The pattern of the NOS isoforms expression in arcuate nucleus of hypothalamus in experimental hypertension

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In is known, that one of the main regulators of the blood pressure (BP) is hypothalamus. The efficiency of the BP regulation in a changeable environment is dependable on its coherence and coordination. This intrahypothalamic coordinator is an arcuate nucleus. One of the main conditions of the proper neuronal function is combination of their functional stability and plasticity, which is strictly dependable on adequate blood supply, metabolic and trophic processes. One of the key regulators of the above mentioned features is nitric oxide (NO).

The purpose was to establish the pathogenetic link of NO synthases isoforms imbalance in Arc of hypothalamus with formation of hypertension in etiologically different models of essential hypertension (SHR) and endocrine-saline model (ESM).

Materials and methods. Study was conducted on 48 mature male rats with weight of 250–270 gram and age of 13–14 months, which were allocated into 3 experimental groups of 16 animals: the 1st group was control with Wistar rats; the 2nd group comprised of Wistar rats with ESM and the 3rd was made of SHR.

Results. Formation of the hypertension in ESM and SHR rats led to similar changes of the pattern of NOS isoforms expression. We found the significant increase of the IRM content to nNOS and iNOS, but decrease to eNOS. In the same time, the constitutive isoforms concentration (nNOS and eNOS) was significantly lower compared with control group, and iNOS concentration in both groups with hypertension was higher than in control group (6 % and 9 %, p<0.05, respectively).

Conclusions: In rats with normal BP in Arc, the most expressed NOS isoform was the endothelial one. We found the typical changes in the pattern of the NOS isoforms allocation. We also found the increase of the content of all isoforms with the decrease of the concentration of constitutional ones (nNOS and eNOS), but the increase of the iNOS concentration.
It is known, that hypothalamus is one of the main central regulators of the blood pressure (BP) [1]. A great number of its nuclei are involved in this function: ventromedial, paraventricular, supraoptic, periventricular, and arcuate (Arc) nucleus [2]. The BP regulation in changing conditions is dependable from coordination and consistency of these nuclei. The intrahypothalamic coordinator is arcuate nucleus [3]. Due to the topographical features and the abundance of the projections to other nuclei, this structure is considered as integrative and commutative center, which maintains interaction of both intra- and extrahypothalamic structures, which regulate autonomic function. Peruzzo called Arc the “window to hypothalamus” [4]. The important precondition of the effective neuronal activity is the combination of functional stability and plasticity provided by the adequate blood supply, metabolic and trophic processes [5].

It is proved for now, that one of the key regulators of the processes stated above in central nervous system (CNS) is nitric oxide (NO) [2,5,6]. There are data about the NO involvement into the sympathetic regulation, neurotransmission, vasodilatation and apoptosis induction [2,6,7]. The implementation and direction of the NO effects depends not only on the amount and place of its synthesis, but also on the enzyme isoform mediated this synthesis. There are three isoforms of NO synthases: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). Thus, nNOS modulates a lot of physiological processes including learning, memory formation, and neurotransmission. Additionally, there are data about the involvement of nNOS into central BP regulation [8–10]. eNOS is a powerful vasodilator, also well-known inhibitor of platelets adhesion and aggregation, thus playing an important role in adequate blood supply [11]. In CNS, iNOS produced by activated macrophages and glial cells provides the toxic effect on microorganisms and tumor cells, but in the case of hyperactivity it also induces apoptosis and necrosis [12,13]. Thus, it was proved the NO produced with the iNOS activity causes peroxynitrite-mediated neuronal apoptosis [12–14].

We assume the change of the balance of NOS isoforms in the Arc may lead to violation of central hypothalamic domain of BP regulation and, probably, to the formation and development of hypertension (HT).

Considering stated above we believe that deep and detailed study of the NOS isoforms balance in Arc in HT caused by different factors will contribute to understanding the pathophysiological mechanisms of HT formation and to the search of new molecular targets of antihypertensive therapy.

The purpose of our study was to find out the pathogenetic link between the NOS isoforms imbalance and the formation of hypertension in etiologically different models: the essential in spontaneously hypertensive rats (SHR) and endocrine-saline model (ESM).

Materials and methods

Study was performed on 48 mature male rats with weight of 250–270 gram in age of 13–14 month, which were allocated to 3 experimental groups of 16 animals: the 1st control group comprised of Wistar rats; the 2nd consisted of Wistar rats with endocrine-saline model of hypertension; the 3rd group – of SHR. Experiment was performed according to the national “General ethical principles of the animal experiments” (Ukraine, 2001), concerted with Council Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

For modelling of ESM the Wistar rats were treated with prednisolone intramuscularly at 7 o’clock in dose of 2 mg/kg and at 20 o’clock in dose of 4 mg/kg during 30 days combined with the force watering with 5 ml of saline (2.3 %). From the beginning of the experiment and each 7 days we performed the BP assessment using the non-invasive system BP-2000 (Visitech Systems, USA). Rats of the 1st group showed the systolic pressure of 110±5 mm Hg. The 2nd group showed 110±5 mm Hg at the beginning of experiment, 145±5 mm Hg on the 7th day and 165±5 since

21 days of the modelling. The systolic pressure in the 3rd
group was 165 ± 5 mm Hg.

The object of the study was hypothalamus of experi-
mental animals. We performed the immunofluorescence
assessment of the NOS isoforms expression in serial
paraffin-embedded slices, which were incubated with
rabbit IgG (Santa Cruz Biotechnology, USA) to nNOS,
iNOS and eNOS, respectively in dilution of 1:200. Then
slices were incubated in plastic chambers during 24
hours (T = +4 °C), then we applied mouse anti-rabbit IgG
conjugated with FITC (Santa Cruz Biotechnology, USA) in
dilution of 1:200 and incubated them 2 tweis (T = +37 °C)
during 45 minutes, and then covered them in glycerol/FBS
(9:1). The specificity control was performed as described,
but with previous application of blocking peptide to the
corresponding antibodies in dilution of 1:50.

