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NITRIC OXIDE SYSTEM PARAMETERS IN THE BASAL MAGNOCELLULAR NUCLEUS OF THE RAT BRAIN FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF COLCHICINE

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Background. *The basal magnocellular nucleus (BMN) is a brain structure that provides cholinergic innervation of the neocortex. Disruption of BMN functional activity is observed in many neurodegenerative diseases. One of the key regulatory systems of neuronal activity in the brain is the nitric oxide (NO) system, however, the interactions among NO system components during neurodestruction have not yet been fully elucidated.* ***Objective.*** *The aim of the study was to determine L-arginine, nitrites, nitrotyrosine levels, iNOS, and nNOS expression in the rat BMN in a model of colchicine-induced neurodestruction.* ***Methods.*** *The study involved 30 male Wistar rats divided into three groups: intact, sham-operated with intracerebroventricular (ICV) administration of 3 µl of NaCl, and colchicine group with ICV injection of colchicine (15 µg/3 µl NaCl). ICV injections were carried out using a digital stereotaxic apparatus. Two weeks after colchicine administration, the animals developed cognitive deficits evidenced by behavioral testing. Following euthanasia, the brain tissue was rapidly removed for further processing. iNOS and nNOS expression was assessed via immunofluorescence microscopy, nitric oxide metabolites were measured using the Griess method, L-arginine concentration via HPLC-MS, and nitrotyrosine level via ELISA.* ***Results.*** *Intracerebroventricular administration of colchicine was followed by a decrease in L-arginine level accompanied by a significant increase in NO metabolites and nitrotyrosine levels in the BMN of rats. Immunofluorescent analysis revealed increased density of iNOS-positive and a progressive reduction of nNOS-positive cells in the BMN cell population of colchicine-injected rats. Correlation analysis confirmed that NO-system imbalance plays a significant role in neurodestruction.* ***Conclusions.*** *Colchicine-induced neurodestruction in the rat BMN is associated with imbalance of the nitric oxide system characterized by decreased L-arginine levels, increased NO metabolites and nitrotyrosine content, elevated iNOS expression, and reduced nNOS-positive cell density. These alterations may contribute to neurodegenerative processes in the BMN.*

K e y w o r d s: nitric oxide system, L-arginine, nitrites, nitrotyrosine, iNOS, nNOS, colchicine, neurodegeneration, brain.

The basal magnocellular nucleus (BMN) is a brain structure (Ch4 group) that provides cholinergic innervation of the neocortex and plays a key role in cortical activation, attention, and memory formation. Disruption of its structural integrity and functional activity is observed in many neurodegenerative diseases, including Alzheimer's and Parkinson's diseases [1]. One of the key regulatory systems of neuronal activity and intercellular communication in the brain is the nitric oxide (NO) system. The substrate for NO synthesis is the amino acid L-arginine, which, under the action of different nitric oxide synthase (NOS) isoforms – neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) – is converted to citrulline with the formation of nitric oxide. This gaseous neurotransmitter performs important physiological functions related to neuromodulation, maintenance of vascular tone, synaptic plasticity, and metabolic adaptation of nervous tissue [2].

However, the physiological effects of NO largely depend on its concentration and redox environment. Under conditions of oxidative stress, this gasotransmitter becomes one of the major contributors to nitrosative cellular damage, including damage to brain structures. Excessive NO production may occur following nNOS activation in response to NMDA receptor-mediated Ca^{2+} influx, which represents one of the mechanisms of cell death in glutamate excitotoxicity. In addition, activation of iNOS leads to excessive nitric oxide production and sustains prolonged nitrosative imbalance, thereby promoting the progression of neurodegeneration. One of the principal mechanisms of NO-associated neurodestruction is the formation of reactive nitrogen species (RNS), primarily peroxynitrite, which is generated through the interaction of NO with the superoxide anion. Peroxynitrite is capable of nitrating amino acid residues, including tyrosine, resulting in the formation of nitrotyrosine – a stable marker of nitrosative stress. Protein nitration disrupts their functional properties, damages cytoskeletal structures, induces mitochondrial dysfunction, and activates apoptotic cascades [3].

Thus, the balance between physiological and pathological concentrations of NO in brain structures involved in cognitive functions, including the BMN, determines the direction of the neuronal response – from adaptive changes to irreversible damage manifested as cognitive impairments of varying severity. Therefore, experimental modeling of neurodegenerative conditions represents a modern and effective approach to expanding our understanding of the role of the nitric oxide system in the pathogenesis and progression of neurodegeneration. One of the most widely used agents for this purpose is colchicine, which, when administered intracerebroventricularly (ICV), inhibits microtubule polymerization, leading to impaired axonal transport, neuronal death, and activation of glial cells. The colchicine model reproduces key features of neurodegenerative pathology, including disturbances of cytoskeletal dynamics, oxidative-nitrosative stress, alterations in neurotransmitter systems, and impairment of long-term potentiation (LTP) mechanisms [4, 5].

