NITRIC OXIDE SYNTHASE INHIBITORS PROTECT CHOLINERGIC NEURONS AGAINST QUINOLINIC ACID TOXICITY IN THE RAT BRAIN

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Abstract - The aim of this study was to examine the effects of intrastriatally injected nitric oxide synthase (NOS) inhibitors, Nω-nitro-l-arginine methyl ester (L-NAME) and 7-nitroindazole (7-NI), on quinolinic acid (QA)-induced toxicity in selective vulnerable brain regions of adult Wistar rats. QA was administered into the striatum unilaterally, in a single dose of 150 nM/L with a stereotaxic instrument. The other two experimental groups were pretreated with L-NAME and 7-NI, respectively. The control group of animals was treated with 0.154 mM/L saline solution. The animals were decapitated seven days after the treatment. Samples of both striatum and forebrain cortex were prepared for measurement of acetylcholinesterase (AChE) activity. QA injection revealed a significant increase in AChE activity in both the ipsi- and contralateral striatum and forebrain cortex compared to the control animals. Treatment with NOS inhibitors, followed by QA, very clearly demonstrated lower levels of AChE bilaterally in these brain structures, compared to the QA-treated group.

Key words: Acetylcholinesterase, Huntington's disease, quinolinic acid, Nω-nitro-l-arginine methyl ester, 7-nitroindazole

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 (Beister et al., 2004). Cognitive decline consists of spatial learning deficits, working memory and retrieval dysfunction and perseverative behavior. Our studies have indicated that acute intrastriatal injections of the endogenous N-methyl-D-aspartate (NMDA) receptor agonist, quinolinic acid (QA), can mimic some of the neuroanatomical and behavioral deficits of HD (Maksimović et al., 2002). Intrastriatal injections of QA have been shown to produce an HD-like pattern of neurodegeneration, including loss of striatal projection neurons (Armstrong et al., 2000). The disease causes widespread loss of cells from the corpus striatum, which causes the motor disturbance (Watts and Dunnett, 1998).

HD is characterized by the loss of neurons in the basal forebrain cholinergic cells that project to the cerebral cortex and hippocampus (Smith et al., 2006). These impairments have been correlated with the memory loss noted in the dementia of HD. This “cholinergic hypothesis” has led to the rational design of drugs to enhance or stimulate acetylcholine (ACh)-mediated neurotransmission (Walker et al., 2011).

The nitric oxide (NO) synthase (NOS) inhibitors L-NAME (Nω-nitro-l-arginine methyl ester) and 7-NI (7-nitroindazole) are widely used to study the role of NO in physiological and pathological process (Boje, 2004). The aim of this study was to examine the effects of intrastriatally injected L-NAME and 7-NI on QA-induced striatal toxicity, as well as the role of ACh in the pathogenesis in the QA-induced model of HD.
MATERIALS AND METHODS

Animals

The experimental animals were treated according to the Guidelines for Animal Study, No. 282-12/2002 (Ethics Committee of the Military Medical Academy, Belgrade).

The experiments were performed on adult Wistar rats, weighing between 200-250 g, randomly divided into one control group (n = 10) and three experimental groups (n = 9, each). Animals were housed (five rats per cage) under standardized housing conditions (ambient temperature of 23±2°C, relative humidity 55±3% and a light/dark cycle of 13 and 11 h, respectively) and had free access to standard laboratory pellet food and tap water. All the experiments were performed after a 7-day period of adaptation to laboratory conditions, and were carried out between 9 a.m. and 1 p.m. Experimental protocols were approved by the Local Animal Care Committee and conformed to the recommendations given in "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington, D.C., 1996).

Experimental procedure

Before treatment, the Wistar rats were intraperitoneally (i.p.) anesthetized with pentobarbital-Na in doses of 40.5 mg/kg body weight. QA was administered into the striatum of adult Wistar rats, unilaterally in a single dose of 150 nM/L using a Hamilton syringe, with a stereotaxic instrument for small laboratory animals (coordinates: 8.4 A; 2.6 L and 4.8 V) (Paxinos and Watson, 1986). The second and third experimental groups were treated with L-NAME and QA (L-NAME in a dose of 1 x 10^-4 g) and 7-NI and QA (7-NI in a dose of 1 x 10^-4 g). 7-NI was applied immediately before the neurotoxin, in contrast to L-NAME, which was given thirty minutes before QA. The control group of animals was treated with 0.154 mM/L saline solution likewise. A liquid overload (amounts greater than 10 µL) could provoke a brain tissue edema (Fukushima et al., 1994).

