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EXPERIMENTAL ARTICLES

Pharmacological Modulation of Endogenous Neuroprotection after Experimental Prenatal Hypoxia

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Abstract—Prenatal hypoxia (PH) causes pathological changes in the brain and can lead to irreversible long-term disorders of brain development and the emergence of neuropsychiatric pathologies in children. Pharmacological correction of post-hypoxic disorders of the central nervous system is a priority in modern medicine. The aim of this work was to study the neuroprotective effects of Angiolin, Thiotriazoline, Tamoxifen, Glutoredoxin, Cerebrocurin, an IL-1b antagonist (RAIL), Mexidol, and L-arginine in comparison with the reference drug Piracetam in terms of their effect on the expression of endogenous neuroprotection factors for further substantiation of their use for treating prenatal CNS lesions in a model of chronic hemic PH. The expression of HSP_{70}, HIF-1, and c-fos mRNAs and the content of HSP_{70} in the cytoplasmic and mitochondrial fractions of the brain of 60 day old rat pups subjected to PH were studied using real-time PCR and enzyme-linked immunosorbent assay. We found that chronic PH leads to the inhibition of transcriptional processes in neurons and the suppression of HIF1a, HSP_{70}, and c-fos synthesis. The studied drugs modulated the HSP_{70}-mediated mechanisms of endogenous neuroprotection. The most active among HSP_{70} modulators in chronic PH were Cerebrocurin (150 μL/kg) and Angiolin (50 mg/kg) which surpass the other drugs studied in the level of HSP_{70} and HIF-1α mRNA expression, as well as HSP_{70} protein concentration in the brain of experimental animals, and can be viewed as promising neuroprotective agents in complex therapy after PH.

Keywords: prenatal hypoxia, CNS, endogenous neuroprotection, HSP_{70}, c-fos mRNA, HSP_{70} mRNA, HIF-1a mRNA, HSP_{70} modulators

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INTRODUCTION

Hypoxic brain damage is a leading factor in perinatal pathology of the nervous system in newborn children and, despite extensive studies, the problem remains relevant since the pathogenesis and etiology are still unclear and treatment demands development of new drugs [1]. Prenatal hypoxia (PH) and asphyxia are the main causes of perinatal mortality accounting for 20 to 50% of cases [2]. The developing brain of a newborn is extremely sensitive to hypoxia which causes not only focal damage of the brain tissue but hampers angiogenesis and cell differentiation [3]. Newborns who suffered PH demonstrate various clinical metabolic, immune, and endocrine disorders while adapting to postnatal life, along with a high frequency of infections, neuropsychiatric disorders and significant developmental deviations later in life [2, 4].

An important breakthrough in studying the molecular mechanisms of brain functioning under normal and pathological conditions was made in recent years due to advances in molecular biology, neurochemistry, and fundamental pharmacology [5]. Hypoxic damage to the fetal CNS results from a decrease in the partial pressure of oxygen due to deterioration of utricular blood circulation, placental insufficiency, or fetal pathologies [5, 6]. Different mechanisms of neurodestruction in the period of acute PH include transmitter autocoidosis, glutamate-calcium cascade, disruptions in the nitroxidergic system, and energy deficit. During a prolonged post-hypoxic period, mechanisms of neurodestruction include oxidative stress, secondary mitochondrial dysfunction, neuroapoptosis, neuroinflammation, and deficiency of neurotrophic and cytoprotective factors [2, 5, 7]. Evolution resulted in the formation of compensatory mechanisms in the mother–placenta–fetus system that increase the stability of the fetal CNS, these are, activation of compensatory energy shunts, expression of endogenous

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cytoprotective factors, genes responsible for the synthesis of antioxidative enzymes, and factors regulating circulation [4]. Studies in the first two decades of the 21-st century showed the role of heat-shock proteins (HSP) and hypoxia-induced factor (HIF-1) in the realization of endogenous neuroprotection mechanisms in hypoxic brain damage [7–10]. Currently, nootropics, antihypoxants, and vasoactive substances are used after PH as a means of neuroprotection [11–19]. However, they do not meet clinical demands. Based on numerous works of members of the Department of Pharmacology and Medical Formulation of the Zaporizhzhia State Medical University (ZSMU), a promising approach to neuroprotection after PH is activation of HSP_{70}-dependent mechanisms of endogenous neuroprotection. In this respect, drugs such as Angiolin, Thiotriazoline, Tamoxifen, Glutoredoxin, Cerebrocurin, and RAIL which demonstrate an ability to regulate HSP_{70} expression in models of ischemia of the brain and myocardium, and chronic alcoholism, are of interest [7, 11–16]. Further, evaluation of the HSP_{70}-dependent mechanism of the neuroprotective effect of Mexidol, L-arginine, and Pirocetam that demonstrate different mechanisms of cytoprotective and anti-ischemic action is also of interest [17–19].