To date, changes in the levels of individual components of the NO system in the BMN following ICV colchicine administration have been described [6]; however, studies providing a comprehensive assessment of the substrate, enzymes, and reaction products of this system remain scarce. Consequently, the complex nature of interactions among the components of the NO system during neurodestruction has not yet been fully elucidated.

The aim of this study was to determine the specific alterations in the components of the nitric oxide system (L-arginine, nitrites, nitrotyrosine, and the expression of iNOS and nNOS) in the basal magnocellular nucleus of the rat brain under conditions of established colchicine-induced neurodegeneration.

Materials and Methods

The study involved 30 male Wistar rats aged 10-11 months and weighing 250–350 g. Animals were housed under standard vivarium conditions at the Educational and Scientific Medical Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University (ZSMPhU). All experimental procedures involving animals were conducted in accordance with internationally recognized bioethical standards and principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), and after approval by the Bioethics Committee of ZSMPhU (Protocol No 2, March 15, 2023) and in accordance with the national General Ethical Principles for Experiments on Animals (Ukraine, 2001), which comply with EU Directive 2010/63/EU of September 22, 2010 on the protection of animals used for scientific purposes.

Experimental groups. Rats were randomly assigned to three experimental groups (n = 10 per group). Group 1 consisted of intact animals that were not subjected to any interventions. Group 2 included sham-operated rats that received ICV administration of 3 μl of warm (37°C) physiological saline (NaCl). Group 3 comprised rats that received an ICV injection of colchicine (15 μg/3 μl NaCl, 37°C).

ICV injection procedure. All surgical procedures were performed in the operating room of the Educational and Scientific Medical Laboratory Center ZSMPhU under aseptic conditions. Anesthesia was induced by intraperitoneal administration of Telazol (a combination of tiletamine hydrochloride and zolazepam hydrochloride, 250 mg per vial) at a dose of 0.1 ml per 100 g of body weight.

ICV injections were carried out using a digital stereotaxic apparatus (World Precision Instruments, USA). Either physiological saline or colchicine solution (depending on the experimental group) was injected into the lateral ventricle of the brain using the following stereotaxic coordinates: 9.5 mm anterior

to the interaural line; 1.5 mm lateral to the midline (right); 6.0 mm ventral depth.

The precise injection site was determined using a stereotaxic orientation system based on external cranial landmarks and their correspondence with a rat brain stereotaxic atlas [7]. No mortality was observed among the experimental animals following the procedure, as the toxin dosage was low, the intervention was minimally invasive, and the procedure was conducted over a short duration under adequate anesthetic support. Consequently, the animals developed cognitive deficits after two weeks, as evidenced by behavioral testing in the 8-arm radial maze and the "Open Field" test. These findings indicate that the neurodegeneration followed an Alzheimer's disease-like pattern [8].

Preparation of brain tissue for immunofluorescence analysis. Following euthanasia, the brains were rapidly removed. The obtained tissue was subjected to standard histological processing, including fixation, stepwise dehydration in a graded series of ethanol solutions, subsequent treatment in mixtures of absolute ethanol and chloroform at ratios of 2:1, 1:1, and 1:2, as well as in pure chloroform and a chloroform-paraplast medium. Thereafter, samples were infiltrated and embedded in paraplast (McCormick, USA). Serial brain sections 7 μm thick were cut from paraffin blocks using a Microm-325 microtome (Microm Corp., Germany). Anatomical localization of sections corresponding to the basal magnocellular nucleus was determined using a rat brain stereotaxic atlas [7].

Preparation of brain homogenates and differential centrifugation. Brain tissue was rinsed with physiological saline and homogenized in buffer (pH 7.4) containing (in mmol/l): sucrose 250, Tris-HCl 20, and EDTA 1 (at +4°C). Following homogenization, large cellular debris was removed by primary centrifugation in a refrigerated Eppendorf 5804 R centrifuge (Germany) at +4°C for 7 min at 1000×g. The resulting supernatant was carefully collected and subjected to secondary centrifugation under identical temperature conditions using a Sigma 3-30K centrifuge (Germany) for 20 min at 17,000×g. The final supernatant was separated and stored at -80°C until analysis.

Immunofluorescence analysis of iNOS and nNOS expression. Expression of iNOS and nNOS in BMN neurons was assessed by immunofluorescence microscopy. Histological sections were deparaffinized, rehydrated, and subjected to antigen retrieval using a PT module (Thermo Scientific, USA) with citrate buffer (pH 6.0; Thermo Scientific, USA). Sections were then incubated with specific mouse monoclonal antibodies conjugated with FITC (Santa Cruz Biotechnology, USA) according to the manufacturer's recommendations [9].

