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EFFECT OF L-NAME ON $AlCl_3$-INDUCED TOXICITY IN RAT BRAIN

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The present experiment was carried out to determine the effectiveness of nitric oxide synthase (NOS) inhibitor (L-NAME) in elevating the toxicity of $AlCl_3$ on nitrite concentration and acetylcholine esterase activity of Wistar rats. Animals were killed 10 min and 3 days after the treatment and the forebrain cortex and striatum were removed. The results show that $AlCl_3$ exposure promotes oxidative stress in different neural areas. The biochemical changes observed in neuronal tissues show that aluminium acts as a pro-oxidant, while NOS inhibitor exerts as antioxidant action in $AlCl_3$-treated animals. In the present study, active avoidance learning was significantly impaired after $AlCl_3$ injection, while pretreatment with L-NAME prevented the behavioural deficits caused between the 8^{th} and 12^{th} day after intrahippocampal application of neurotoxin. Our data suggest that aluminium may cause learning and memory deficits, while the treatment with L-NAME may decrease the oxidative stress and prevent learning and memory deficits caused by $AlCl_3$.

Key words: acetylcholine esterase, aluminium, behavior, L-NAME, nitric oxide

INTRODUCTION

Aluminium (Al) compounds are neurotoxic and have been shown to induce experimental neurodegeneration although the mechanism of this effect is unclear (Griffioen *et al.*, 2004). Animals loaded with Al displayed a massive cellular depletion in the hippocampal formation, particularly, the CA1 field, and also in the temporal and parietal cortex (Exley, 1999). Cortical nitroxidergic neurons and granule cells were a specific target of Al neurotoxicity (Rodella *et al.*, 2001). Prolonged exposure of rats to Al can result in numerous ghost-like neurons with cytoplasmic and nuclear vacuolations, and with Al deposits. The senile plaques are generated by brain deposition of fibrils of amyloid beta (Abeta), a fragment derived from the proteolytic processing of the amyloid precursor protein (APP). Tau protein is the major component of paired helical filaments (PHFs), which form

a compact filamentous network described as neurofibrillary tangles (NFTs) (Maccioni, 2001). Previous studies have shown that Al^{3+} alters the phosphorylation of tau by neuronal cdc2-like kinase and dephosphorylation of phosphorylated tau by phosphatase 2B (Li *et al.*, 1998; Falke *et al.*, 2003).

Aluminium is transported by the iron-carrier protein, transferrin (Tf) that enters the brain by binding to Tf receptors. The interaction between Tf and its receptor may function as a general metal ion regulatory system in the CNS. Brain Al entry may involve Tf-receptor mediated endocytosis and a more rapid process transporting small molecular weight Al species. There appears to be an Al efflux from the brain, probably as Al citrate (Yokel, 2000).

Nitric oxide (NO) is an enzymatic product of NO synthase (NOS), which exists in three isoforms. Nitric oxide exerts significant neurophysiological functions. However, NO can also be neurotoxic primarily due to its free radical properties, and it has been implicated in neurodegenerative diseases (Law *et al.*, 2001). In CNS, the activation of N-methyl-D-aspartic acid (NMDA) type of glutamatergic receptor induces Ca^{2+}-dependent NOS activity and NO release, which then activates soluble guanylate cyclase for the synthesis of cGMP. Both compounds appear to be important mediators in long-term potentiation and long-term depression, and thus may play important roles in the mechanisms of learning and memory. Aluminium can decrease the ability of rats to learn and memorize, inducing their synaptic configuration changes, which may be related to synaptic efficacy and may be one of the mechanisms for Al-induced neurodegenerative changes (Jing *et al.*, 2004). The dysfunction of cholinergic neurons is belived to be primarily responsible for cognitive deficits after intracerebral Al intoxication (Nebeshima and Yamada, 2000). Cholinergic neurons, unlike other brain cells utilize acetyl-CoA not only for energy production but also for acetylcholine (ACh) synthesis. Studies *in vitro* have suggested that acetylcholine esterase (AchE), a marker of cholinergic system function, may interact with Abeta to promote deposition of amyloid plaques in the brain after Al intoxication (Rees *et al.*, 2003; Zatta *et al.*, 2002).

Acetylcholine and NO are important neuromodulators implicated in brain plasticity and disease (Scheiner *et al.*, 2000). *In vivo* and *in vitro* studies have shown that, in all brain structures investigated, endogenous NO modulates the release of several neurotransmitters, such as ACh, catecholamines, excitatory and inhibitory amino acids, serotonin, histamine, and adenosine (Prast and Philippu, 2001).

In view of the above, the present study was undertaken to examine whether the production of NO, activity of AchE, as well as the task of active avoidance after receiving intracerebral injections of $AlCl_3$ can be modulated by the pretreatment with N-nitro-L-arginine methyl ester (L-NAME), the non-specific NOS inhibitor (Vasiljević *et al.*, 2002).

