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The balance of pressor and depressor substances in the neurons of arcuate nucleus of hypothalamus in etiologically different arterial hypertension

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Abstract

It has been proved that arterial hypertension is not associated exclusively with an increase in vascular tone but also with a complex of pathological processes that results in cardiovascular pathology and lesions of the target organs. The central and peripheral contours of blood pressure regulation are well studied. Nevertheless, despite the presence of two regulatory contours the key structure that integrates and controls their function is the hypothalamus, in particular its arcuate nucleus. It is proved that the effectiveness of blood pressure regulation depends on those neuromodulators and neurohormones that are synthesized in the hypothalamus or transported to it. The most important neurohormones in the regulation of arterial blood presssure are brain natriuretic peptide, β-endorphin, neurotensin and angiotensin II. Equally important factor is the functional state of arcuate nucleus neurons, which depends on adequate blood supply, innervation and inter-neuronal relationships. The link between the above described processes is nitrogen monoxide system (NOS) and its universal messenger – nitric oxide NO.

The purpose of the work was to determine the nature of the balance of pressor (angiotensin II and neurotensin) and depressor (brain natriuretic peptide and β-endorphin)

neurohormones in the neurons of the arcuate nucleus of the hypothalamus and the ratio of NOS isoforms with hypertension of different etiology.

Materials and methods. The studies were conducted on 32 mature male Wistar rats and 16 male spontaneously hypertensive rats. To study the content of neuropeptides and NOS enzyme isoforms, an immunohistochemical method was used, followed by digital image processing by Image J. The results that were obtained were calculated using the "Statistica 11.0" program.

Conclusions

1. The same changes of neuropeptides' content in hypothalamic arcuate nucleus are observed in the rats with arterial hypertension which are characterized by a decrease in the brain natriuretic peptide, β-endorphin and angiotensin II content with a significant increase in neurotensin content regardless of arterial hypertension etiology and pathogenesis. The peculiarities of the balance between depressor and pressor substances depend on the etiopathogenetic mechanisms – with the endocrine-salt model of arterial hypertension there is a significant predominance of depressor neuropeptides (in 4.3 times) whereas in essential hypertension this balance is similar to the control.

2. The changes of the NOS isoform ratio in the arcuate nucleus of hypothalamus are unidirectional, both with endocrine-salt model of arterial hypertension and essential hypertension – the content of eNOS decreases while nNOS and iNOS content increases.

Key words: hypothalamus, arcuate nucleus, neuropeptides, nitric oxide, rats, arterial hypertension

To date, pathogenesis of arterial hypertension (AH) is not associated exclusively with an increase in vascular tone. It has been proved that a steady increase in blood pressure (BP) is accompanied by a complex of pathological processes, namely increase of sympathetic nervous system activity, metabolic disorders, hormonal imbalance, insulin resistance and rapid progression of atherosclerosis. Above mentioned processes result in cardiovascular pathology development with the lesions of target organs [1].

It should be noted that the contours of BP regulation are well studied. According to classical concepts, they can be divided into central and peripheral. The central mechanisms include the cerebral cortex, the limbic system, the hypothalamic nuclei, the supra-segmental and segmental centers of the autonomic nervous system. Peripheral contour is presented by renin angiotensin aldosterone system, the renal mechanism, in particular, prostaglandins, and

a family of natriuretic peptides of the heart, which provide adequate regulation of arterial pressure. The key structure that integrates and controls the function of these contours is the hypothalamus and its nuclei. The effectiveness of BP regulation and maintenance depends on the concordance and coordination of hypothalamic nuclei action with higher and peripheral regulatory systems [2, 3, 4].

Most of the researchers consider the arcuate nucleus (ARC) of the hypothalamus to be an important structure that coordinates and modulates the work of the hypothalamic centers. This function is realized due to its topography, variety of receptors on its neurons (NPY1, leptin, insulin, corticosteroids, estradiol) and possibility of synthesizing a large number of neuropeptides (dopamine, dinorphine, β-endorphine, neuropeptide Y, neurotensin, melanostimulating hormone, etc.) [5, 6, 7]. A number of researchers have found that the effectiveness of BP regulation mostly depends on those neuromodulators and neurohormones that are synthesized in the hypothalamus or transported to it. It has been proved that the most significant neurohormones in the regulation of BP are brain natriuretic peptide (BNP), β-endorphin, neurotensin and angiotensin II [8, 9].