Immunofluorescent visualization was performed in the ultraviolet range using a 38HE high-emission filter on an Axio Imager M2 microscope (Carl Zeiss, Germany) equipped with an AxioCam ERc 5s camera (Carl Zeiss, Germany). Digital micrographs were analyzed using ImageJ software to determine the following parameters:

– corrected total cell fluorescence (CTCF), reflecting the amount of immunoreactive material per cell. Values were expressed in arbitrary immunofluorescence units (AIFU) and calculated using the formula: $CTCF = ID_1 - (S \times ID_0)$, where ID_1 is the integrated density of the immunopositive cell signal (automatically determined), S is the cell area exhibiting fluorescence, and ID_0 is the integrated density of background fluorescence (cells without specific signal).

–Density of immunopositive cells (cells/mm^2), calculated as the number of immunopositive cells per unit area of the image field (mm^2).

Determination of nitric oxide metabolites (NOx). NOx levels in the cytosolic fraction of brain homogenates were measured spectrophotometrically using the Griess reaction with a Libra S32 spectrophotometer (Biochrom Ltd., USA) at a wavelength of 540 nm. Quantification was performed using a linear calibration curve constructed over a sodium nitrate concentration range of 0–50 μmol/l. NOx concentrations in tissue samples were expressed in μmol/l and subsequently recalculated as nmol/mg protein.

Protein concentration assay. Protein content in the cytoplasmic fraction of brain homogenates was determined using the biuret reaction. Optical density was measured at 546 nm using a Libra S32 spectrophotometer (Biochrom Ltd., USA).

Determination of L-arginine concentration. Larginine levels in the cytoplasmic fraction of brain tissue were measured by high-performance liquid chromatography coupled with mass spectrometric detection (HPLC–MS) using an Agilent 1260 system equipped with a single quadrupole mass spectrometer (G6120B Single Quad LC/MS; Agilent Technologies, Singapore) with electrospray ionization (ESI). The HPLC system included a G4225A vacuum degasser, G1312B binary pump, G1329B autosampler, G1316C column thermostat, and G4212B diode-array detector (all Agilent Technologies). Nitrogen was

supplied by a NiGen LCMS 40-1 generator (Claind, Italy). Chromatographic separation was performed on a Zorbax SB-C18 column (30 mm × 4.6 mm, 1.8 μm) under isocratic conditions at a column temperature of 40°C. The mobile phase consisted of phase A (water with 0.1% trifluoroacetic acid) and phase B (acetonitrile with 0.1% formic acid) in a 95:5 (v/v) ratio at a flow rate of 0.4 ml/min. Detection was carried out using a diode-array detector at 200 nm. The total analysis time was 5 min, and the injection volume was 1 μl. Prior to injection, samples were filtered through a 0.22 μm nylon filter. Quantification was performed by comparison with calibration curves constructed from standard samples prepared in intact rat plasma.

Determination of nitrotyrosine levels. Nitrotyrosine concentration in blood serum was determined using a commercial enzyme-linked immunosorbent assay kit (Hycult Biotech, The Netherlands) according to the manufacturer's instructions. Absorbance was measured using a Sirio S spectrophotometer (Seac, Italy), and results were expressed in nM/ml and subsequently recalculated per mg of protein.

Statistical analysis. All experimental data were processed using STATISTICA software (license No. JPZ804I382130ARCN10-J) and Microsoft Excel 10.0 (Microsoft Corp., USA). Normality of data distribution was assessed using the Shapiro–Wilk test. Statistical significance of differences between experimental groups was evaluated using Student's *t*-test for normally distributed data or the Mann–Whitney U test for non-normally distributed data. Relationships between parameters were analyzed using correlation analysis with the calculation of Kendall's correlation coefficient.

Results

The conducted study made it possible to identify alterations in the parameters of the NO system in the BMN of rats following ICV administration of colchicine. Specifically, in brain homogenates of animals from group 3, the L-arginine content was significantly lower compared to rats from groups 1 and 2, by 55.5 and 58.1%, respectively. At the same time, this experimental group exhibited markedly elevated levels of NO metabolites (NOx) and nitrotyrosine (Table 1).

