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Arterial hypertension as a predictor of morpho- densitometric changes development in rats` solitary-vagal complex

M. V. Danukalo, O. V. Melnikova

Zaporizhzhia State Medical University
Department of Pathological Physiology

Abstract

The components of the dorsal vagal complex: the nucleus of the solitary tract (NST) and the dorsal motor nucleus (DMN) are key structures in BP regulation. The maintenance of BP normal level depends on functional state of these structures. it is possible to estimate the neuron morpho-functional state not only with direct electrophysiological methods but also analyzing the nucleus size and nucleic acid (NA) concentration in it. Therefore, **the aim** of our work was to determine the features of morpho-densitometric indices in the nuclei of the NST and DMN neurons in AH of various origins in experimental animals and to give a comparative analysis of these data. **Materials and methods:** the study was carried out on 30 mature male rats: 10 control Wistar rats, 10 Wistar rats with modeled endocrine-salt AH (ESAH) and 10 spontaneously hypertensive rats (SHR) which are well-established model of human essential arterial hypertension (EAH). The histochemical method of staining with gallocyanine-chrome alum by Einarson was used for the evaluation of morpho-densitometric characteristics of the DMN and NTS neurons` nuclei: the nucleus area, the content and concentration of nucleic acids in the nucleus.

Conclusions: The following conclusions can be made as a research result: 1. Morpho-densitometric changes occur in NTS and DMN in arterial hypertension, regardless of its pathogenesis.

2. The most distinct changes of the studied indices of neurons` nuclei were observed in DMN of the rats with EAH. Although only the neurons` nuclei area significantly changed in the NTS. The distribution of neurons` nuclei by their area in DMN and NTS in SHR rats showed a downward trend.

3. Significant decrease in all morpho-densitometric characteristics of the neurons` nuclei is observed in the DMN in the rats with an ESAH. The distribution of neurons` nuclei by their area showed a tendency to decrease. In the nuclei of NST neurons, only the content and concentration of NA were reliably low in comparison with control animals. The distribution curve of neurons by the area of their nuclei in ESAH rats is characterized by wider variability of size than in the control group.

Key words: nucleus of the solitary tract, dorsal motor nucleus, arterial hypertension, rats.

Introduction. Understanding of arterial hypertension (AH) pathogenesis is centered around the concept of the regulatory systems function alterations that is accompanied with the imbalance between the pressor and depressor factors of blood pressure (BP) regulation. The centers of the autonomic nervous system (ANS) which play the key role in BP homeostasis are of great scientific interest. The nucleus of the solitary tract (NST) and the dorsal motor nucleus (DMN) which are the components of the dorsal vagal complex are the leading structures in this process [1]. Particular attention is paid to NST in numerous studies dedicated to research of the functional state of brainstem structures in the condition of AH [2-4]. It is explained by the fact that NST is able to modulate the activity of both the sympathetic and parasympathetic sections of the ANS, depending on the incoming signal from the baro- or chemoreceptors in the blood vessels. NST influences indirectly on the ANS sympathetic part tone through the caudal and rostral ventrolateral regions. Also NTS acts on the intermediolateral group of spinal cord cells, which is the segmental center of the sympathetic nervous system through them. The presence of neuronal projections from NTS to DMN determines the possibility of the first to modulate the tone of the parasympathetic nervous system [4]. Thus, it can be argued that NST is an important relay link in the regulation of BP, and its functional state will determine the maintenance of BP normal level.

All of the foresaid contributed to the development of interest among researchers to NST as a primary link in the BP regulation.