The brain tissue preparation

The animals were decapitated seven days after the treatment and the heads were frozen immediately (-70°C). The brain structures – striatum and forebrain cortex, were dissected on ice, and each tissue slice (approximately 0.1 g) was transferred into a tube of 1 ml cold, buffered sucrose medium (0.25 M/L sucrose with 0.1 mM/L EDTA in 50 mM K-Na phosphate buffer, pH 7.2). Homogenization of the tissue in the sucrose medium was performed by a homogenizer (Tehtnica, Zelezniki, Slovenia) at 800 rotation/min on ice. The homogenates were centrifuged at 1000 x g, for 15 min at 4°C. The precipitates were redispersed in sucrose medium and centrifuged again. The supernatants were centrifuged at 2500 x g for 30 min at 4°C and the obtained precipitates were redispersed in 1.5 mL of deionized water. The samples were centrifuged at 2000 x g for 15 min at 4°C and the supernatants (crude mitochondrial fractions) were stored at -70°C (Gurd et al., 1974). Total protein concentration was estimated with bovine serum albumin as a standard (Lowry et al., 1951).

Biochemical analyses

Acetylcholine esterase (AChE) activity was measured in the ipsi- and contralateral striatum and forebrain cortex. The method is based on the ability of AChE to degrade acetylthiocholine iodide into the product that binds DTNB (5,5-dithiobis-2-nitrobenzoev acid), thus forming a yellow color compound. The change in absorbance was followed in 3-5 min intervals at 412 nm (Micic and Petronijevic, 2000). The results were expressed as µM acetylthiocholine per g proteins.

Reagents

All used chemicals were of analytical grade. The following compounds were used in this study: quinolinic acid (QA), No-nitro-l-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI) and acetylthiocholine iodide, and were purchased from Sigma-Aldrich (Sr. Louis, USA). 5,5-dithiobis-2-nitrobenzoev acid (DTNB) was purchased from Merck.
Statistical analysis

The program STATISTICA 5.0 was used to perform one-way ANOVAs and post hoc Turkey tests (0.05 confidence value).

RESULTS

The obtained results of biochemical analysis showed that AChE activity was significantly elevated in the ipsi- \((p < 0.01)\) and contralateral \((p < 0.01)\) striatum of QA-injected animals in comparison with control groups (Fig. 1). The treatment with L-NAME followed by QA significantly decreased AChE activity in the contralateral striatum compared to the QA group \((p < 0.01)\). In addition, the activity of AChE significantly decreased bilaterally in the forebrain cortex in the 7-NI+QA group compared to the QA-injected animals (Fig. 1).

DISCUSSION

The bilateral increase in AChE activity in both the striatum and forebrain cortex seven days after QA application suggests a direct involvement of this neurotoxin in the modulation of enzyme activity. NOS

Fig. 1. The AChE activity (µM acetylthiocholine/g proteins) in the ipsi- and contralateral striatum. CG – control group animals treated with saline solution in dose 0.154 mM/L, QA – quinolinic acid in dose 150 nM/L (for HD induction protocol see Section Animals and Methods), L-NAME+QA – L-NAME in dose of 1 x 10⁻⁴ g was given 30 min before QA, 7-NI+QA – 7-NI in dose of 1 x 10⁻⁴ g was given just before the neurotoxin. Bars on the graph represent mean ± SEM from 9-10 animals for each group. Labels of statistical significance: c – compared to control group; q – compared to QA group. Statistical significance was considered at: * \(p < 0.05\) (L-NAME+QA vs CG, 7-NI+QA vs CG and 7-NI+QA vs QA in ipsilateral striatum, 7-NI+QA vs CG in contralateral striatum), ** \(p = 0.01\) (QA vs CG in ipsilateral striatum, QA vs CG, L-NAME+QA vs CG, L-NAME+QA vs QA and 7-NI+QA vs QA in contralateral striatum), one-way ANOVA, Turkey test.
inhibitors (L-NAME and 7-NI, respectively) with neurotoxin lead to reduced AChE activity bilaterally in both the striatum and forebrain cortex.