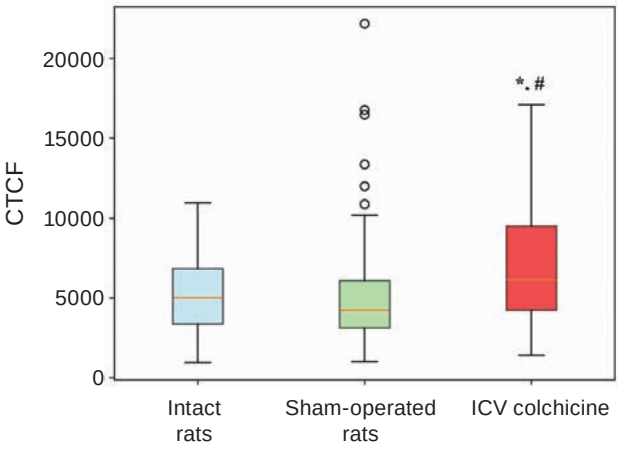

*Fig. 1. Corrected total cell fluorescence (CTCF) of iNOS⁺ cells in the BMN of rats from experimental groups (Uif). *Statistically significant difference compared with the intact group (P_U <0.05). #Statistically significant difference compared with the sham-operated group (P_U <0.05)*

Evaluation of the fluorescence intensity of immunopositive cells (CTCF) demonstrated that iNOS⁺ cells in the BMN of rats subjected to ICV colchicine administration exhibited 22.7% significantly higher fluorescence compared to intact animals and 45.3% greater fluorescence intensity compared to iNOS⁺ cells in sham-operated rats ($P < 0.05$) (Fig. 1).

At the same time, CTCF in nNOS⁺ cells of the BMN in colchicine-treated rats was 40.5% significantly lower than in the corresponding structure of intact rats and 51.3% lower than in sham-operated rats (Fig. 2).

Table 1. Parameters of the nitric oxide system in brain homogenates of experimental rats (M ± m)

Experimental group	L-arginine, nm/mg protein	NOx, nm/mg protein	Nitrotyrosine, nm/mg protein
Intact rats	40.7 ± 3.8	0.40 ± 0.03	0.0030 ± 0.0003
Sham-operated rats	43.2 ± 3.7	0.8 ± 0.2	0.005 ± 0.001
ICV colchicine	18.1 ± 2.5[1,2]	4.70 ± 0.83[1,2]	0.010 ± 0.001[1,2]

Note. [1]Statistically significant difference (P_{st} < 0.05) compared with the intact group. [2]Statistically significant difference (P_{st} < 0.05) compared with the sham-operated group

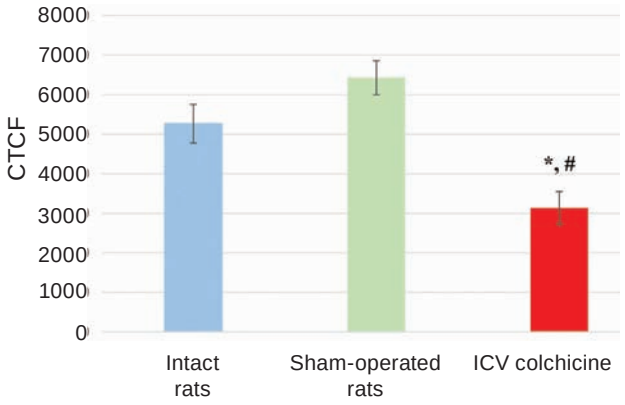

*Fig. 2. Corrected total cell fluorescence (CTCF) of nNOS⁺ cells in the BMN of rats from experimental groups (Uif). *Statistically significant difference compared with the intact group ($P_{st} < 0.05$). #Statistically significant difference compared with the sham-operated group ($P_{st} < 0.05$)*

The density of immunopositive cells for both nNOS and iNOS in the BMN of rats treated with ICV colchicine differed from that in intact and sham-operated animals. Notably, ICV administration of colchicine caused a pronounced reorganization of NOS-expressing cell populations in the BMN, specifically: 53% decrease in nNOS⁺ cell density and 85% increase in iNOS⁺ cell density relative to intact animals. Compared with sham-operated rats, nNOS⁺ cell density in colchicine-treated animals decreased by approximately 41%, whereas the density of iNOS⁺ cells increased by nearly 127% (Fig. 3).

In turn, Kendall's correlation analysis revealed a number of statistically significant associations between NO metabolism parameters and the activity of the corresponding enzymes.

Relationships between L-arginine and enzymatic systems. The analysis demonstrated that L-arginine levels exhibit a strong positive correlation with indicators of the neuronal isoform of nitric oxide synthase: CTCF of nNOS ($\tau = 0.63$) and the density of nNOS⁺ cells ($\tau = 0.60$). At the same time, an inverse relationship was observed between arginine and markers of nitrosative stress as well as the inducible isoform of the enzyme: moderate negative correlations were found with CTCF of iNOS ($\tau = -0.48$), iNOS⁺ cell density ($\tau = -0.50$), and NO metabolites (NOx) levels ($\tau = -0.50$), with the strongest negative correlation observed with nitrotyrosine content ($\tau = -0.61$).