The aim of this work was to evaluate the effects of Angiolin, Thiotriazoline, Tamoxifen, Glutoredoxin, Cerebrocurin, RAIL, Mexidol, L-arginine, and the reference drug Piracetam on the expression of endogenous neuroprotection factors and experimentally confirm the need for further studies on drugs modulating this system as components of complex therapy for prenatal hypoxic damage to the CNS.

MATERIALS AND METHODS

Characteristics of laboratory animals and the experimental model of PH. The experiments were conducted on 50 outbred white rat females and 10 males weighing 220–240 g aged 4.5 months obtained from the vivarium of the Institute of Pharmacology and Toxicology of the National Medical Academy of Ukraine. The rats were kept under standard vivarium conditions at 20–25°C, humidity 50–55%, natural light, diet recommended for this species of laboratory animals, and water ad libitum. Here, we used the chronic hematic nitrite-induced PH model [20, 21]. For a fixed term of pregnancy, mature male rats were placed with virgin female rats with a ratio of 2 males per 4 females. The Pregnancy period was counted starting from the discovery of spermatozoids in the vaginal smear (day 1 of the pregnancy). Modelling hematic hypoxia was performed in the prenatal period of development by daily intraperitoneal administration of sodium nitrite solution to pregnant female rats from day 16 to day 21 of the pregnancy at 50 mg/kg (the dose causing moderate hypoxia) [20]. Control pregnant rats received physiological solution in the same regime. The progeny was divided into groups: (1) healthy pups from females with physiologically normal pregnancy which received physiological solution; (2) control group of pups after PH which received physiological solution daily; 3–11 groups of pups after PH that received drugs daily from postnatal day 1 to day 30.

Justification for the selected drugs and their characteristics. We chose drugs with experimentally proven ability to modulate the expression of HSP_{70}:

(1) Thiotriazoline (Morpholinium-3-methyl-1,2,4-triazolyl-5-thioacetic acid) (2.5% solution for injections, Arterium, Ukraine), metabolitotropic cardioprotector and antioxidant. There is evidence that the drug increases HSP_{70} expression in the myocardium in ischemia, chronic cardiac insufficiency, and alcohol-induced heart damage [11].

(2) Tamoxifen (pills, Finland, intranasal gel (1 mg/1 mL) extemporaneously prepared at the Department of Medicine Technology of Zaporizhzhia State Medical University) at low doses is an estrogen receptor beta agonist (β-ER). It increases the concentration of HSP_{70} in the mitochondria and cytosol of the brain and myocardium in acute ischemia by regulating mechanisms involving reactive oxygen species and reduced glutathione (ROS/GSH-dependent mechanisms) [12].

(3) Angiolin ([S]-2,6-diaminohexane acid 3-methyl-1,2,4-triazolyl-5-thioacecate) (substance, NPO Farmatron, Ukraine), anti-ischemic, endothelium protective drug. It increases the concentration of HSP_{70} in the mitochondria and cytosol of the brain and myocardium in acute and chronic ischemia and alcoholism. It also normalizes the expression of the early-response gene (*c-fos*) and vascular endothelial growth factor (VEGF) [13].

(4) Glutoredoxin (Sigma-Aldrich, United States). Earlier data indicated that glutoredoxin influences GSH-dependent mechanisms of HSP_{70} expression in the mitochondria and cytosol of the brain and myocardium in acute cerebral ischemia [14].

(5) Cerebrocurin (solution for injections, NPO NIR, Ukraine). Cerebrocurin contains neuropeptides, S-100 proteins, reelin, nerve growth factor (NGF) (not less than 2 mg/mL) and amino acids. It exerts pronounced neuroprotective effects, increases neuronal plasticity, and stimulates the expression of transcription factors and protective proteins including HSP [15].

Among promising drugs for neuroprotection, we also studied:

(6) RAIL, a selective IL-1b antagonist (substance provided by the Federal State Unitary Enterprise State Research Institute of Highly Pure Biopreparations (Russia, Saint Petersburg, LSR-007452/1-0300710)). The substance was obtained biotechnologically from *E. coli* TG1 (pTAC-hlL-1ra) and contains 153 fragments of amino acids. Its molecular weight is 17.906 kDa. Intranasal RAIL gel (5 mg/mL) was developed at the

Department of Medicine Technology of Zaporizhzhia State Medical University). RAIL disrupts IL-b-dependent cascade mechanisms of ischemic neurodestruction [16]. There is evidence that RAIL normalizes glutathione (GSH)-dependent mechanisms of HSP_{70} expression in the mitochondria and cytosol of the brain in acute ischemia [22].