Equally important factor that plays a significant role in regulating the activity of neurons is their functional state, which depends on adequate blood supply, innervation and interneuronal relations, which are provided by adequate microcirculation and neuroglia.

The link between the above described processes is the system of nitrogen monoxide and its universal messenger NO. Currently three isoforms of the nitric oxide synthase (NOS) are known: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) [10]. It has been proved that the synthesis of NO and its effect, both quantitatively and topographically, depends on the amount and type of the enzyme that generates it. With the predominant generation by eNOS improvement of blood supply is expected, nNOS - will stimulate neurotrophic processes, while iNOS will trigger local inflammation and cell death [11]. Therefore, there is an assumption that the functional activity of the ARC will directly depend on the presence, ratio and quantitative characteristics of NOS isoforms in its neurons. Whereas the balance of pressor and depressor neurohormones will characterize the state and activity of the central – hypothalamic – contour of BP regulation [12].

The purpose of the work was to determine the nature of the balance of pressor (angiotensin II and neurotensin) and depressor (BNP and β-endorphin) neurohormones and the ratio of NOS isoforms in the neurons of the ARC of the hypothalamus with hypertension of different etiology.

Materials and methods. The studies were carried out on 32 mature male Wistar rats and 16 adult male spontaneously hypertensive rats (SHR). Animals that were used in experiments were obtained from the kennel of the Veterinary Medicine Association of Biomodelservice (Kyiv, Ukraine). All studies were conducted in the autumn-winter period in the vivarium of the Zaporizhzhia State Medical University. The animals were at air temperature 20-25^0 C, with a daylight hours 7-00 – 19-00, with free access to food and water and a standard diet.

The experimental part of the study was carried out exactly in accordance with the National "General Ethical Principles of Animal Experimentation" (Ukraine, 2001), in agreement with the Directive 2010/63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Animals were divided into three groups, with two subgroups in each:

Group 1a was a control group; it consisted of 10 mature Wistar rats.

Group 1b was an additional control group of 6 adult male Wistar rats. Fivefold pressure measurement showed stable indices of BP. P_{syst}/P_{diast} was 110/75 ± 5 mmHg, which made it possible to attribute them to a group of normotensive animals.

Group 2a consisted of 10 mature rats of the SHR line which are considered as a model of essential arterial hypertension (EAH).

Group 2b was an additional group and included 6 mature SHR. BP measurement showed steady high figures during all studies P_{syst}/P_{diast} was 165/110 ± 10 mm. Hg. that confirmed the development of spontaneous hypertension.

Rats of 3a and 3b groups were modeled with secondary AH for 30 days by forming a daily cortisosteroid imbalance and salt load - endocrine-salt arterial hypertension (ESAH). At the beginning of the modeling BP in rats was 110/75 ± 5 mmHg, steady increase of BP was reached for 14th day: 160/100 ± 10 mmHg and it remained constantly high until the end of the simulation.

Group 3a was formed from 10 mature Wistar rats to study the ratio of NOS isoforms.

Group 3b was additional (6 mature Wistar rats), which was formed to study the content of neuropeptides in the ARC of the hypothalamus.

All the rats of additional groups were injected with 120 μg colchicine (SIGMA Chemical, USA) diluted in 20 μl of 0.9% NaCl solution. Colchicine was injected into the lateral brain ventricle on the 28th day of research 48 hours before removal from the experiment [13].

Animals on the 30th day of the simulation were deprived of food from 18-00 and the next day from 10-00 they were removed from the experiment by one-stage decapitation under anesthesia (sodium thiopental 40 mg/kg intraperitoneally).

The animals' brain was placed into the Bouin's solution for 20 hours (t = 23-25^{0}C) immediately after the decapitation. After 2 hours of washing out of picric acid in running cold water, the brain was dehydrated in ascending concentrations of ethanol (50%-100%) at the t=+37^{0}C and then put at liquid paraplast (t= +56^{0}C) for 1 hour, and finally it was placed in paraplast blocks.

The frontal serial sections (14 μm) of the brain were made on the rotary microtome MICROMHR-360 (Microm, Germany). The histological sections were deparaffinized and rehydrated in descending solutions of ethyl alcohol and washed three times for 10 minutes in 0.1 M phosphate buffer (pH 7.4) before the application of the primary antibodies.