Correlations among nitrosative stress markers. Parameters characterizing pathological activation of the NO system showed a tight positive interrelationship. In particular, iNOS⁺ cell density in the BMN positively correlated with NOx levels ($\tau = 0.73$) and nitrotyrosine content ($\tau = 0.67$) and negatively with CTCF of nNOS⁺ cell ($\tau = -0.41$). A high degree of

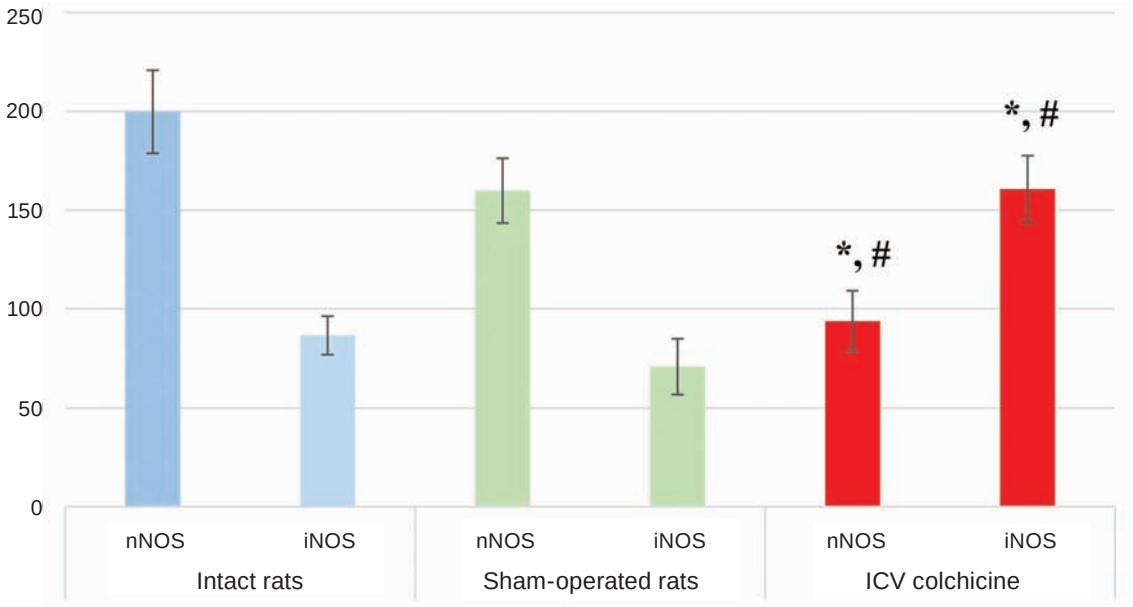

*Fig. 3. Parameters of nNOS⁺ and iNOS⁺ cell density in the BMN of rats from experimental groups (cells/mm²). *Statistically significant difference compared with the intact group ($P_{st} < 0.05$). #Statistically significant difference compared with the sham-operated group ($P_{st} < 0.05$)*

positive correlation was also found between nitrite levels and nitrotyrosine ($\tau = 0.73$).

Notably, $nNOS^+$ cell density exhibited statistically significant negative correlations with markers of nitrosative stress. Specifically, negative correlations were observed with $iNOS^+$ cell density ($\tau = -0.51$), NOx concentration ($\tau = -0.63$), and nitrotyrosine content ($\tau = -0.67$) (Fig. 4).

Discussion

It is currently established that the intensity of NO production by specific NOS isoforms is determined by substrate availability, the presence of cofactors, and multilevel regulation of enzyme expression (including transcriptional, post-transcriptional, translational, and post-translational mechanisms). Under conditions of adequate cofactor supply, the key limiting factor becomes the concentration of L-arginine and its bioavailability. The latter depends directly on the rate of transmembrane amino acid transport into the cell and on the activity of competing metabolic pathways, particularly the arginase reaction within the urea cycle [10].

The results obtained indicate that intracerebroventricular administration of colchicine leads to the development of substrate deficiency. The strong positive correlation identified between L-arginine levels and nNOS expression suggests that, in this model, arginine deficiency acts as a limiting factor for this isoform, ultimately contributing to impaired neuronal plasticity and synaptic transmission. These findings are consistent with the results reported by Kan et al., who demonstrated that a murine model of Alzheimer's disease (CVN-AD) is characterized by increased arginine utilization and reduced bioavailability in the brain [11].