(7) L-Arginine (42% solution for injection in vial, Tivortin, Yuria-pharm, Ukraine), an NO precursor; it mitigates disruptions in the nitroxidergic system in ischemia and exerts an endotheliotropic antioxidant effect [17].

(8) Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate), solution for injections, 50 mg/mL, NPO Farmasoft, Ellara, Russia. Antioxidant with neuroprotective properties. It inhibits the mechanisms of ischemic neurodestruction involving reactive oxygen species (ROS-dependent mechanisms) [18].

(9) We selected Piracetam as a reference drug (200 mg/mL, JCS Farmak, Ukraine). Piracetam is a nootropic drug with a pronounced anti-ischemic effect [19]. It was selected as a reference drug due to the fact that pre-clinical studies gave comprehensive information on the molecular biochemical mechanism of its nootropic, neuroprotective, and anti-ischemic effects, along with the evidence base for its clinical efficacy [2, 7]. In Ukraine, the Russian Federation, the United States, and the European Union, Piracetam is included as a neuroprotective drug in the treatment protocols of patients with acute cerebrovascular disorders, traumatic brain injuries, fetoplacental insufficiency, and prenatal encephalopathy [7, 19].

Drugs were administered at the following doses: Piracetam 500 mg/kg, i/p; Angiolin 50 mg/kg, i/p; Thiotriazoline 50 mg/kg, i/p; Mexidol 100 mg/kg, i/p; L-arginine 200 mg/kg, i/p; Glutoredoxin 200 μg/kg, i/p; Tamoxifen 0.1 mg/kg, intranasally; RAIL 1 mg/kg, intranasally; Cerebrocurin 150 μL/kg i/p. To determine dosage, we used the instructions for the drugs and the results of our previous studies [7, 11, 13, 22].

Rats were withdrawn from the experiment on day 60 under thiopental anesthesia (40 mg/kg). The brain and blood from the celiac artery were harvested for studies. Blood and meninges were quickly removed from the brain and the studied fragments were placed in liquid nitrogen. Then they were ground to powder in liquid nitrogen and homogenized at 2°C in 10 volumes of medium containing (mmol): sucrose 250, Tris-HCl-buffer 20, and EDTA 1 (pH 7.4). The mitochondrial fraction was separated by differential centrifugation at +4°C in a Sigma 3-30k refrigerated centrifuge (Germany). The brain of experimental animals was placed in Bouin's fluid for 24 hours and, after standard histological processing, the tissue was embedded in paraplast. To conduct PCR, 5μm thick sections of sensorimotor cortex layer V–VI were made using a rotation microtome. The ability of the studied drugs to positively modulate the mechanisms of endogenous neuroprotection was estimated by the expression and concentration of HSP_{70}, as well as the expression of HIF-1 and c-fos. One of the neuroprotective effects of HSP_{70} is to increase the "lifespan" of HIF-1 which under hypoxia is capable of activating and regulating compensatory cytosolic mitochondrial bypasses (including malate-aspartate bypass) [7]. Transcription factor c-fos (sometimes in the literature called an early-response gene) is quickly and temporarily activated in response to a wide range of factors, including hypoxia, and is able, depending on the level of expression, to participate in processes from apoptosis to neuroprotection through initiation of HSP_{70} transcription [42].

Real-time PCR. The mRNA expression of HSP_{70}, HIF-1, and c-fos was measured by polymerase chain reaction in real time. Tissues were deparaffined by incubation in two consecutive baths of xylol and 100% ethanol. Following deparaffination and centrifugation, the residue was dried in air to remove residual ethanol. Extracting total RNA from tissue was performed using "Trizol RNA Prep 100" kit (Isogen, Russia) which contains the following reagents: Trizol reagent and ExtraGene E. RNA was extracted according to the kit protocol. For reverse transcription (synthesis of DNA), we used "Reverse transcription reagent kit (RT-1)" (Syntol, Russia). To measure the level of expression of studied genes, we used CFX96 ™ Real-Time PCR Detection Systems (Bio-Rad Laboratories, Inc., United States) and reagent kit for conducting PCR-RT in the presence of SYBR Green R-402 (Syntol, Russia). Specific pairs of primers (5'–3') for the analysis of studied and reference genes were selected using Primer Blast software (www.ncbi.nlm.nih.gov/tools/primer-blast) and made by ThermoScientific (United States). Fluorescence intensity was registered automatically at the end of each elongation stage in the SybrGreen channel. We used beta-actin (Actb) as a reference gene to determine the relative value of change in the level of expression of the studied genes.

Enzyme-linked immunoassay. The technique is based on solid-phase enzyme-linked sandwich immunosorbent assay. The level of the heat-shock protein HSP_{70}, was measured in the mitochondrial and cytoplasmatic fractions of the brain by enzyme-linked immunoassay using AMP'D® HSP70 high sensitivity ELISA kit, Enzo (Sweden). The concentration of HSP_{70} was expressed in ng/mL.