MATERIAL AND METHODS

Animals

Male adult Wistar rats, with body mass 500±50 g, were used for the experiments. Groups of two or three rats per cage (Erath, FRG), were housed in an airconditioned room at a temperature of 23±2 °C with 55±10% humidity and with lights on 12 h/day (07.00-19.00 h). The animals were given a commercial rat diet and tap water *ad libitum*.

Experimental procedure

Animals were anesthetized by intraperitoneal injections of sodium pentobarbital (0.04 g/kg b.w.). A single dose of aluminium chloride ($AlCl_3$) (Sigma, USA) (3.7×10^{-4} g/kg b.w. in 0.01 mL of deionizied water), was injected into CA1 sector of the hippocampus, by using a Hamilton microsyringe, using stereotaxic instrument for small animals (coordinates: 2.5 A; 4.2 L; 2.4 V) (König and Klippel, 1963). The second and third group were treated with L-NAME (Sigma Chemical Co. USA; 100 μg dissolved in saline solution)+$AlCl_3$ and L-NAME+saline solution. The fourth group (n=10) received the same volume of 0.9% saline solution only and it served as control-sham-operated. In all treated animals the injected intracerebral volume was 10 μL and it was always injected into the left side.

For biochemical analysis the rats were divided into four basic groups (according to drug treatment). Each basic group consisted of two different subgroups (according to survival times - 10 min and 3 days) and each subgroup consisted of 10 animals. All animals were decapitated and the brains immediately removed. Ipsi- and contralateral forebrain cortex and striatum from individual animals were quickly isolated and homogenized in ice-cold buffer containing 0.25 M sucrose, 0.1 mM EDTA, 50 mM K-Na phosphate buffer, pH 7.2. Homogenates were centrifuged twice at 1580 x g for 15 min at 4°C. The supernatant obtained by this procedure was then frozen and stored at -70°C.

For the test of acquisition and expression of active avoidance, the 8th day after treatment (saline solution, $AlCl_3$, L-NAME+$AlCl_3$, L-NAME), the animals were subjected to behavioral tests (two-way active avoidance) over five consecutive days. Animals were then sacrificed by decapitation 12 days after surgery, their brains were removed and flash-frozen in liquid nitrogen.

Biochemical analysis

Forebrain cortex and striatum were dissected bilaterally from each frozen brain and crude mitochondrial fraction was prepared from each region as previously described (Gurd *et al*., 1974).

After deproteinization the production of NO was evaluated by measuring nitrite and nitrate concentrations. Nitrites were assayed directly spectrophotometrically at 492 nm, using the colorimetric method of Griess (Griess reagent: 1.5% sulfanilamide in 1 M HCl plus 0.15% N-(1-naphthyl) ethylendiamine dihydrochloride in distilled water). However, nitrates were previously transformed into nitrites by cadmium reduction (Navarro-Gonzalez *et al*., 1998).

The determination of acetylcholine esterase (True cholinesterase; Acetylcholine acetylhydrolase EC 3.1.1.7, AchE) activity was based on degradation of acetyl thiocholine iodide by AchE into a product which binds to 5,5-dithiobis-2-nitrobezoic acid (DTNB), developing a yellow colour (Mićić and Petronijević, 2000). Kinetics of the enzymatic reaction was followed over 3-5 minutes at 412 nm. Values of AchE activity were calculated from the linear part of the reaction curve and were expressed as μmol acetyl thiocholine/min/g prot.

The protein content in the rat brain homogenates (forebrain cortex and striatum, ipsi- and contralateral) was measured by the Lowry method using bovine serum albumin (Sigma) as standard (Lowry *et al*., 1951).

Active avoidance test

Apparatus. Two-way active avoidance (AA) was studied in a series of automatically operated commercial shuttle-boxes and programming-recording units (Campden Instruments, USA). Boxes (48x21x22.5 cm) were used without the central partition.

Procedure. Measurements of AA responses were achieved by using spaced trials behavioral procedures (20-trial sessions daily for five consecutive days). A conventional two-way AA schedule was used with trials starting at 30 sec intervals. Each trial began with a conditioned signal (CS) (broad-band noise of 68 dB lasting seven seconds), followed by an unconditioned stimulus (US) (foot shock of 1.5 mA, three seconds duration) which was delivered through the floor grid. Crossing responses during the conditioned stimulus (AA response) terminated the conditioned stimulus and prevented the onset of unconditioned stimuli. A response after the onset of an unconditioned stimulus (escape response) terminated both conditioned and unconditioned stimuli. Inter-trial crossings were not punished.

Data presentation and analysis

Data are expressed as means ±S.D. Statistical significance was determined as $p<0.05$ using either the Student's t-test or ANOVA followed by Tukey's t-test.

Materials

Chemicals were purchased from Sigma (St. Louis, MO, USA). All used chemicals were of analytical grade. All drug solutions were prepared on the day of the experiment. Animals used for the procedure were treated in strict accordance with the NIH Guide for Care and Use of Laboratory Animals (1985).