The assessment of immunofluorescence was per-
formed in ultraviolet spectrum of emission 390 nm using
38HE light filter with high emission (Carl Zeiss) in Axio-um
microscope (Carl Zeiss). The images obtained using 8-bit
camera AxioCam-ERC5s (Carl Zeiss) were analyzed with
ImageJ in interactive way. We marked the regions of interest
in the field of view of 15 000 µm², where we calculated the
contain of the immunoreactive material (IRM) in conditional
units of immunofluorescence (Uif) as well as IRM concentra-
tion in 1 µm² (Uif/µm²). We assessed no less than 200 areas
of view in each series. The obtained data was analyzed in
EXCEL-7.0 (Microsoft Corp). We counted the mean and its
standard error. With the aim to assess the differences in the
study results we used Student’s t-criterion with evaluation
of significant differences according to Student’s tables.
Statistically significant difference was considered at p < 0.05.

Results and discussion

During the immunofluorescence analysis of hypothalamus
of rats with topographic identification of anterior hypo-
thalamic field [15] we found that IRM to NOS isoforms were
allocated predominantly in dorsolateral of Arc diffusely in
neuronal cytoplasm and axons. High intensity of fluores-
cence was found in granules of IRM in neurons of control
group and ESM on the periphery of cytoplasm and in
axons (Fig. 1–3), whereas in SHR we found no granules
to nNOS (Fig. 1B).

Fig. 1. Pattern of the nNOS expression in neurons of Arc in Wistar (A), SHR (B) and ESM (C). Indirect immunofluorescence, 400×. Arrows show IRM to nNOS.

Fig. 2. Pattern of the eNOS expression in neurons of Arc in Wistar (A), SHR (B) and ESM (C). Indirect immunofluorescence, 400×. Arrows show IRM to nNOS.

Fig. 3. Pattern of the iNOS expression in neurons of Arc in Wistar (A), SHR (B) and ESM (C). Indirect immunofluorescence, 400×. Arrows show IRM to nNOS.
After the statistical analysis of the expression of NOS isoforms in control group we found that the highest figures of IRM contain and concentration were for eNOS. The lowest IRM contain was for nNOS compared with eNOS and iNOS (45.8% and 24.7% respectively, p<0.05). The lowest IRM concentration was found for iNOS (Table 1).

Hypertension in rats of SHR and ESM groups leads to the same type of changes in the expression pattern of NOS isoforms. We observed significant increase of IRM concentration and contain both to nNOS and iNOS, but decrease to eNOS (Table 1). The concentration of constitutive isoforms of nNOS and eNOS were significantly lower than in the control group, and iNOS concentration in both hypertensive groups were higher than in Wistar (9% for SHR and 6% for ESM, p<0.05, Table 1).

Comparison of the NOS isoforms expression in SHR and ESM groups showed the absence of significant of iNOS contain of all isoforms, whereas the IRM concentration to nNOS was significantly lower (4 %, p<0.05) in ESM compared with SHR (Table 1).

The predominance of the eNOS expression in Arc seems to be logic and expected. It is well known, that the main role of its isoform is local production of vasodilative nitric oxide [5], which is necessary for the regulation of cerebral blood supply and neurotrophic processes [5,6].

The fact of the same type changes of the expression of all isoforms in etiologically different hypertension became discussible. Both in essential and endocrine-saline models we found increase of both neuronal and inducible isoforms. However, in majority of clinical and bench studies the another consistent pattern was found: the forms of hypertensive systems are compensatory with aim to provide adequate blood supply and interneuronal interactions [5,6].

Table 1. Indexes of the expression of NOS isoforms in Arc in rats of experimental groups (M±m)

<table>
<thead>
<tr>
<th>Expression figures</th>
<th>Groups</th>
<th>Wistar, n=16</th>
<th>SHR, n=16</th>
<th>ESM, n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>nNOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRM contain, U</td>
<td>967.7±21.3</td>
<td>1304.5±16.7</td>
<td>1272.6±18.4&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>concentration, U&lt;sub&gt;con&lt;/sub&gt;</td>
<td>78.6±1.8</td>
<td>73.3±1.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>70.2±0.9&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRM contain, U</td>
<td>1207.5±25.1</td>
<td>1371.3±26.1</td>
<td>1395.4±15.3&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>concentration, U&lt;sub&gt;con&lt;/sub&gt;</td>
<td>74.5±1.7</td>
<td>81.6±1.7</td>
<td>78.8±0.9&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRM contain, U</td>
<td>1410.1±46.4</td>
<td>1289.7±18.1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1296.7±20.5&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>concentration, U&lt;sub&gt;con&lt;/sub&gt;</td>
<td>80.9±2.6</td>
<td>73.3±1.1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>72.9±1.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> significant differences (p<0.05) compared with control group;
<sup>2</sup> significant differences (p<0.05) compared with SHR group.

Conclusions

1. In normotensive rats, the most predominant form of NOS in Arc is endothelial.

2. In etiologically different hypertensions, there are the same type changes in the expression pattern of NOS isoforms to be observed in Arc. The increase of contain of all the isoforms with the decreased concentration to constitutive isoforms but the increase of iNOS is typical.

Perspectives. We plan to study the features of expression of pressor and depressor neuropeptides in Arc and find out the pathogenetical link of their state with hypertension formation.

References


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