Statistical analysis. Experimental data were statistically analyzed using "Statistica® for Windows 6.0" (StatSoft Inc., no. AXXR712D833214FAN5), "SPSS 16.0," and "Microsoft Office Excel 2010" software. Prior to statistical tests, we checked the results for normality (Shapiro–Wilk and Kolmogorov–Smirnov tests). In normal distribution, intergroup differences were considered statistically significant based on the parametric Student's *t*-test . If the distribution was not normal the comparative analysis was conducted using

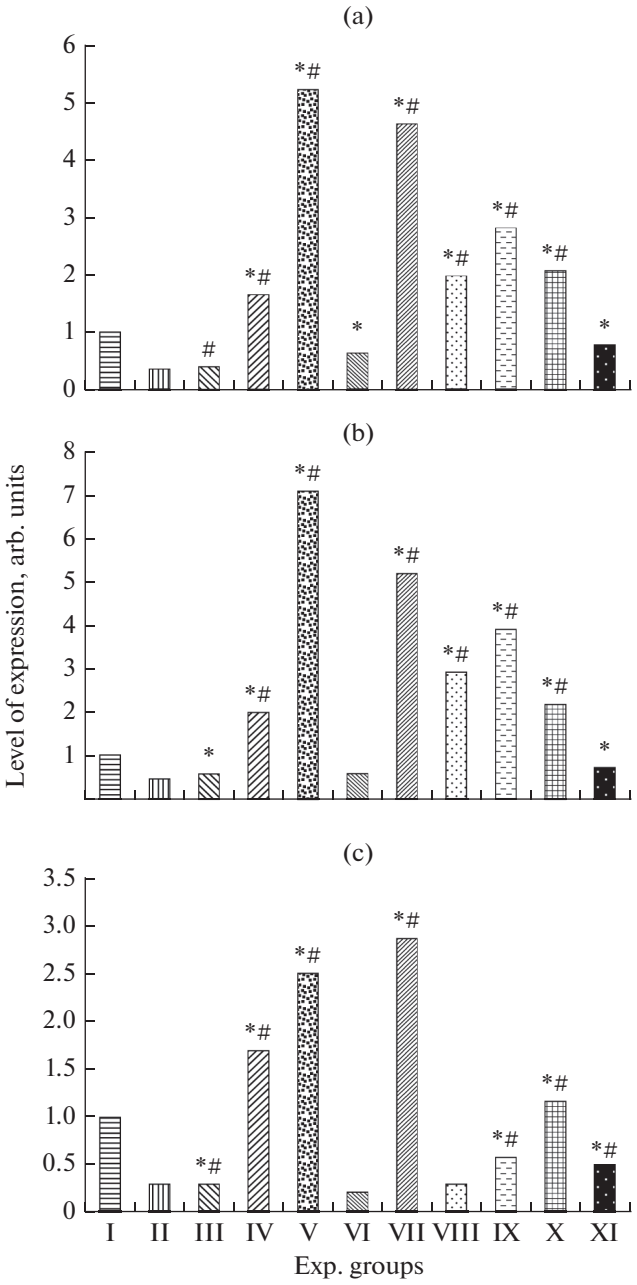

Fig. 1. The character of expression of mRNA of HIF1-α (a), HSP_{70} (b) and c-fos (c) in the sensorimotor cortex of rats subjected to prenatal hypoxia, after a 30-day pharmacological correction on day 60 of life ($M \pm m$). Experimental groups: I, healthy animals (intact), $n = 10$; II, animals subjected to PH (control), $n = 10$; III, PH + L-arginine (200 mg/kg), $n = 10$; IV, PH + Tamoxifen (0.1 mg/kg), $n = 10$; V, PH + Cerebrocurin (150 μL/kg), $n = 10$; VI, PH + Piracetam (500 mg/kg), $n = 10$; VII, PH + Angiolin (50 mg/kg), $n = 10$; VIII, PH + RAIL (1 mg/kg), $n = 10$; IX, PH + Glutoredoxin (200 μg/kg), $n = 10$; X, PH + Thiotriazoline (50 mg/kg); XI, PH + Mexidol (100 mg/kg), $n = 10$. *Statistically significant differences compared to the control group (PH), $p < 0.05$; # statistically significant differences compared to the Piracetam control group, $p < 0.05$.

the non-parametric Mann–Whitney U-test. To compare independent variables in more than two selections, we applied ANOVA dispersion analysis for normal distribution and the Kruskal–Wallis test for non-normal distribution. To analyze correlations between parameters, we used correlation analysis based on the Pearson or Spearman correlation coefficient. For all types of analysis, the differences were considered statistically significant at $p < 0.05$ (95%).