RESULTS

Nitrite levels in the rat forebrain cortex

The results presented in Fig. 1 show the nitrite levels (nM/mg proteins), bilaterally in the rat forebrain cortex homogenates at 10 min (A) and 3 days (B) after the treatment. At the early tested time (10 min) $AlCl_3$ injection resulted in an increase of nitrite production bilaterally in the forebrain cortex, compared to control groups, with statistically significant differences (Student's t-test; $p<0.05$).

Also, L-NAME application resulted in increased nitrite levels bilaterally in the forebrain cortex after 10 min, compared to control groups, as well as compared to L-NAME+$AlCl_3$-treated groups. However, L-NAME+$AlCl_3$ injection resulted in lower nitrite levels, compared to the neurotoxin-treated group ($p<0.05$). At 3 days after L-NAME application levels of nitrite production increased bilaterally in the forebrain cortex, compared with controls ($p<0.05$) (Fig. 1A,B).

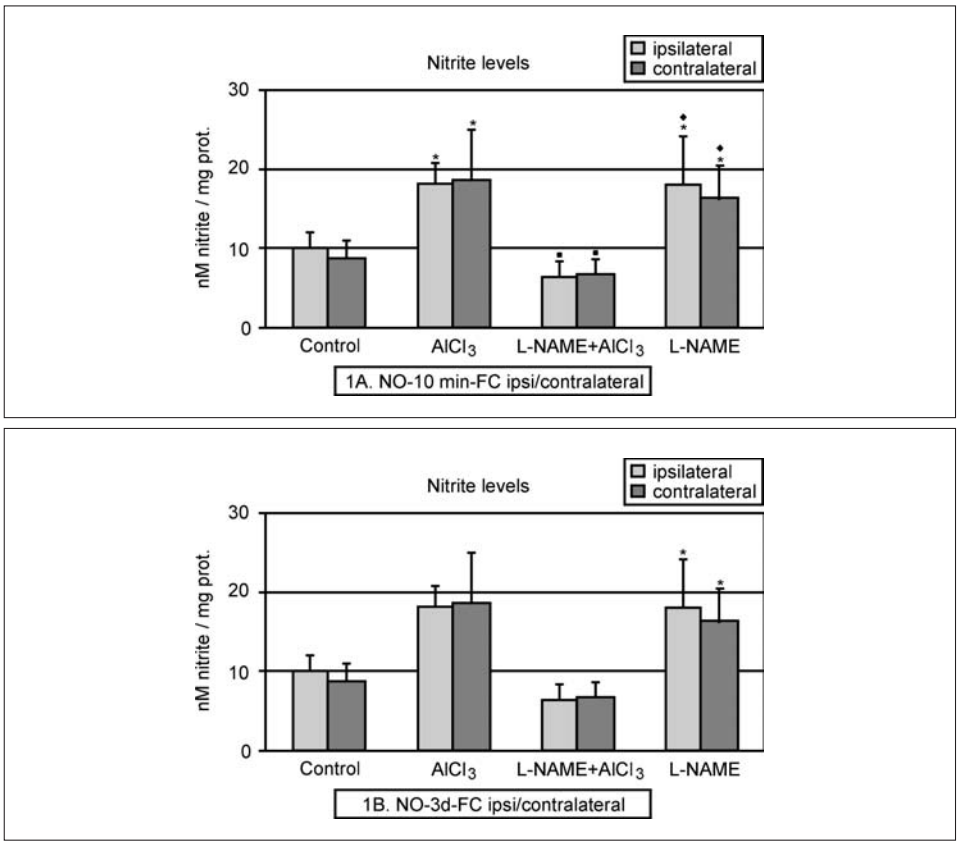


Figure 1A,B. The effect of intrahippocampal drug injection on nitrite levels (nM nitrite/mg proteins) in the rat ipsilateral and contralateral forebrain cortex at different survival times: 10 min (A) and 3 days (B). Data are means ± S.D. of 10 animals. *Indicates a statistically significant difference between treated ($AlCl_3$- and L-NAME-treated) and control (sham-operated) animals ($P<0.05$). •Indicates a statistically significant difference between L-NAME+$AlCl_3$-treated and $AlCl_3$-treated animals ($P<0.05$). ♦Indicates a statistically significant difference between L-NAME-treated and L-NAME+$AlCl_3$-treated animals ($P<0.05$)

Nitrite levels in the rat striatum

The results presented in Fig. 2 show the nitrite levels (nM/mg proteins), bilaterally in the rat striatum homogenates at 10 min (A) and 3 days (B) after treatment. $AlCl_3$ injection resulted in higher nitrite levels after 10 min in the contralateral and after 3 days in both the ipsi- and contralateral striatum, compared to control animals ($p<0.05$). In L-NAME+$AlCl_3$ group at 10 min, nitrite levels decreased bilaterally in the striatum, compared to $AlCl_3$-treated group. At 10 min after NOS inhibitor injection, nitrite production increased bilaterally in the striatum, compared to L-NAME+$AlCl_3$-treated animals (Fig. 2A, B).

