FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.10, No 2, 2003, pp. 88 - 91 UC 616-092.9:612.017]:611.813

NERVE GROWTH FACTOR AND FIBROBLAST GROWTH FACTOR PREVENTS ACUTE QA-EXCITOTOXICITY IN RAT BASAL FOREBRAIN

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Summary. *Adult Wistar rats were treated with unilateral intrastriatal injection of quinolinic acid (QA) in one single dose of 150 nM/L. The other two group of animals were pretreated with nerve growth factor (NGF) and fibroblast growth factor (FGF), respectively. Control group was treated with 0.154 mmol/L saline solution likewise. Activity of cytochrome c oxidase (COX) was decreased in the basal forebrain of neurotrophins (NTF)-treated animals. Striatal lesions led to the loss of tonic inhibitory inputs to the globus pallidus with consequent increase changes due to transsynaptic degeneration in the basal forebrain, will be also less extensive in the NTF-treated animals.*

Key words: *Huntington disease, quinolinic acid, nerve growth factor, fibroblast growth factor, basal forebrain, cytochrom c oxidase*

Introduction

Huntington's disease (HD) is a neurodegenerative disorder with autosomal dominant inheritance (1, 2). In early stages of the disease neuropathological changes are mainly confined to the caudate nucleus of the striatum where a subpopulation of striatal projection neurons has been found to degenerate preferentially (3, 4, 5).

The N-methyl-D-aspartate (NMDA) class of glutamate receptors is believed to play a prominent role in the pathogenesis of CNS excitotoxicity (6). Excessive release of glutamate, activation of NMDA receptor mechanisms, excessive influx of Na^+, Cl^-, water and Ca^{2+}, as well as prolonged burst firing throughout excitatory pathways represent mechanisms that have been identified as a clue for the regional vulnerability of certain populations of neurons. Mitochondrial energy metabolism provides a main line of defence (7, 8). Mitochondrial ATP production fuels both the sodium/ potassium pump and calcium uptake by the endoplasmic reticulum, both of which provide protection against excitotoxicity (9). It may be that impaired ATP production is a common precipitating factor in HD, and that it works by rendering neurons vulnerable to excitotoxic damage.

Excessive activation of glutamate receptors is considered a key step in HD. The synthesis of NAD (or NADP) from tryptophan involves a series of enzymes and the formation of a number of intermediates which are collectively called "kynurenines" (10). One of the "kynurenines", 2,3-pyridine dicarboxilic acid (quinolinic acid- QA), was an agonist of a subpopulation of NMDA receptors caused excitotoxic neuronal death (11, 12). QA interacts with a subgroup of NMDA receptors and when directly injected into brain areas, it destroys most neuronal cell bodies and neuronal terminals (13).

Neurotrophic factors (NTF) may be defined as proteins which promote the survival, morphological differentiation and gene expression of specific neuronal populations (14). The nerve growth factor (NGF) and the fibroblast growth factor (FGF) markedly protect striatal neurons from NMDA-receptor induced neurotoxicity (15).

Our aim was to examine whether NGF and FGF could protect the basal forebrain after intrastriatal application of QA in a rat model of HD.

Material and methods

Animals

Adult rats of Wistar strain (Rattus norvegicus) of both sexes, with body weight 250 g, were used for experiments. Groups of two or three rats per cage (Erath, FRG), were housed in an air-conditioned room at room temperature of 23±2 °C with 55±10% humidity and with lights on 12 h/day (07.00-19.00). The animals were given a commercial rat food and tap water ad libitum. These animals were anaesthetized by giving intraperitoneal injections of pentobarbital sodium (0.0405 g/kg b.w.) and were placed in a stereotaxic frame.

Experimental procedure and intracerebral injection of drug

The rats were divided into four groups (according to drug treatment) and each group consisted of 8 animals. The first group received an unilateral injection of QA (Aldrich Chemical Company, Inc.) in the single dose of 250.7 mg (dissolved in H_2O) using a stereotaxic instrument for small animals and coordnate for the striatum (8.4; 2.4; 5.0 mm). The second and third group were treated with NGF (Sigma, Aldrich Chemie, Germany; 7×10^{-9} g dissolved in saline solution) + QA and FGF (Sigma, Aldrich Chemie, Germany; 4×10^{-9} g dissolved in saline solution) + QA. NGF and FGF was immediately applicated before the neurotoxin. For all treated animals the injected intracerebral volume was 10×10^{-6} ml. The control group received the same volumen of 154 mmol/L saline solution and served as a control-sham-operated.

All animals were sacrificed by decapitation 7 days after the treatment and the brains immediately removed. Ipsi- and contralateral basal forebrains from individual animals were quickly isolated and homogenized in an 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 (16).

Cytochrome c oxidase activity

The cytochrome c oxidase activity (COX) was measured as a decrease of absorbance during oxidation of ferrous cytochrome c to ferric cytochrome c. Kinetics was followed in the potassium-phosphate buffer (0.05 M, pH=7.1) during 3-5 minutes at 550 nm. Samples were pretreated with deoxycholate (7.5%). Sodium dithionite (1mM $Na_2S_2O_4$) was used for the reduction of cytochrome c. The reaction started with addition of prepared sample (0.05 ml) to the reduced solution of citochrome c (0.95 ml) (17).

Protein measurement

The content of protein in the rat brain homogenates (ipsi- and contralateral basal forebrain) was measured by the method of Lowry et al. using bovine serum albumin (Sigma) as standard (18).