The study of NOS isoform expression of was performed in rats of 1a, 2a and 3a groups. Serial histological sections of the hypothalamus were processed by a standard immunohistochemical study [14].

An immunohistochemical study of neuropeptides (BNP, β-endorphine, angiotensin II, and neurotensin) was performed in 1b, 2b, and 3b groups using the procedure described before [15, 16].

The stereotactic atlas of the rat brain was used for the identification of the ARC neurons and further detection of the neuropeptide complex (BNP, β-endorphine, angiotensin II, and neurotensin) and the isoform profile of the NOS enzyme in the studied structure [17].

The histological sections were examined with ultraviolet microscopy using a high-power filter 38 NE (Zeiss, Germany) on a microscope of AxioImager-M2 (Zeiss, Germany) with a help of highly sensitive video camera AxioCam-5HRm (Zeiss, Germany). The image analysis was carried out in semi-automatic mode using the open source Image J (National Institutes of Health, USA) software.

Zones with statistically significant fluorescence were identified while analyzing the images in the interactive mode using the "mask". This method allowed us to determine the content of the immunoreactive material (IRM) in the relative units (Uif) to the studied neuropeptide, or NOS isoform in the corresponding region of the nucleus, and to distinguish the neurons of ARC from other hypothalamic nuclei and blood vessels.

The number of studied fields of view with masks in the sections of ARC was at least 170-200 in each series of the experiment.

Using the obtained digital data "depressor-pressor coefficient" (K_{DP}) and the "isoform ratio of the enzyme NOS" were calculated.

The "depressor-pressor coefficient" (K_{DP}) was calculated according to the formula: the sum of BNP and β-edorphine content was divided by the sum of angiotensin II and neurotensin content. The isoform ratio was calculated using the proportion in which the content of the eNOS was taken as a unit.

The calculation of the obtained results was carried out using the program "Statistica 11.0" (Stat Soft Ins, USA). To compare the indices of groups with normal data distribution, the Student t-criterion was used. The data is presented as a mean arithmetic and standard error of representativeness of the mean value. The differences were considered to be statistically reliable at a value of $p<0,05$ [18].

Results of research and discussion

In the course of the study it was found that in the rats with developed AH the same changes of neuropeptides' content in ARC are observed: a decrease in the BNP, β-endorphin and angiotensin II content with a significant increase in the neurotensin content regardless of AH etiology and pathogenesis (Fig. 1).