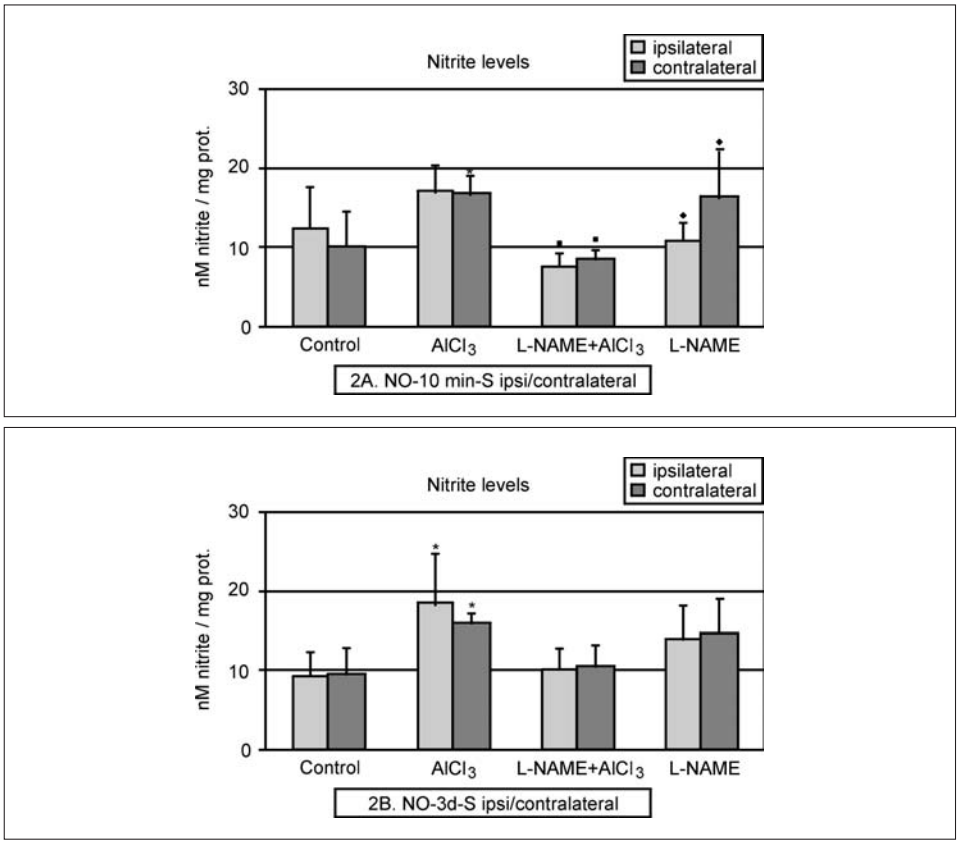
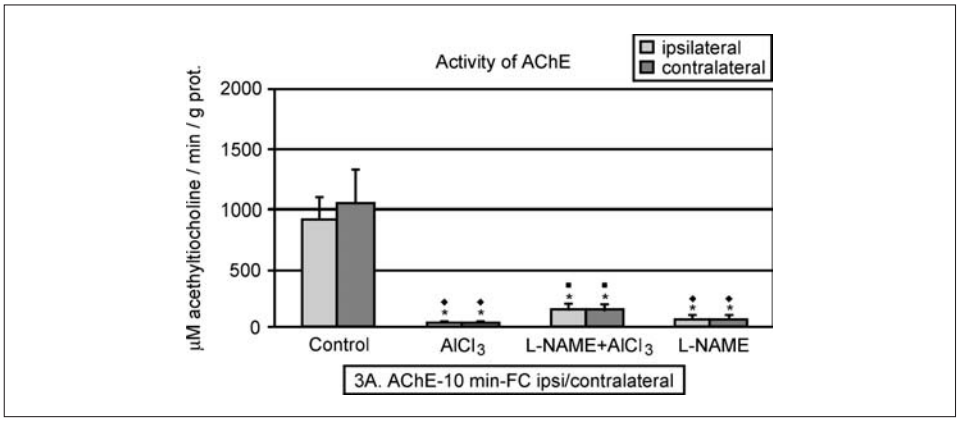


Figure 2A,B. The effect of intrahippocampal drug injection on nitrite levels (nM nitrite/mg proteins) in the rat ipsilateral and contralateral striatum at different survival times: 10 min (A) and 3 days (B). Data are means ± S.D. of 10 animals. *Indicates a statistically significant difference between $AlCl_3$-treated and control (sham-operated) animals ($P<0.05$). •Indicates a statistically significant difference between L-NAME+$AlCl_3$-treated and $AlCl_3$-treated animals ($P<0.05$). ♦Indicates a statistically significant difference between L-NAME-treated and L-NAME+$AlCl_3$-treated animals ($P<0.05$)