RESULTS AND DISCUSSION

The data presented in Fig. 1 characterizing the expression of HIF-1α and mRNA of HSP_{70} and c-fos on postnatal day 60 in the sensorimotor cortex of rats that were subjected to PH suggest that the expression of mRNA of HIF-1α in the control group of rats that were not treated was 67% lower than in the intact group. We also registered a decrease in the expression of HSP_{70} mRNA by 59% and c-fos mRNA by 72%. Enzyme-linked immunoassay showed a decrease in the concentration of HSP_{70} by 26% in mitochondria and almost 2-fold reduction in the cytosol of the brain in pups subjected to PH compared to healthy animals of similar age (Fig. 2). The revealed facts indicate inhibition of transcription processes in neurons and suppression of HSP_{70}-dependent mechanisms of endogenous neuroprotection.

A 30-day course of treatment with the selected drugs resulted in various effects on the expression of HIF-α, HSP_{70}, and c-fos mRNAs, as well as the concentration of HSP_{70} in the brain of pups subjected to PH.

Studies on the expression of HIF-α mRNA showed that, in all groups except animals that received L-arginine, the level of HIF-α protein synthesis was higher than in controls (Tamoxifen by 4.9 times, Cerebrocurin by 15.8 times, Piracetam by 82%, Angiolin by 13.9 times, RAIL by 6 times, Glutoredoxin by 8.5 times, Thiotriazoline by 6.2 times, and Mexidol by 2.3 times). The highest significant ($p < 0.05$) values of HIF-α mRNA expression were observed in animals after Cerebrocurin and Angiolin; the level of HIF-α mRNA expression in animals that received L-arginine did not differ from controls.

Changes in the level of HSP_{70} mRNA expression after administration of drugs are in general similar to the changes in HIF-α mRNA. Most drugs caused a considerable increase in this parameter (Tamoxifen by 4.8 times, Cerebrocurin by 17.4 times, Angiolin by 12.7 times, RAIL by 7 times, Glutoredoxin by 9.5 times, and Thiotriazoline by 5.2 times). Application of L-arginine, Mexidol, and Piracetam did not result in a considerable (compared to other drugs) increase in the level of HSP_{70} mRNA expression (by 39, 37.7, and 66%, respectively) whereas Cerebrocurarin and Angiolin are characterized by the highest results among the studied drugs, similar to the results of HIF-α mRNA expression (Fig. 1a).

The drugs studied led to an increase in the level of c-fos mRNA expression in the sensorimotor cortex of the brain in most experimental groups (Tamoxifen by 6 times, Cerebrocurin by 9 times, Angiolin by 10 times, Glutoredoxin by 2 times, and Thiotriazoline by 4 times, Mexidol by 75%). Administration of L-arginine and RAIL did not provoke statistically significant changes in the level of c-fos mRNA expression whereas a course of Piracetam led to a 25% decrease in this parameter.

The HSP_{70} concentration in the cytosol fraction of the brain homogenate in the experimental groups generally correlated with the values of its mRNA expression. Thus, administration of Angiolin and Cerebrocurarin led to increases in HSP_{70} concentration in the cytosol by 3 and 3.8 times, respectively, whereas Piracetam and Mexidol had minimum effect. The rest of the drugs induced a statistically significant increase by 1.9–2.3 times compared to control (Fig. 2b).

The changes in HSP_{70} concentration in the mitochondrial fraction in the groups of animals subjected to PH that received treatment were different after the drugs studied. Thus, HSP_{70} concentration in mitochondria of the neurons in animals that received L-arginine and Thiotriazoline did not differ from the animals that did not receive treatment, whereas in the groups treated with Piracetam and Mexidol the results were insignificantly lower (by 24 and 14%, respectively). A course of Tamoxifen increased the concentration of mitochondrial HSP_{70} by 85%, RAIL, by 48%, and Gluthoredoxin, by 57% (Fig. 2a). As with the other studied parameters, maximum HSP_{70} concentration in the mitochondrial fraction were observed after Cerebrocurarin and Angiolin (an increase by 2.4 and 2.2 times, respectively).