A possible mechanism underlying this phenomenon is the lower Michaelis constant (K_m) of iNOS compared to nNOS, reflecting a higher substrate affinity of the inducible isoform and its ability to produce large amounts of NO autonomously, without the requirement for Ca^{2+} ions [12]. Our

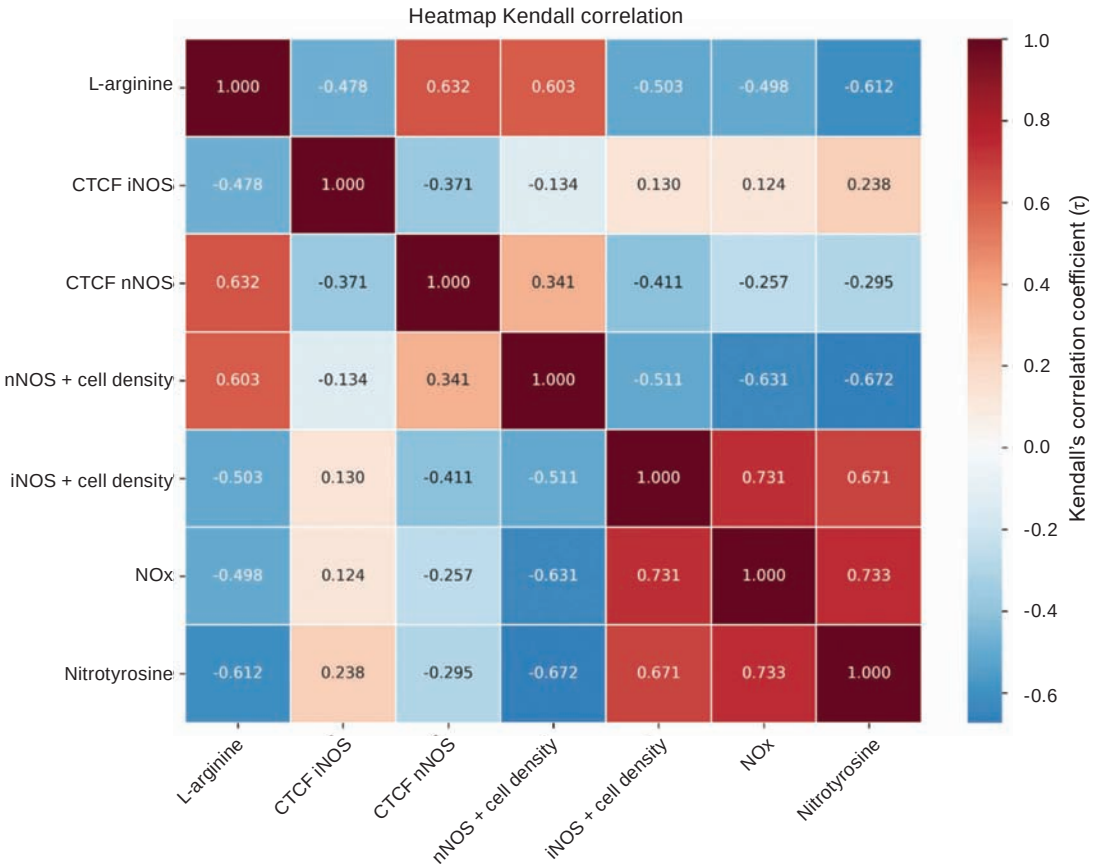

Fig. 4. Kendall's correlation coefficients between the investigated parameters of the NO system in the BMN of experimental rats

data regarding the negative correlation between the density of iNOS-positive cells and the fluorescence intensity (CTCF) of nNOS in BMN neurons may indicate a profound disorganization of the nitric oxide system under conditions of colchicine-induced neurodegeneration. Increased iNOS expression against the background of neuroinflammation leads to excessive NO production, which, in combination with colchicine-initiated oxidative stress, creates conditions for nNOS uncoupling. The mechanism of uncoupling involves the disruption of electron transfer within the enzyme (likely due to L-arginine deficiency or oxidation or impaired availability of the cofactor tetrahydrobiopterin – BH4), resulting in nNOS switching from NO production to the generation of superoxide anion (O_2^-). Under conditions of oxidative destruction, a non-enzymatic interaction occurs between the superoxide and the excess NO generated by iNOS. The rate of this reaction is extremely high and leads to the formation of peroxynitrite ($ONOO^-$) – a potent oxidizing and nitrating agent. Thus, the described biochemical properties of iNOS and the process of enzyme uncoupling provide a mechanistic basis for the shift from physiological neurosignaling to nitrosative damage of BMN structures [13-17].

In turn, excessive iNOS expression in the brain following ICV colchicine administration has been repeatedly demonstrated by several investigators and has been associated with elevated levels of inflammatory mediators, including COX-2, TNF-α, IL-1β, IL-6, as well as increased nitrite content and reactive oxygen species production [18-20]. It should be noted that among proinflammatory mediators, nuclear factor kappa B (NF-κB) acts as a transcriptional activator of genes responsible for iNOS synthesis [21]. Therefore, even in the absence of direct cytotoxic effects, colchicine exerts a distant destructive impact and, through mechanisms of secondary alteration involving reactive oxygen and nitrogen species, contributes to damage of BMN neurons.