Activity of acetylcholine esterase in the rat forebrain cortex

The results presented in Fig. 3 show the activity of AchE (μM acethyltiocholine/min/g proteins), a marker of cholinergic system function, bilaterally in the rat forebrain cortex homogenates at 10 min (A) and 3 days (B) after treatment. Activity of AchE was significantly lower bilaterally in the forebrain cortex after $AlCl_3$-, L-NAME+$AlCl_3$-, and L-NAME-injection, compared to controls. $AlCl_3$ injection resulted in lower nitrite levels after 10 min and 3 days bilaterally in the forebrain cortex, compared to L-NAME+$AlCl_3$-treated animals. Intrahippocampal L-NAME+$AlCl_3$ injection resulted in generally higher levels of



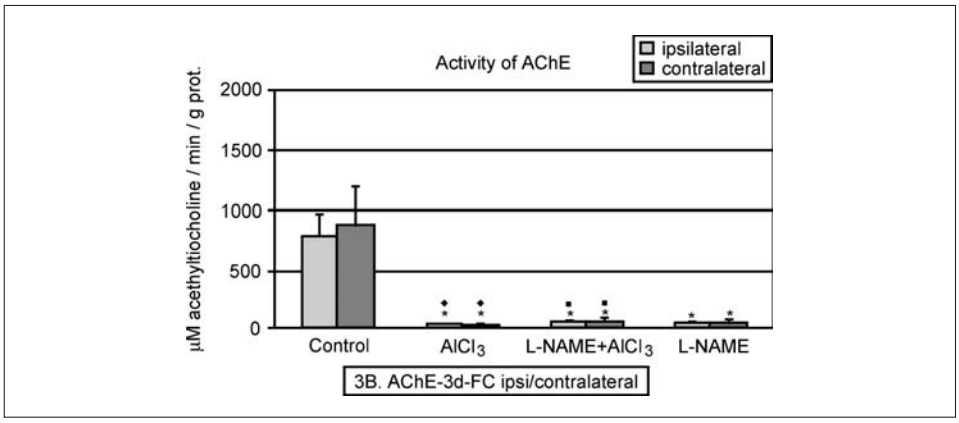


Figure 3A, B. The effect of intrahippocampal drug injection on AchE activity (μM acethyltiocholine/min/g proteins), in the rat ipsilateral and contralateral forebrain cortex at different survival times: 10 min (A) and 3 days (B). Data are means ± S.D. of 10 animals. *Indicates a statistically significant difference between treated ($AlCl_3$-, L-NAME+$AlCl_3$- and L-NAME-treated) and control (sham-operated) animals ($P<0.05$). •Indicates a statistically significant difference between L-NAME+$AlCl_3$-treated and $AlCl_3$-treated animals ($P<0.05$). ♦Indicates a statistically significant difference between treated ($AlCl_3$- and L-NAME-treated) and L-NAME+$AlCl_3$-treated animals ($P<0.05$)

AChE activity compared to neurotoxin-treated animals, with a significant difference ($p<0.05$), at all tested times. At 10 min after L-NAME injection, the AchE activity decreased in the ipsi- and contralateral forebrain cortex, compared to L-NAME+$AlCl_3$-treated animals (Fig. 3A, B).

Activity of acetylcholine esterase in the rat striatum

The results obtained for the ipsi- and contralateral striatum were similar. Activity of AchE was lower bilaterally in the striatum after $AlCl_3$-, L-NAME+$AlCl_3$-, and L-NAME-injection, compared to controls. $AlCl_3$ injection resulted in lower