It can be suggested that the mechanism of neurodestructive processes followed by cognitive deficit in rats subjected to PH is largely orchestrated by the suppression of early-response *c-fos* gene expression, which is attributed to its transcription role in the synthesis of regulatory proteins participating in the mechanisms of endogenous neuroprotection, angiogenesis, and memory consolidation [5, 7]. This suggestion is substantiated by other studies showing that transcription factors (c-fos and c-jun) have a role in the initiation of mechanisms of endogenous neuroprotection by triggering the expression of heat-shock proteins (HSP_{70} and HSP_{72}) and hypoxia-inducible factor (HIF-1) [23, 24]. The products of *c-fos* genes play a vital role in cell cycle control, development, growth, and cell differentiation; they also determine the fate of differentiated neurons and participate in transferring information from cell to cell. When adaptative capabilities are compromised, ROS sharply increase whereas reduced thiols are deficient, leading to the inactivation of transcription factors followed by inhibition of *c-fos* gene expression [23]. It is known that inhibiting c-fos mRNA translation in the brain disrupts short-term

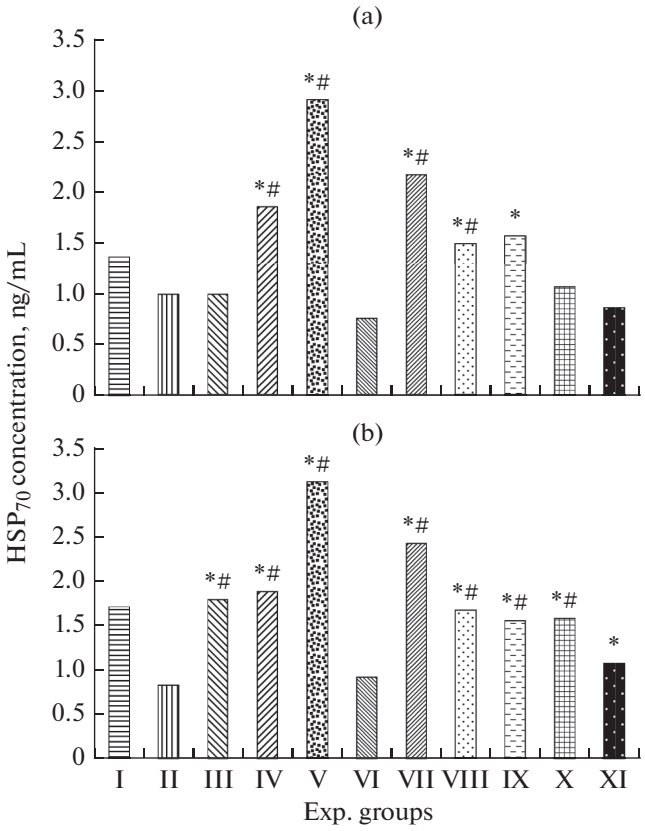

Fig. 2. Quantitative indices of HSP_{70} content in mitochondrial (a) and cytosolic (b) fractions of the brain homogenate of rats subjected to prenatal hypoxia after a 30-day pharmacological correction on day 60 of life ($M \pm m$). Experimental groups: I, healthy animals (intact), $n = 10$; II, animals subjected to PH (control), $n = 10$; III, PH + L-arginine (200 mg/kg), $n = 10$; IV, PH + Tamoxifen (0.1 mg/kg), $n = 10$; V, PH + Cerebrocurin (150 μL/kg), $n = 10$; VI, PH + Piracetam (500 mg/kg), $n = 10$; VII, PH + Angiolin (50 mg/kg), $n = 10$; VIII, PH + RAIL (1 mg/kg), $n = 10$; IX, PH + Glutoredoxin (200 μg/kg), $n = 10$; X, PH + Thiotriazoline (50 mg/kg); XI, PH + Mexidol (100 mg/kg), $n = 10$. *Statistically significant differences compared to the control group (PH), $p < 0.05$, #statistically significant differences compared to the Piracetam control group, $p < 0.05$.

memory in various models of learning in different animal species [24]. Thus, the above-mentioned experimental studies led us to conclude that the *c-fos* gene is a bridge from the animal's individual experience to its entire genome [25, 26]. However, increased expression of c-fos does not have a physiological function. When the gene's expression is increased by over 20 times, c-fos acquires negative effects. Hyperexpression of the *c-fos* gene, as was observed in the CA1 region of the hippocampus of 1—14-day-old rat pups that suffered from PH, leads to a considerable increase in the concentration of c-fos protein which takes an immediate part in the process of DNA fragmentation and initiation of apoptotic cell death [5, 27]. Based on the above, we can conclude that *c-fos* gene activation

occurs in any type of exposure. The character of the gene expression will determine the cell's fate: either translation and transcription will be activated or it will undergo apoptotic cell death.