It should be noted that most researchers consider NST as the main regulator of the sympathetic nervous system, depriving attention of DMN which is an important link in the efferent vagal influence on the heart. Today, most of DMN studies relate to gastrointestinal pathology [5], and only few studies focus on DMN in arterial hypertension. Whereas, DMN has multiple connections with the overlying centers involved in the regulation of the cardiovascular system: hypothalamic and limbic nuclei as was shown by other researchers [6]. Also, it was found with the methods of anterograde tracing and confocal microscopy that the axons of DMN neurons are projected onto each heart ganglion in a number of experiments in the 2000s. Electrostimulation of DMN C-fibers selectively causes bradycardia with a decrease in myocardial contractility according to electrophysiological studies. In addition, it has been found that DMN neurons are baro-sensitive [7]. In earlier studies, a number of scientists showed that the administration of angiotensin II in DMN resulted in a decrease in blood pressure, which further demonstrates the significant contribution of DMN in maintaining BP homeostasis [8]. Among other things, it should be noted that studies of the functional state of NST and DMN as they are two closely related key regulatory structures of the ANS in hypertension are very rare. It is logical to assume that the morpho-functional state of DMN neurons, as the main efferent link of the vagus nerve, will affect the adequate regulation of BP. Therefore, **the aim** of our work was to establish the features of morpho-densitometric indices of the nuclei of the NST and DMN neurons in AH of various origins in experimental animals and to give a comparative analysis of these data.

Materials and methods: The study was carried out on 30 male rats. 1st group – 10 intact Wistar rats, 2nd group – 10 Wistar rats with ESAH. In third group was 10 spontaneously hypertensive rats (SHR) with genetically determined AH. To date, the SHR line is the well-established model of human essential arterial hypertension (EAH) [9].

ESAH was induced by intraperitoneal injection of prednisolone (twice a day for 30 days: at 7 am – 2 mg/kg, at 20 pm – 4 mg/kg) with 5 ml of 2,3% NaCl solution forced intake) [10]. This model is similar to the secondary human AH caused by endocrine disorders. To confirm the development of a persistent increase in BP in all groups of animals, BP was repeatedly measured on a BP-2000 apparatus (Visitech Systems, USA) [11]. The average BP level in the control was 110/75 ± 5 mm. Hg., in ESAH – 155/90 ± 5 mm. Hg., in EAH – 165/100 ± 5 mm. Hg. At the end of experiment the animals were immediately sacrificed via decapitation after being anesthetized with aethaminalum-natrium (40 mg/kg body weight intraperitoneally). Medulla oblongata was the object of study in all experimental animals. 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.

The topographical identification of the NTS and DMN neurons was performed using the rat brain stereotactic atlas [12]. To study morpho-densitometric characteristics of NTS and DMN neurons` nuclei the 5 μm sections were stained in a gallocyanine-chrome alum by Einarson [13] and mounted in Eukitt (O.Kindler GmbH & Co, Freiburg, Germany).

The study of sections stained for nucleic acids (NA) was performed in a visible spectrum on the AXIOSKOP microscope (Carl Zeiss, Germany). The images taken with the COHU 4722 (COHU Inc., USA) sensitive camera were recorded as a computer file. The image was digitized according to densitometric scale with 256 shades of gray. The interactive mode was used to identify nucleus-containing neurons and the zone of "interest" wheres morphometric and densitometric parameters of the nucleus were calculated automatically – its area (μm^2) and optical density of NA (Uif), which characterizes the NA content in the cell nucleus section, concentration of NA in the nucleus (Uif /μm^2), which indirectly reflects the neuron functional activity. All these parameters were measured for each neuron with the nucleus. At least 100 cells from each series were subjected to analysis. Microphotographs of the neurons were processed using the Image J software [14].

Statistical analysis. All experimental data obtained were processed using STATISTIKA (license №JPZ804I382130ARCN10-J), EXCEL 7.0 (Microsoft Corp., USA). The arithmetic mean value (M), its variance and the standard error of the mean (m) were calculated for all parameters. Parametric statistical methods (Student t-test for the sample with normal distribution) and non-parametric (Mann-Whitney test for the sample with non-normal distribution) were used to define the reliability of differences between the experimental and control groups of rats. The differences were considered to be statistically reliable at a value of $p<0,05$ [15].