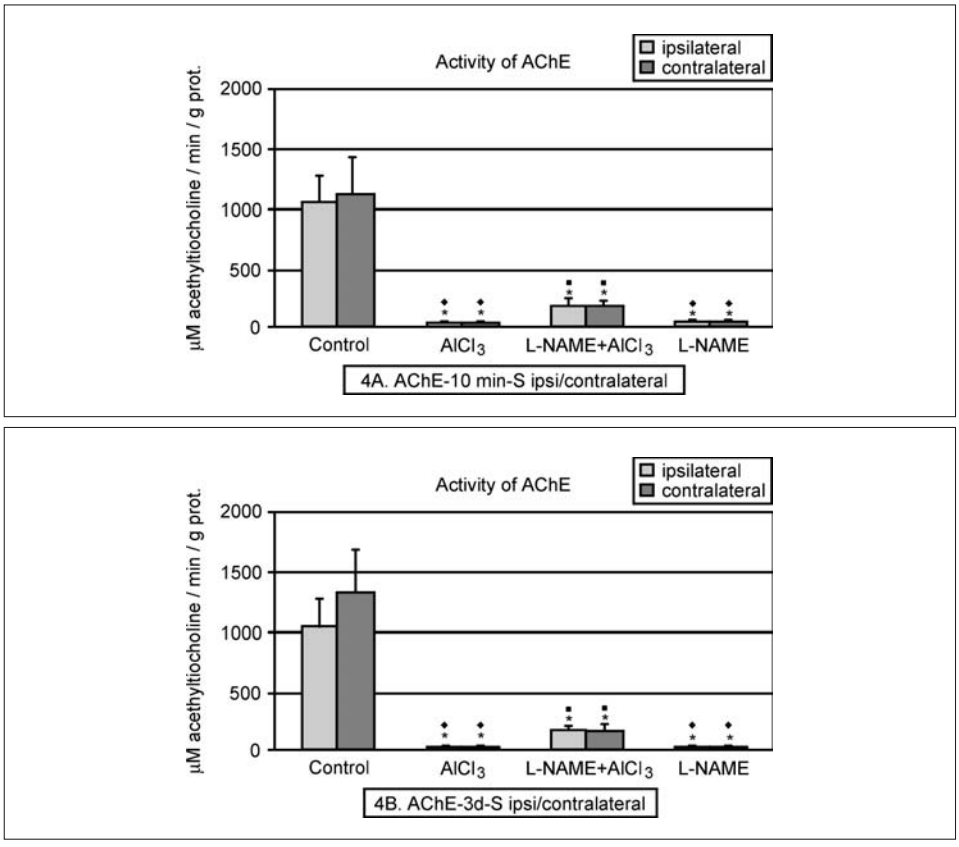


Figure 4A, B. The effect of intrahippocampal drug injection on AchE activity (µM acethyltiocholine/min/g proteins), in the rat ipsilateral and contralateral striatum at different survival times: 10 min (A) and 3 days (B). Data are means ± S.D. of 10 animals. *Indicates a statistically significant difference between treated ($AlCl_3$-, L-NAME+$AlCl_3$- and L-NAME-treated) and control (sham-operated) animals ($P<0.05$). •Indicates a statistically significant difference between L-NAME+$AlCl_3$-treated and $AlCl_3$-treated animals ($P<0.05$). ♦Indicates a statistically significant difference between treated ($AlCl_3$- and L-NAME-treated) and L-NAME+$AlCl_3$-treated animals ($P<0.05$)

nitrite levels after 10 min and 3 days in both ipsi- and contralateral striatum, compared to L-NAME+$AlCl_3$-treated animals. $AlCl_3$ injection followed with L-NAME, clearly increased activity of AchE in this brain structure, compared to $AlCl_3$-treated animals, at all tested times. At 10 min and 3 days after L-NAME injection, AchE activity decreased in the ipsi- and contralateral striatum, compared to L-NAME+$AlCl_3$-treated animals (Fig. 4A, B).

Behavioral changes after treatment

As a part of active avoidance model, we determined the number of active avoidance (AA) of aversive unconditioned stimuli during 5 consecutive days (8-12 days after treatment), at 20 trials per day, as a measure of acquisition of positive reactions. Differences in the number of correct responses first became evident 10 days after $AlCl_3$ injection (3rd day of examination) and progressively widened over the subsequent three days. At the end of the 12th day (5th day of examination), $AlCl_3$-treated animals showed two-fold reduction in correct responses compared to the control group. From the 4th until the 5th day there was an obvious progressive depression in positive reactions in the group treated with neurotoxine, compared to L-NAME+$AlCl_3$ group (Fig. 5).

