurol*, 30(11), 1016-21.
6. *Gurd JW, Jones LR, Mahler HR, Moore WJ,* 1974, Isolation and partial characterization of rat brain synaptic membrane. *J Neurochem*, 22: 281-90.
7. *Guzowski JF, Mc Gaugh JL,* 1997, Antisense oligodeoxynucleotide-mediated disruption of hippocampal CREB protein levels impairs memory of a spatial task. *Proc Natl Acad Sci USA,* 94, 2693-8.
8. *Hansson O, Guatteo E, Mercuri NB, Bernardi G, Li XJ, Castilho RF, Brundin P,* 2001, Resistance to NMDA toxicity correlates with appearance of nuclear inclusions, behavioural deficits and changes in calcium homeostasis in mice transgenic for exon 1 of the huntington gene. *Eur J Neurosci*, 14(9):1492-504.
9. *Harris CA, Miranda AF, Tanguay JJ, Boegman RJ, Beninger RJ, Jhamandas K,* 1998, Modulation of striatal quinolinate neurotoxicity by elevation of endogenous brain kynurenic acid. *British J Pharmacol*, 124, 391-9.
10. *Jain KK,* 2000, Evaluation of memantine for neuroprotection in dementia. *Expert Opin Investig Drugs*, 9(6), 1397-406.

11. *Jiang H, Movsesyan V, Liu X W, Katagiri Y, Monshipoyri M, Lazarovici P,* 2001, A double cysteine trkA mutant exhibiting reduced NGF binding and delayed Erk signaling. *J Mol Neurosci*, 17(3):293-302.
12. *Lee TH, Kato H, Pan LH, Ryu JH, Kogure K, Itoyama Y*, 1998, Localization of nerve growth factor, trkA and p75 immunoreactivity in the hippocampal formation and basal forebrain of adult rats. *Neurosci*, 83(2), 335-49.
13. *Lowry OH, Passonneau JV,* 1974, In: A flexible system of enzymatic analysis. Academic Press, New York.
14. *Martinez JL, Derrick BE,* 1998, Long- term potentiation and learning. *Annu Rev Psychol,* 47, 173-203.
15. *Mićić VD, Petronijević DN,* 2000, Acetycholinesterase activity in the Mongolian gerbil brain after acute poisoning with aluminum, *J Alzh Dis*, 2, 1-6.
16. *Mink JW,* 1999, Basal ganglia. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR, editors. Fundamental neuroscience. San Diego: Academic Press; p. 951-72.
17. *Moroni F,* 1999, Trytophan metabolism and brain function: focus on kynurenine and other indole metabolites. *European J Pharmacol*, 375, 87-100.
18. *Nakamura H, Nakamura K, Yodoi J,* 1999, Redox regulation of cellular activation. *Annu Rev Immunol*, 15, 351-69.
19. *Popoli P, Pintor A, Domenici MR, Frank C, Tebano MT, Pezzola A, Scarchilli L, Quarta D, Reggio R, Malchiodi-Albedi F, Falchi M, Massotti M,* 2002, Blockade of striatal adenosine A2A receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. *J Neurosci*, 22(5):1967-75.
20. *Tkac I, Keene CD, Pfeuffer J, Low WC, Gruetter R,* 2001, Metabolic changes in quinolinic acid-lesioned rat striatum detected non-invasively by in vivo (1)H NMR spectroscopy. *J Neurosci Res,* 66(5):891-8.
21. *Wu C, Lai CF, Mobley WC,* 2001, Nerve growth factor activates persistent Rap1 signaling in endosomes. *J Neurosci*, 21(15):5406-16.

NERVNI FAKTOR RASTA SPREČAVA EKSCITOTOKSIČOST HOLINERGIČKIH NEURONA IZAZVANU HINOLINSKOM KISELINOM KOD WISTAR PACOVA

VASILJEVIĆ IVANA, JOVANOVIĆ MARINA, ČOLIĆ M, MIŠIĆ D, NINKOVIĆ MILICA i MALIČEVIC Ž

SADRŽAJ

Tačna etiologija selektivnog umiranja neurona u neurodegenerativnim bolestima, uključujući i Hantingtonovu bolest (HB), još uvek je nepoznata. Postoji mogućnost da je to kompleks između izmenjenog energetskog metabolizma, ekscitotoksičnosti i oksidativnog stresa. Ekscitotoksično oštećenje strijatuma indukovano hinolinskom kiselinom (HK) korišćeno je za određivanje neuroprotektivnog dejstva nervnog fatora rasta (NGF) na holinergičke i GABAergičke neurone strijatuma. HK je endogeni ekscitotoksin koji deluje preko N-metil-D-aspartatnih receptora i uzrokuje neurotoksično oštećenje neurona koje

odgovara promenama opisanim u HB. Unilateralna aplikacija HK u strijatum pacova u pojedinačnoj dozi od 150 nM/L, korišćena je kao eksperimentalni model HB. Druga grupa životinja tretirana je HK u istoj dozi i NGF-om u dozi od $7x10^{-9}$ g. NGF je aplikovan odmah nakon neurotoksina. Kontrolna grupa primila je 0.154 mmol/L fiziološkog rastvora natrijum hlorida na isti način. Aktivnost acetilholin esteraze (AChE) značajno je povećana, kako u ipsi-, tako i u kontralateralnom strijatumu, kori prednjeg mozga, hipokampusu i bazalnom prednjem mozgu HK-tretiranih životinja. Postoje podaci da interakcija između neurotrofičkih receptora i glutamatergičke aktivnosti može imati veoma važnu ulogu u plastičnim promenama i sinaptičkoj reorganizaciji strijatnih krugova nakon ekscitotoksičnog oštećenja. Pored sposobnosti da moduliše aktivnosti koje se ostvaruju preko glutamatergičke sinapse, NGF ima i antioksidativne efekte, te naši rezultati pokazuju njegovu protektivnu ulogu na funkcionalne defekte uzrokovane HK.