Data presentation and analysis

All experiments were done with n=8. Data are expressed as means ± SD. Differences between groups were examined using a Student′s independent t-test. Statistical signifikance was accepted at $p<0.05$.

Results

The effect of different intrastriatal drug injection on content of COX activity (mg cyt c/mg prot.) in the ipsi- and contralateral basal forebrain is shown in Figure 1. QA injection results show a significant increase in COX production in the ipsi- and contralateral basal forebrain (ipsilateral basal forebrain = 0.504±0.065; contralateral basal forebrain = 0.475±0.071) compared to the control animals (ipsilateral basal forebrain = 0.161±0.039; contralateral basal forebrain = 0.207±0.050).

NGF treatment followed with QA, very clearly represent the lower levels of COX in the basal forebrain ipsi- and contralateral (ipsilateral basal forebrain = 0.202± 0.072; contralateral basal forebrain = 0.239±0.041) compared to QA-treated group.

The COX activity was decreased in the ipsi- and contralateral basal forebrain FGF+QA animals (ipsilateral basal forebrain = 0.254±0.075; contralateral basal forebrain = 0.210±0.051) compared with QA-treated group.

There was not statistically significant difference in content of COX activity obtained from each hemisphere in all examined experimental group of animals.

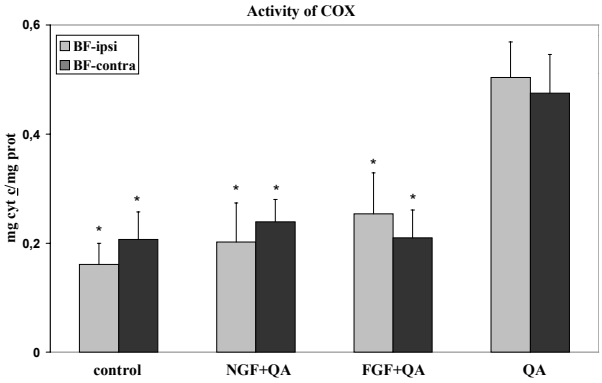

Fig. 1. Activity of COX in the ipsi- and contralateral basal forebrain of QA- and NTF-treated Wistar rats. (BFi, BFc = basal forebrain ipsi-, contralateral). Values are given as mg cyt c/mg prot. Mean ± S.D. ∗ - Significance to corresponding values of QA-treated group. (Student′s t-test, $p < 0.05$)

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Discussion

Mitochondria are essential to the cell for maintaining the normal voltage gradient across the cell membrane as well as a number of processes that controlling intracel-

lular calcium (19). Complex IV, cytochrome c oxidase (COX), is the last component of mitochondrial electron-transport chain. It catalyzes the four-electron reduction of molecular oxygen to water, what is accompanied with protons translocation into intermembrane space of mitochondria. In the case of oxidative stress the main place of relief of incomletely reduced forms of oxygen is the Complex I of respiratory chain because of that connections with these intermediates and this transporter are not so tigh, compared to the Complex IV (20).

Mitochondrial dysfunction may contribute to the localized hypometabolism and progressive atrophy of the HD caudate. In the group of QA-treated animals activity of COX was increased in the ipsi- and contralateral basal forebrain (Fig. 1). These results indicate a temporal and spatial propagation of oxidative stress in the basal forebrain, the structure distant, but tightly connected with striatum, the place of direct neurotoxic damage.

It is well established that the activity of COX, a mitochondrial enzyme, reflects the long-term, steady-state levels of neuronal activity (21). The present results indicate that striatal lesions induce changes in the functional activity of basal ganglia nuclei and that the NTF partly reverse the alterations in the functional state of the basal ganglia circuitry. Activity of COX was mutually decreased in the basal forebrain of NTF-treated animals (Fig. 1). It could be proposed that lesion-induced morphological changes in the striatum and atrophic changes due to transsynaptic degeneration in the basal forebrain, will be also less extensive in the NGF- and FGF-treated animals (22, 23).

Interplay between defects in energy metabolism, excitotoxicity and oxidative damage may play a role in the pathogenesis of HD. In our study the *in vivo* cytoprotective effects of NTF against striatal excitotoxic lesions suggest that neurotrophic molecules could be used as potential neuroprotective agents in HD, which has been suggested to involve excitotoxicity.

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NERVNI I FIBROBLASTNI FAKTOR RASTA SPREČAVAJU AKUTNU EKSCITOTOKSIČNOST IZAZVANU HINOLINSKOM KISELINOM U BAZALNOM PREDNJEM MOZGU PACOVA

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Kratak sadržaj: *Adultni Wistar pacovi su jednostrano dobili intrastrijatnu injekciju hinolinske kiseline (HK) u pojedinačnoj dozi od 150 nM/L. Druge dve grupe životinja su pre neurotoksina tretirane nervnim (NGF), odnosno fibroblastnim faktorom rasta (FGF). Kontrolna grupa je dobijala 0,154 mmol/L fiziološki rastvor na isti način. Aktivnost citohrom c oxidase (COX) je snižena u bazalnom prednjem mozgu kod životinja tretiranih neurotrofinima. Oštećenja strijatuma koja vode u gubitak toničnih inhibitornih aferenci u globus palidus sa konsekventnim povećanjem promena, a povezane su sa međusinaptičkom degeneracijom u bazalnom prednjem mozgu, biće manje izražene kod životinja tretiranih neurotrofinima.*

Ključne reči: *Hantingtonova bolest, hinolinska kiselina, nervni faktor rasta, fibroblastni faktor rasta, bazalni prednji mozak, citohrom c oksidaza.*