Another important finding of the present study is the identification of a pathogenetic cascade of nitrosative stress. Specifically, an increase in the density of $iNOS^+$ cells and their fluorescence intensity was accompanied by the accumulation of NO metabolites

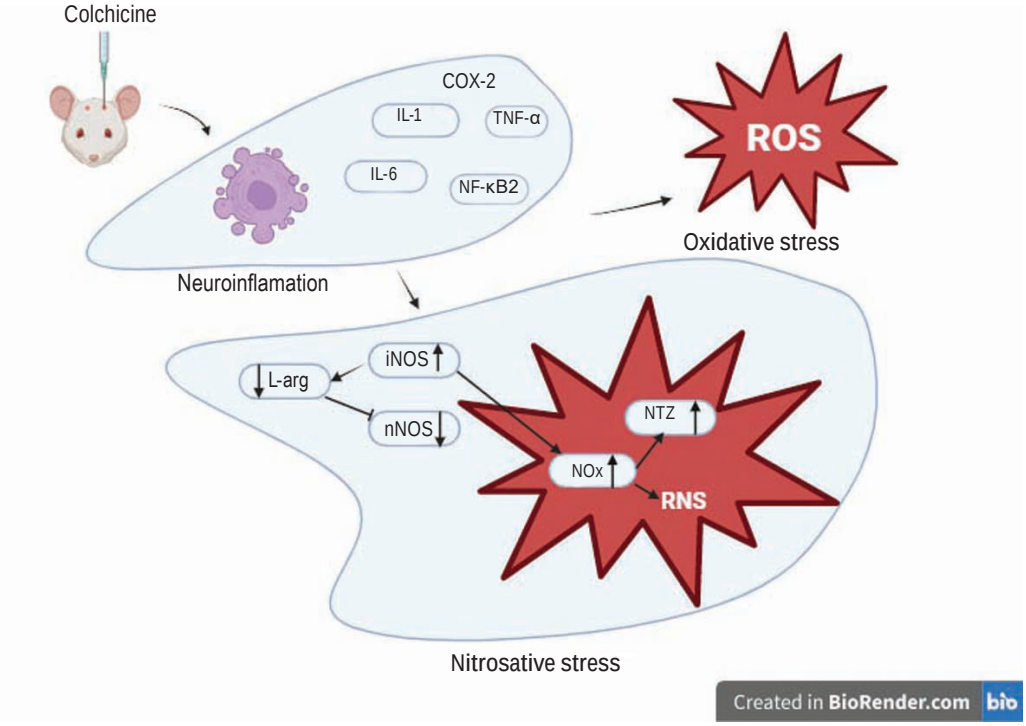

Fig. 5. Hypothetical mechanism of nitrosative stress development in the BMN 14 days after ICV colchicine administration. IL-1 – interleukin 1; IL-6 – interleukin 6; COX-2 – cyclooxygenase 2; TNF-α – tumor necrosis factor α; NF-κB – nuclear factor κB; L-arg – L-arginine; NTZ – nitrotyrosine; NOx – NO metabolites; RNS – reactive nitrogen species; ROS – reactive oxygen species; iNOS – inducible nitric oxide synthase isoform; nNOS – neuronal nitric oxide synthase isoform

and nitrotyrosine. A strong direct relationship between iNOS$^+$ cell density, NOx levels, and nitrotyrosine concentration forms a stable pathological axis:

– excessive iNOS expression leads to uncontrolled NO production;

– elevated NO levels under oxidative stress conditions are converted into peroxynitrite;

– the final marker of this process is nitrotyrosine, indicating irreversible protein nitration and cellular destruction (Fig. 5).

The presence of nitrosative stress under conditions of experimental neurodegeneration has also been demonstrated in the studies of Sil et al. [6].

On the other hand, the present study revealed evidence of functional antagonism and isoform switching. The negative correlation between L-arginine levels and markers of nitrosative stress indicates that substrate depletion may occur in parallel with the progression of colchicine-induced toxic effects. Of particular interest is the inverse relationship between the density of nNOS$^+$ cells and the density of iNOS$^+$ cells/NOx levels. These findings suggest that the loss of nNOS$^+$ neurons and the decline in their functional activity occur simultaneously with compensatory (or pathological) hyperactivation of the iNOS system. Thus, a state of "nitrosative decompensation" develops in the BMN, in which the physiological role of NO is overridden by its cytotoxic effects.