Hypoxia-inducible factors (HIFs) are one of the adaptive mechanisms activated during hypoxia/ischemia. Hypoxia stabilizes the HIF-1 subunit which binds the HIF-1α subunit and induces the transcription of target genes regulating oxygen homeostasis. Some of these HIF-1-regulated genes include erythropoietin playing an important role in cell survival, endothelial growth factor, which activates endothelial cells and provides capillary growth [5, 28, 29], and glucose transporter-1, (Glut-1) which influences cell metabolism of glucose [7]. HIF plays a crucial role in stimulating angiogenesis and metabolic adaptation in the process of brain development. The expression of HIF-1 gene depends not only on hypoxia but on other regulatory factors, including genetically determined factors, which is why it is not always adequate for the level of hypoxia. In this case, as well as when hypoxia/ischemia are too durable and strong, a cascade may be unfolded of transcription and epigenetic reactions and structural metabolic shifts mediated by them which can mark reprogramming with a high readiness for CNS perinatal damage [8].

Studies in the past decade showed the ability of heat-shock proteins (HSP_{70}) to exert protective effects in brain and heart ischemia [7, 30]. The neuroprotective action of HSP_{70} was demonstrated under the conditions of oxidative and nitrosative stresses, glutamate excitotoxicity, oxygen and glucose deprivation, and various models of brain ischemia [11, 14, 31]. Increased HSP_{70} in the brain tissues points to the activation of endogenous neuroprotection mechanisms whereas its deficiency indicates neurodegenerative processes. Apart from that, there is recent evidence of its role in stabilizing HIF-1a protein which in ischemia is responsible for the expression of genes whose products take part in proliferation, apoptosis, stabilizing protein molecules under oxidative stress, and initiation of the folding of oxidatively modified functionally active macromolecules [5, 7, 32]. In the view of data on the ability of HSP_{70} to increase neuronal resiliency under hypoxia and the fact that changes in the level of HSP- and HIF-proteins in the brain are unidirectional, we can suggest that HSP_{70} takes part in the regulation of signaling pathways of cell response to hypoxic stress through regulation of HIF-protein stability [7], which is in accord with our previous studies [5, 7, 16, 22]. Our results do not contradict the data obtained by other researchers who demonstrated a regulatory influence of HSP_{70} on the processes of mitochondrial dysfunction, oxidative stress, and the expression of HIF-proteins in the brain cells under ischemia and hypoxia [6, 9, 27].

Piracetam that plays a special role in influencing metabolic processes and blood circulation in the fetal brain, which is why using it to treat the consequences of PH in the CNS is possible. The drug stimulates redox reactions, intensifies glucose utilization, and improves regional blood circulation in ischemic brain regions. Piracetam increases the body's energy potential by accelerating ATP turnover. Improvement of energy processes under its influence increases resilience to hypoxia [19, 33]. However, piracetam has side effects: in children under 1 year with hypoxia in the CNS, it can cause seizures and aggravate lactate-acidosis [2]. The known mechanisms of the anti-ischemic action of Piracetam do not fully explain its influence on the studied aspects of endogenous neuroprotection. L-arginine, as a NO precursor, exerts endothelial protection, vasodilatation, antioxidant effects which make it effective in premature delivery, fetal growth arrest, and fetoplacental insufficiency [2]. However, as our studies show, restoration of NO production does not influence the expression of HSP_{70}, though there is evidence on NO-dependent regulation of HSP_{70} [7, 34]. This may be connected with a quick transformation of NO into peroxynitrite in ischemia and a loss of its physiological functions [35].

The neuroprotectors Mexidol and Thiotriazoline had some success in the treatment of PH. Mexidol inhibits oxidative modification of proteins, nucleic acids, and phospholipids; as a scavenger of oxygen and fatty acid radicals, it preserves the physical and chemical constants of the phospholipid bilayer of the membrane [18, 36]. Mexidol exerts cerebrovascular effects by decreasing oxidative damage to GABA-receptors of the brain vessels [36]. Mexidol has a positive influence on energy exchange in newborns after PH and accelerates the restoration of locomotor functions [37]. However, "direct" antioxidant properties do not give Mexidol a full influence on HSP_{70}-dependent mechanisms of endogenous neuroprotection. Thiotriazoline reduces ROS formation in mitochondria preserving oxidative energy production. By decreasing hyperproduction of NO and ROS, Thiotriazoline prevents oxidative modification of the protein structures of receptors, ion channels, enzymes, and transcription factors (regulates SH/SS-mechanisms of expression in ischemia and nuclear transcription factor kappa B (NF-kB)) and restores the expression of redox-sensitive genes necessary to protect cells from toxic effects of oxidative stress, including HSP_{70} [7, 11, 38]. Using Thiotriazoline in primiparas over 30 years old with a possibility of miscarriage provides a considerable decrease in the frequency of complications associated with pregnancy and birth, and improves health (in Apgar score) and adaptation in newborns [2].