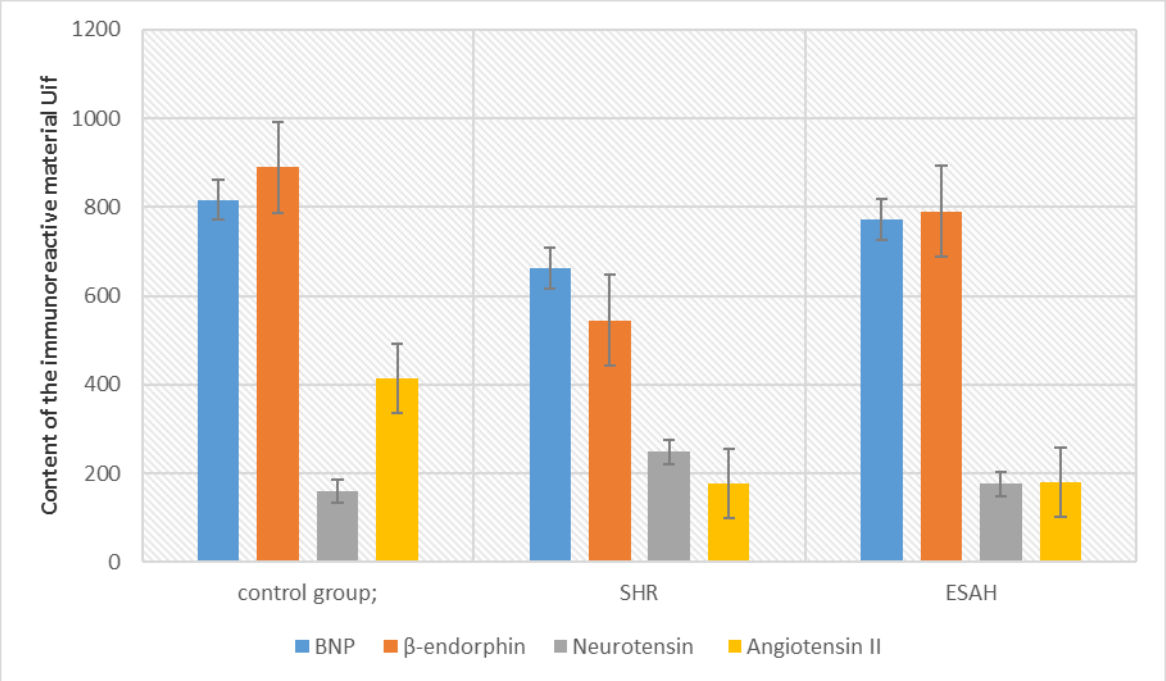

Figure 1 - Quantitative characteristic of neuropeptides content in the ARC of the hypothalamus.

However, when calculating the depressor-pressor coefficient, significant differences were found in the groups of rats with different models of hypertension. Thus, in the EAH

model, this parameter (K_{DP} = 2.82) did not differ significantly from the values of control group animals (K_{DP} = 2.97) with normal BP. A significant prevalence of depressor neuropeptides in 4.3 times (K_{DP} = 4.3), which was associated with a higher content of β-endorphin, and a lower neurotensin value in the ESAH model if compared with the corresponding indice of the EAH group (see Figure 1).

An analysis of the NOS isoforms content has shown that the development of AH, regardless of the AH model, leads to the same changes in the isoform profile. The increase of the IRM content both to the nNOS and to the iNOS was noted, at the same time eNOS content decreased (Fig. 2).

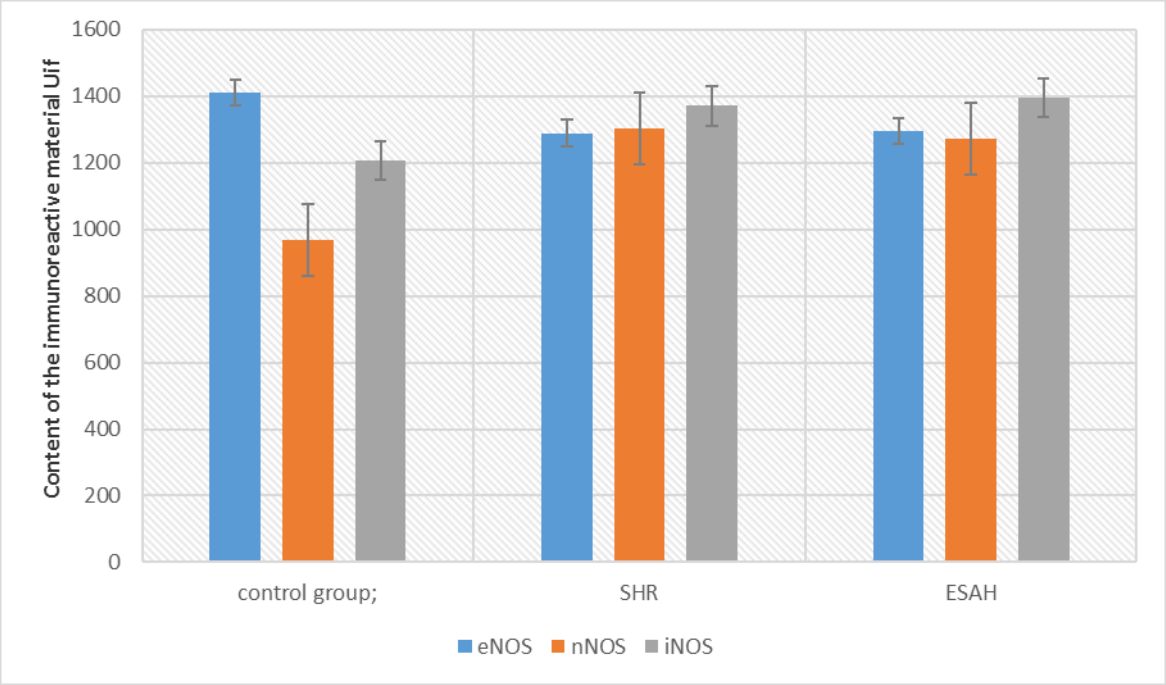

Figure 2 - Quantitative characterization of the content of ARC in the hypothalamus of the endothelial NOS, neuronal NOS and inducible NOS.