Endogenous ACh exerts a complex modulation of striatal synaptic transmission, which produces both short-term and long-term effects. ACh-mediated mechanisms might be of crucial importance in processing the cortical input to the striatum. Striatal neurons receive a myriad of synaptic inputs originating from different sources. Massive afferents from all areas of the cortex represent the most important source of excitatory amino acids, whereas the nigrostriatal pathway and intrinsic circuits provide the striatum with dopamine, ACh, GABA, NO and adenosine (Perluigi et al., 2005). All these neurotransmitter systems interact with each other and with voltage-dependent conductances to regulate the efficacy of the 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 (Bonelli et al., 2004). During pathological conditions, striatal synaptic transmission is altered, depending on the presynaptic inhibition of transmitter release and opposite membrane potential changes that occur in projection neurons and in cholinergic interneurons. These ionic mechanisms might partially explain the selective neuronal vulnerability observed both in the striatum and forebrain cortex during HD (Nopoulos et al., 2010).

Fig. 2. The AChE activity (µM acetylthiocholine/g proteins) in the ipsi- and contralateral forebrain cortex. CG – control group animals treated with saline solution in dose 0.154 mM/L, QA – quinolinic acid in dose 150 nM/L (for HD induction protocol see Section Animals and Methods), L-NAME+QA – L-NAME in dose of 1 x 10⁻⁴ g was given 30 min before QA, 7-NI+QA – 7-NI in dose of 1 x 10⁻⁴ g was given just before the neurotoxin. Bars in the graph represent mean ± SEM from 9-10 animals for each group. Labels of statistical significance: c – compared to control group; q – compared to QA group. Statistical significance was considered at: *p <0.05 (L-NAME+QA vs QA in ipsilateral forebrain cortex, L-NAME+QA vs QA and 7-NI+QA vs QA in contralateral forebrain cortex), **p <0.01 (7-NI+QA vs QA in ipsilateral forebrain cortex, QA vs CG, 7-NI+QA vs CG in contralateral forebrain cortex), ***p <0.001 (QA vs CG and L-NAME+QA vs CG in ipsilateral forebrain cortex, L-NAME+QA vs CG in contralateral forebrain cortex) one-way ANOVA, Turkey test.
Intrastriatal application of QA, an endogenous NMDA agonist, bilaterally in the striatum resulted in increased AChE activity, a biochemical marker for cholinergic neurons (Fig. 1). Studies have shown that QA interacts with a subgroup of NMDA receptors and when directly injected into brain areas, it destroys most neuronal cells bodies sparing fibers and neuronal terminals (Kalonia et al., 2012). However, other studies have shown that cells in the nucleus basalis remain intact in HB, as well as cholinacetyl transferase activity. Also, cortical NMDA receptors are more sensitive to QA than those located in the cerebellum or the spinal cord, which suggests the existence of multiple forms of NMDA receptors can probably be explained by considering that QA is a relatively poor agonist for the NMDA receptor complex containing either NR2C or NR2D subunits, while it interacts with a relatively high affinity with the NMDA receptor complex containing NR2B subunits (Zeron et al., 2004). Our study also showed an increase in AChE activity bilaterally in the forebrain cortex after administration of QA (Fig. 2). A possible explanation for the increased AChE activity after QA injection may be that in neurons with increased release of ACh, its synthesis is increased from membrane choline phospholipids. Phospholipids are degraded, as the plasma membrane, whose properties change, leading to the formation of free radicals in the form of lipid peroxides (Zou et al., 1999; Samuel et al., 2000).

To assess the intermediary role of NO in the neurotoxicity elicited by this toxin, its action was tested in animals treated with L-NAME and 7-NI. Decreased AChE activity after L-NAME+QA administration contralaterally in the striatum and bilaterally in the forebrain cortex, compared to the QA group, is probably due to the reduced release of ACh, which is a substrate of the enzyme (Fig. 1, 2). On the other hand, a selective inhibitor of neuronal NOS (7-NI) with QA decreased the activity of AChE bilaterally in both brain structures; this is probably due to reduced ACh release (Figs. 1, 2). One of the most prominent cholinergic losses after the application of NOS inhibitors is the reduced number of nicotinic ACh receptors in the hippocampus and cortex (Buonomano and Merzenck, 1998). This deficit results in reduced nicotinic cholinergic excitation, which may impair not only postsynaptic depolarization but also presynaptic neurotransmitter release and Ca2+-dependent intracellular signaling, including transcriptional activity (Foskett, 2010).

In conclusion, our results indicated that NO-mediated neurotoxicity spread temporally and spatially in the forebrain cortex after intrastriatal QA application with changes of cholinergic transmission. NOS inhibitors, like both L-NAME and 7-NI could protect the cells in the striatum and forebrain cortex from QA-induced damage and may therefore limit the retrograde and anterograde spread of neurotoxicity.

REFERENCES