Limitations. This study has several limitations. First, the sample size was relatively small, and the evaluation was restricted to male Wistar rats within a specific age range, potentially excluding the influence of biological sex, hormonal variation, or different genetic backgrounds on the observed effects. Second, the study was conducted using an animal model and may not fully reflect human physiological conditions. Third, only selected parameters were evaluated, and specifically, the endothelial isoform of nitric oxide synthase (eNOS) was omitted from the analysis, leaving out its potential contribution to the overall pool of nitric oxide metabolites and vascular tone during neurodegeneration. These limitations should be considered when interpreting the results.

Conclusions.

1. Intracerebroventricular administration of colchicine induces profound destabilization of the nitric oxide system in the brain, characterized by a decrease in L-arginine concentration accompanied by a significant increase in NO metabolites (NOx) and nitrotyrosine levels.

2. The development of experimental neurodegeneration is associated with differential alterations in NOS isoform activity within the basal magnocellular nucleus, including a marked increase in corrected total fluorescence in iNOS-positive cells alongside a simultaneous decrease of this parameter in nNOS-positive neurons.

3. Colchicine administration results in an inversion of the cellular composition of the basal magnocellular nucleus, manifested by an increased density of iNOS-positive cells and a progressive reduction in the population of nNOS-positive neurons.

4. Correlation analysis confirmed the pathogenetic significance of NOS isoform imbalance following colchicine administration. A strong positive association was identified between L-arginine content and nNOS expression, as well as between NOx levels, nitrotyrosine concentration, and the density of iNOS$^+$ cells. At the same time, strong negative correlations were observed between arginine concentration and iNOS expression, and between markers of nitrosative stress (NOx, nitrotyrosine) and the density of nNOS$^+$ structures.

Author contributions. MVD conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing - original draft; DLU investigation, data curation; AHK supervision, validation, writing - review and editing; YMK conceptualization, supervision, funding acquisition, writing - review and editing.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПОКАЗНИКИ СИСТЕМИ ОКСИДУ АЗОТУ В БАЗАЛЬНОМУ МАГНОЦЕЛЮЛЯРНОМУ ЯДРІ ГОЛОВНОГО МОЗКУ ЩУРІВ ПІСЛЯ ІНТРАЦЕРЕБРО-ВЕНТРИКУЛЯРНОГО ВВЕДЕННЯ КОЛХІЦИНУ

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Вступ. Базальне магноцелюлярне ядро (BMN) є структурою головного мозку, що забезпечує холінергічну іннервацію неокортексу. Порушення функціональної активності BMN спостерігається під час багатьох нейродегенеративних захворюваннях. Однією з ключових регуляторних систем нейрональної активності в мозку є система оксиду азоту (NO), проте взаємодія між компонентами системи NO в умовах нейродеструкції досі залишається недостатньо з'ясованою. **Мета.** Метою дослідження було визначити рівні L-аргініну, нітритів, нітротирозину, а також експресію iNOS та nNOS у BMN щурів за моделювання колхіцин-індукованої нейродеструкції. **Методи.** У дослідженні було задіяно 30 самців щурів лінії Вістар, розділених на три групи: інтактну, хибнооперовану з внутрішньошлуночковим (ICV) введенням 3 мкл NaCl та колхіцинову з ICV ін'єкцією колхіцину (15 мкг/3 мкл NaCl). ICV ін'єкції проводили за допомогою цифрового стереотаксичного апарату. Через два тижні після введення колхіцину у тварин розвинувся когнітивний дефіцит, підтверджений поведінковими тестами. Після евтаназії тканину мозку швидко вилучали для подальших досліджень. Експресію iNOS та nNOS оцінювали за допомогою імунофлуоресцентної мікроскопії, метаболіти NO вимірювали методом Грісса, концентрацію L-аргініну – методом HPLC-MS, а рівень нітротирозину – методом імуноензимного аналізу ELISA. **Результати.** ICV введення колхіцину супроводжувалося зниженням рівня L-аргініну, що поєднувалося зі значним підвищенням рівнів метаболітів NO та нітротирозину в BMN щурів. Імунофлуоресцентний аналіз виявив збільшення щільності iNOS-позитивних і прогресуюче зменшення nNOS-позитивних клітин у популяції клітин BMN щурів, яким вводили колхіцин. Кореляційний аналіз підтвердив, що дисбаланс системи NO відіграє значну роль у нейродеструкції. **Висновки.** Колхіцин-індукована нейродеструкція в BMN щурів пов'язана з дисбалансом системи оксиду азоту, що характеризується зниженням рівня L-аргініну, підвищенням вмісту метаболітів NO та нітротирозину, підвищенням експресії iNOS і зниженням щільності nNOS-позитивних клітин. Ці зміни можуть сприяти нейродегенеративним процесам у BMN.

Ключові слова: система оксиду азоту, L-аргінін, нітрити, нітротирозин, iNOS, nNOS, колхіцин, нейродегенерація, головний мозок.

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