Calculation of the NOS enzyme isoforms ratio the demonstrated its features in etiologically different AH. In the control group the ratio of eNOS : nNOS : iNOS was 1:0.7:0.86, in the rats with EAH – 1:1.01:1.06, and the rats with ESAH - 1: 0.98: 1.09. It should be noted that hypertension development resulted in a significant increase in the iNOS content by 13% in EAH and by 15.5% at ESAH. Other important feature of AH development was the increase of nNOS content along with the decrease of the eNOS content (see Figure 2).

Analysis and discussion of results

The results of the research demonstrate that AH development leads to to complex and diverse changes of both regulatory neuropeptides balance and NOS enzyme isoforms' profile

in the structure of the ARC of the hypothalamus. Similar changes in the content of all three NOS isoforms were observed both in EAH and in ESAH: the decrease of the eNOS content in the ARC structure was accompanied by a reliable increase in nNOS and iNOS content. The revealed peculiarities can be explained by the nature of isoforms' intracellular compartmentalization, their role in regulating neurotrophic and protective functions, and changes of isoforms' genes expression with a steady increase in BP [18].

The balance of the constitutive NOS isoforms content in AH was characterized by a decrease in eNOS with the increase of nNOS that can be related to a number of factors. In the experiment of Gerasimova A.S., it was found that inhibition of nNOS activity is accompanied by increase in vasopressin concentration, whereas the reverse effect causes its decrease and, accordingly, decreases blood circulating volume and total peripheral vascular resistance [19]. Thus, the described effect can be considered as an important mechanism of elevated BP correction. The decrease in the eNOS content is associated by the most of researchers both with the decrease of gene expression and the influence of endogenous inhibitors such as asymmetric dimethylarginine, the content of which largely increases in blood plasma in cardiovascular disease [20]. The high vulnerability of eNOS to the inhibiting influence is related to the features of its subcellular localization. It was found that the granules of the enzyme are located predominantly in the cell membrane, the Golgi apparatus, the nucleus and the mitochondria [20]. In contrast, nNOS is represented in the cytoplasm, sarcolemma, and endoplasmic reticulum of the cell [21].

The established fact of the high iNOS content confirm the results of numerous researchers which have proved that NO synthesized by iNOS is involved in inflammation and oxidative stress because hypertension, both in subjects with obesity and with cardiovascular diseases, usually includes an inflammatory process. Moreover, there are reports of an initially abnormal increase of the iNOS expression in AH [21]. However, there are alternative versions of the enhanced expression of iNOS. It is believed that it can play a physiological protective role in BP increase by modulating the production of cyclooxygenase and thrombocytes' regulating eicosanoids [21].

The changes of the depressor-pressor relationships in the ARC of the hypothalamus in AH were unidirectional but with specific features for EAH and ESAH. It was revealed that depressor peptides (BNP and β-endorphin) and angiotensin II content decreased while neurotesin content increased (see Fig. 1). Taking into account the established fact of eNOS content decrease in the ARC, it is possible to suppose a decrease in the transport of these neuropeptides to ARC structure from other parts of the brain. While the increase in

neurotensin content that is synthesized in the neurons of ARC can be related on the one side to its genetically determined excessive abnormal synthesis in the SHR line rats and on the other side it can be the component of BP central regulation in the rats with ESAH. Our supposition is related to the established fact of dose-dependent effect of neurotensin with intraventricular administration. In Samers' work, it was shown that high doses of neurotensin (more than 2 mg) contributed to BP increase, while as sub-threshold doses, on the contrary, reduced BP [22, 23]. Genetically determined AH in the rats of the SHR line is probably associated with an abnormally high neurotensin level, whereas in AH, which was formed as a result of endocrine pathology, slight increase of neurotensin to 10% caused the reverse effect – a decrease in BP, working as a compensation mechanism.

Conclusions

1. The same of changes of neuropeptides' content in ARC are observed in the rats with developed AH which are characterized by a decrease in the BNP, β-endorphin and angiotensin II content with a significant increase in the neurotensin content regardless of AH etiology and pathogenesis. The peculiarities of the balance between pressor and depressor neuropeptides depend on the etiopathogenetic mechanisms – with ESAH there is a significant predominance of depressor neuropeptides content (in 4.3 times), whereas in the EAH this balance is similar to the control.

2. The changes of the NOS isoforms' profile in the ARC of the hypothalamus are unidirectional, both with ESAH and EAH – the content of eNOS decreases while nNOS and iNOS content increases.

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