In our opinion, the detected pronounced properties of Cerebrocurin to activate the synthesis of HSP- and HIF-proteins can be explained, first, by their ability to modulate genome response in hypoxia by activation of global transcription factors initiating HSP synthesis [39]. Second, some works demonstrate the abil-

ity of neuropeptides to directly bind HSP-proteins and present them to dendrite cells [5, 7]. Apart from that, Cerebrocurarin, similar to other neuropeptide drugs, shows pronounced antioxidant activity, which results from the ability of neuropeptides to positively influence the expression of genes encoding the synthesis of major enzymes of the antioxidant system—catalase and superoxide dismutase [15]. This property of the studied drugs allows inhibition of oxidative destruction of HSP_{70}-protein under conditions of intensified free-radical oxidation, which prolongs its protective action.

A pronounced effect on the HSP_{70}-mechanisms of endogenous neuroprotection was demonstrated in our study on Angionlin. It shows considerable anti-ischemic, neuroprotective, carpioprotective, and endothelial protective effects mediated by the following mechanisms. First, due to its antioxidative mechanism (ROS and NO scavenger), Angiolin has a positive influence on the expression of genes coordinating the synthesis of superoxide dismutase, glutathione peroxidase, and glutathione reductase [7, 13], regulates ROS/GSH-dependent mechanisms of HSP_{70} expression, and provides increased bioavailability of NO [7, 40]. Second, Angiolin is capable of activating compensatory cytosolic and mitochondrial energetic bypasses under ischemia. Third, Angiolin due to the L-lysin residue in its composition increases the affinity of GABA receptors and reduces excitotoxicity [41].

The neuroprotective activity of the selective estrogen receptor modulator Tamoxifen is explained, in our opinion, by activation of β-estrogen receptors in the brain followed by HSP_{70} dissociation from the latter. The mechanism of this interaction is explained by the ability of HSP_{70} to form complexes with estrogen receptors not bound by estrogen, thus keeping them inactive [7, 40, 42]. When the estrogen receptor interacts with a steroid ligand, conformational changes of the receptor protein take place and HSP is released from the complex; it enters the cell and mitochondria to realize its neuroprotective effects. Also, some researchers hypothesized that selective estrogen receptor modulators (SERMs) can enhance the expression of HSP through stimulation of protein transcription factor, a heat-shock factor (HSF) [43, 44]. Apart from that, Tamoxifen is capable of limiting the development of oxidative and nitrosyl stress against increased glutathione level, thus restoring the thiol-disulfate balance in nerve cells [42].

The neuroprotective effect of RAIL is explained by its ability to disrupt IL-1b-dependent expression of iNOS and the subsequent initiation of NO-associated mechanisms of neurodestruction. RAIL also has a positive influence on thiol-disulfate system parameters and HSP_{70}. It is possible that RAIL, through increasing the level of HSP_{70} during the activation of the redox-sensitive transcription factors AP-1 and NF-kB leads to increased expression of the genes encoding glutathione peroxidase, glutathione reductase, and glutathione transferase, along with the genes encoding enzymes stabilizing the intracellular concentration of reduced glutathione (gamma-glutamyl transferase and gamma-glutamylcysteine synthetase) by synthesizing it de novo [16].

CONCLUSIONS

(1) Chronic PH leads to the inhibition of transcription processes and HIF1α, HSP_{70}, and c-fos synthesis in neurons, which points to deficiencies in the mechanisms of endogenous neuroprotection.

(2) Daily administration of drugs (Piracetam 500 mg/kg, i/p, Angiolin 50 mg/kg, i/p, Thiotriazoline 50 mg/kg, i/p, Mexidol 100 mg/kg, i/p, L-arginine 200 mg/kg, i/p, Glutoredoxin 200 μg/kg, i/p, Tamoxifen 0.1 mg/kg, intranasally, RAIL 1 mg/kg, intranasally, Cerebrocurin 150 μL/kg, i/p) to rats after prenatal chronic hypoxia from day 1 to day 30, has various effects on the expression of mRNA of HIF1α, HSP_{70}, and c-fos, as well as on the concentration HSP_{70} in the brain of experimental animals, which points to the ability of the studied drugs to modulate HSP_{70}-mediated mechanisms of endogenous neuroprotection.

(3) Among the studied drugs in the model of chronic PH, the greatest influence on HSP_{70}-dependent mechanisms of endogenous neuroprotection was demonstrated by Cerebrocurin (150 μL/kg) and Angiolin (50 mg/kg) which surpass the other drugs studied in the level of increase of mRNA of HIF1α and HSP_{70}, as well as HSP_{70} protein concentration.

(4) The results provide an experimental basis for further research of the drugs studied, particularly Angiolin and Cerebrocurin, as promising neuroprotective drugs within complex therapy of the CNS damage caused by chronic PH in newborns.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare no conflicts of interest.

Ethical approval. The experimental part of the study was performed with strict compliance with the national "Common Ethical Principles for the Use of Animals in Research" (Ukraine, 2001) agreed with Council Directive 2010/63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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