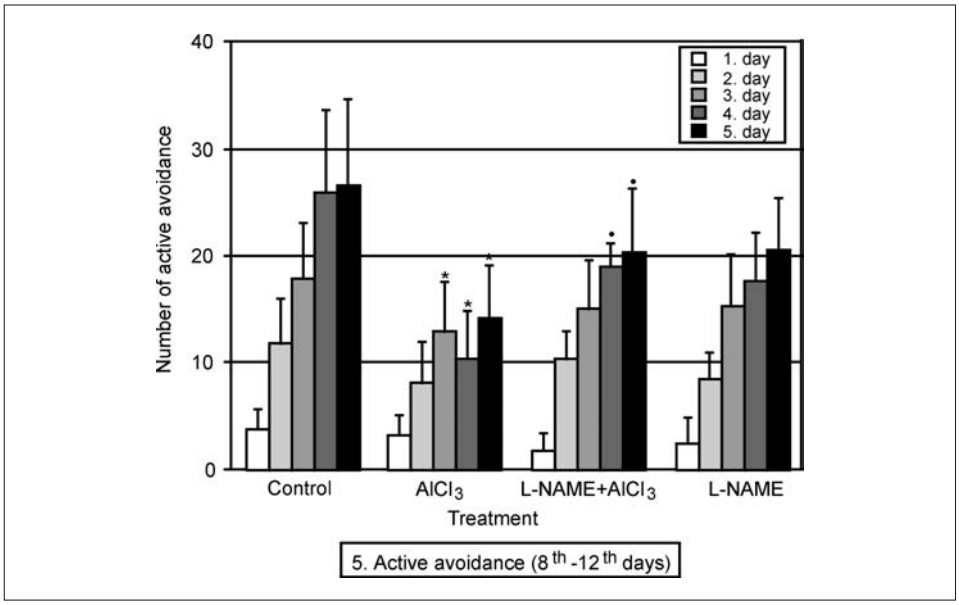


Figure 5. The influence of intrahippocampal drug injection on the active avoidance behavior in rats. Data are means ± S.D. of 10 animals. *Indicates a statistically significant difference between $AlCl_3$-treated and control (sham-operated) animals ($P<0.05$). •Indicates a statistically significant difference between L-NAME+$AlCl_3$-treated and $AlCl_3$-treated animals ($P<0.05$)

DISCUSSION

The application of $AlCl_3$ to the CA1 sector of the hippocampus resulted in the impairment of cognitive functions accompanied with significant bilateral decrease in AChE activity, as well as the increase of NO production in the forebrain cortex and striatum. It suggests that inhibition of NOS by L-NAME protects the cells in these regions from $AlCl_3$-induced damage and, therefore, may limit the retrograde and anterograde spread of neurotoxicity.

Aluminium salts have been shown to cause damage to astroglial and neuronal cells in selective brain regions of the associative cortex and hippocampus (Pelvig *et al.*, 2003). Neuropharmacological data indicate that Abeta toxicity is mediated by an excitotoxic cascade involving the blockade of astroglial glutamate uptake, sustained activation of NMDA receptors and an overt intracellular Ca^{2+} influx. These changes are associated with increased NOS activity in the cortical target areas that may directly lead to the generation of free radicals (Harkany *et al.*, 2000).

In our study, $AlCl_3$ injection resulted in increased NO production after 10 min and unchanged nitrite concentration after 3 days bilaterally, in the forebrain cortex, compared to controls (Fig. 1). It has been previously known (Tohgi *et al.*, 1998) that production and oxidation of NO in the brain increased in the early stage of the disease, while it decreased with elevating loss of neurons.

Also, under the condition of this experiment, $AlCl_3$ application produced a rapid (within 10 min) increase in nitrite levels in the contralateral striatum, and continued to increase gradually throughout the experiment (after 3 days), compared to controls (Fig. 2). 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. The integrative action exerted by striatal projection neurones on this converging information dictates the final output of the striatum to the other basal ganglia structures. During various pathological conditions, striatal synaptic transmission is altered depending on presynaptic inhibition of transmitter release and opposite membrane potential changes occur in projection neurones and in cholinergic interneurones (Calabresi *et al.*, 2000).

Aluminium, by potentiating lipid peroxidation, affects the uptake of choline in nerve endings (Amador *et al.*, 2001). Acetylcholineserase that exists in several molecular forms and catalyzes degradation of Ach is a good marker of the cholinergic system function (Zatta *et al.*, 2002). A significant reduction of AChE activity in $AlCl_3$-treated group in the forebrain cortex and striatum at various times after neurotoxin injection suggests a loss of AChE substrate in cholinergic neurons of basal forebrain and reduced function and/or damage of its cholinergic neurons (Fig. 3 and Fig. 4). Neurochemical disruption of connectivity between the hippocampus and forebrain cortex, as well as striatum, induced by Al injection into CA1 sector could provoke retrograde transneuronal damage of these brain structures that could explain decreased AChE activity in our experiments.

The literature so far published results implicate that prolonged treatment with soluble salts of Al was decreased in the all the components of soluble and

membrane-bound forms of AchE in the brain. In addition, the Al treatment resulted in complete loss of the component II of erytrocyte membrane AchE. These results could represent the mode of action through which Al may contribute to pathological processes in Al-induced neurotoxicity (Dave *et al.*, 2002).

As a part of active avoidance (AA) model, we showed two-fold reduction in correct responses from the 3rd to the 5th day of examination in $AlCl_3$-treated animals compared to the control group (Fig. 5). Aluminium induces stable and reproducible depression of AA reaction, meaning, Al damages rat cognitive function. These results, along with the obtained decreased AchE activity suggest that Al exerts its toxic effects by altering cholinergic transmission, which is ultimately reflected in neurobehavioral deficits (Julka *et al.*, 1995). Aluminium is not a selective cholinergic neurotoxin and could probably also affect noncholinergic neurons involved in spatial learning. Previous studies have shown that attention and spatial learning were disrupted in the Al-treated animals (Kesner *et al.*, 2002; Guzowski and Mc Gaugh, 1997).

Also, it was previously known (Zou *et al.*, 1998) that Al could block the induction of long-term potentiation and decrease the amplitude of population spike potentiated in CA3, as well as that the effect of Al might be antagonized by modulation of L-arginine-NO pathway. The arginine derivate – L-NAME is non-specific NOS inhibitor, which inhibits all three NOS isoforms (Luth *et al.*, 2001). L-NAME+$AlCl_3$ application which produced a rapid (within 10 min) decrease in nitrite levels bilaterally in forebrain cortex and striatum, compared to $AlCl_3$-treated groups suggests that L-NAME is supressing nitrite accumulation and decreasing neuron impairment through NMDA receptors (Fig. 1A and Fig. 2A).

Furthermore, we have shown that in all tested times after intrahippocampal injection, the application of L-NAME+$AlCl_3$ produced approximately a 50% increase in AChE activity bilaterally in the forebrain cortex and striatum, compared to $AlCl_3$-treated animals (Fig. 3 and Fig. 4). Meaning that L-NAME acted cholinergically protective, even though enzyme activity was not restored to control value levels. In the same experimental group, increased AchE activity was followed by an increase of correct responses from the 4th day of testing in AA reaction, compared to the $AlCl_3$-treated group, though values were far below control ones (Fig. 5). That means that L-NAME ameliorated memory impairment induced by $AlCl_3$ injection in AA response task.

L-NAME injection after 10 min and 3 days which can increase NO production bilaterally in the forebrain cortex, compared to control groups suggests that the inhibition of NOS expression prevented the increase in nitrogen intermediates and GABA release, but not a glutamate release (Fig. 1). An inflammatory reaction of the basal forebrain facilitates GABA release through the production of NO (Casamenti *et al.*, 1999).

The literature results implicate that ACh and NO co-release from the same cholinergic-nitrergic nerves, and that ACh acts as a presynaptic transmitter in modulating NO release (Scheiner *et al.*, 2000; Lee *et al.*, 2001).

Our study showed a significant reduction of AChE activity in both forebrain cortex and striatum after L-NAME injection, compared to controls, as well as compared to L-NAME+$AlCl_3$-treated groups (Fig. 3 and Fig. 4). The results show

that L-NAME supresses spontaneous behavioral rat activity causing cholinergic system functional impairment. Decreased release of Ach from basal forebrain is caused by supression of cholinergic basalocortical neurons by nitrergic cortical fibres after L-NAME application (Prast and Philippu, 2001; Tong and Hamel, 2000).

Literature results (Chalimoniuk *et al.*, 1998) implicate that the neurotoxic fragment of Abeta decreased significantly the NMDA receptor-mediated Ca^{2+}, and calmodulin-dependent NO synthesis that may then be responsible for disturbances of the NO and cGMP signalling pathway. cGMP-dependent signal transduction in the hippocampus and cerebellum may become insufficient in senescent brain and may have functional censequences in disturbances of learning and memory processes. Amyloid beta peptide accumulated during brain aging and may be an important factor in decreasing the NO-dependent signal transduction mediated by NMDA receptors.

Our study showed unchanged correct responses from the 1^{st} to the 5^{th} day of testing AA reactions, in L-NAME-treated animals compared to the control group of animals (Fig. 5). Neuropharmacological data indicate that there is no difference in behaviour in the active avoidance task between saline and L-NAME-treated rats (Prickaerts *et al.*, 1998).

In conclusion, our data revealed that NO was included in $AlCl_3$-induced neurotoxicity, resulting in both temporal and spatial spreading of damage to the selective vulnerable brain structures with impairment of cognitive functions and cholinergic transmission and deficits in learning and memory, so that NOS inhibitors, such as L-NAME, could have potentially neuroprotective effect.

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EFEKAT L-NAME NA $AlCl_3$-INDUKOVANU TOKSIČNOST U MOZGU PACOVA

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SADRŽAJ

U eksperimentu je ispitivana efikasnost inhibitora azot oksid sintaze (NOS)–L-NAME na toksičnost $AlCl_3$ i određivana koncentracija nitrita i aktivnost acetilholin esteraze kod Wistar pacova. Životinje su dekapitovane 10 minuta ili 3 dana nakon tretmana i izolovani su kora prednjeg mozga i strijatum. Rezultati ukazuju da $AlCl_3$ pokreće oksidativni stres u različitim regionima mozga. Biohemijske promene opisane u neuronskom tkivu ukazuju da aluminijum deluje kao pro-oksidans, dok inhibitor NOS ima antioksidativno dejstvo kod životinja tretiranih $AlCl_3$. Reakcija aktivnog izbegavanja je bila znatno poremećena nakon aplikacije $AlCl_3$, dok se davanjem L-NAME sprečavaju poremećaji ponašanja uzrokovani između 8. i 12. dana posle intrahipokampusne primene neurotoksina. Naši rezultati ukazuju da aluminijum može dovesti do smetnji u procesima učenja i pamćenja, dok tretman sa L-NAME smanjuje oksidativni stres i sprečava promene u učenju i pamćenju uzrokovane $AlCl_3$.