Results: The study have found that AH is accompanied by pronounced changes in the morpho-densitometric indices of the DMN neurons nuclei in experimental animals (Table 1).

Table 1. Morphometric and densitometric indices of the DMN neurons nuclei in rats of the experimental groups (M ± m)

Characteristic under study	Control (n=10)	EAH (n=10)	ESAH (n=10)
Nuclear area (μm^2)	95,03±1,69	78,58±2,48*	90,07±1,64*#
NA content (U_{if})	35,70±1,21	44,97±2,25*	30,20±0,7*#
NA concentration (U_{if} /μm^2)	0,38±0,01	0,56±0,02*	0,33± 0,05*#

Notes: 1) (*) indicates a significant difference in the parameters (p <0,05) of the experimental group rats relative to the control; 2) (#) indicates a reliable difference in the parameters (p <0,05) between the groups of the rats with different models of experimental AH.

In the rats both with EAH and ESAH a significant decrease in the area of the DMN neurons` nuclei was observed in comparison with the control. Thus, in EAH rats this index decreased by 17,31%, and in the group with ESAH – by 5,21% in comparison to control animals. Whereas, the area of neuron's nuclei in the ESAH rats was significantly higher by 14,62% than in the EAH group (see Table 1).

Densitometric parameters (NA content and concentration) of the DMN neurons` nuclei had their own peculiarities. It was found that in EAH animals NA content in the neurons` nuclei was significantly higher by 25,96%, compared with the control group, while in rats with ESAH this index decreased by 15,4%. Differences in the NA content in neurons` nuclei were also significant between groups with different models of AH. Thus, in rats with ESAH this parameter was lower by 32,84% compared with EAH group. Similar changes were observed in the NA concentration in the nuclei of neurons of DMN. In EAH animals, the NA concentration was higher by 47,36%, and in ESAH ones it was lower by 13,15% compared to the control parameters. The difference in the NA concentration between groups of animals with AH was significant – 58,92% in the ESAH group of rats compared with EAH rats (see Table 1).

Less expressed changes in the studied indices of the NTS neurons` nuclei in the experimental groups were established (Table 2).

Thus, it was found that in EAH animals the area of the NTS neurons` nucleus was significantly lower by 8,28% compared with the control group. There were no reliable changes in this parameter in rats with ESAH and the control ones. It should be noted that the difference in the studied parameter between AH groups was significant. Moreover, in ESAH rats the neurons` nucleus area was 7,95% higher compared to same index in EAH animals. The NA content in the nuclei of NST neurons in ESAH animals was significantly lower both in relation to the normotensive animals and in relation to the EAH group. In the first case, the decrease was 17,74%, in the second – 13,64%. A similar pattern was observed in the NA concentration index in the NTS neurons` nuclei. In animals with ESAH it was decreased by 15,90% in relation to the control group, and in relation to the EAH group - by 21,27% (see Table 2).

Table 2. Morphometric and densitometric indices of the NTS neurons` nuclei in rats of the experimental groups (M ± m)

Characteristic under study	Control (n=10)	EAH (n=10)	ESAH (n=10)
Nuclear area (μm^2)	69,38±1,08	62,52±1,12*	68,69±1,18$^{\#}$
NA content (U_{if})	30,71±0,86	29,25±1,07	25,26±0,46*$^{\#}$
NA concentration (U_{if} /μm^2)	0,44±0,01	0,46±0,01	0,37±0,07*$^{\#}$

Notes: 1) (*) indicates a significant difference in the parameters (p <0,05) of the experimental group rats relative to the control; 2) (#) indicates a reliable difference in the parameters (p <0,05) between rats of the experimental groups with different models of AH.

The analysis of the DMN and NTS neurons` nuclei area distribution by the frequency of occurrence in the studied samples was made for comprehensive understanding of obtained data. The results are presented in graphs (Figure 1 and 2).

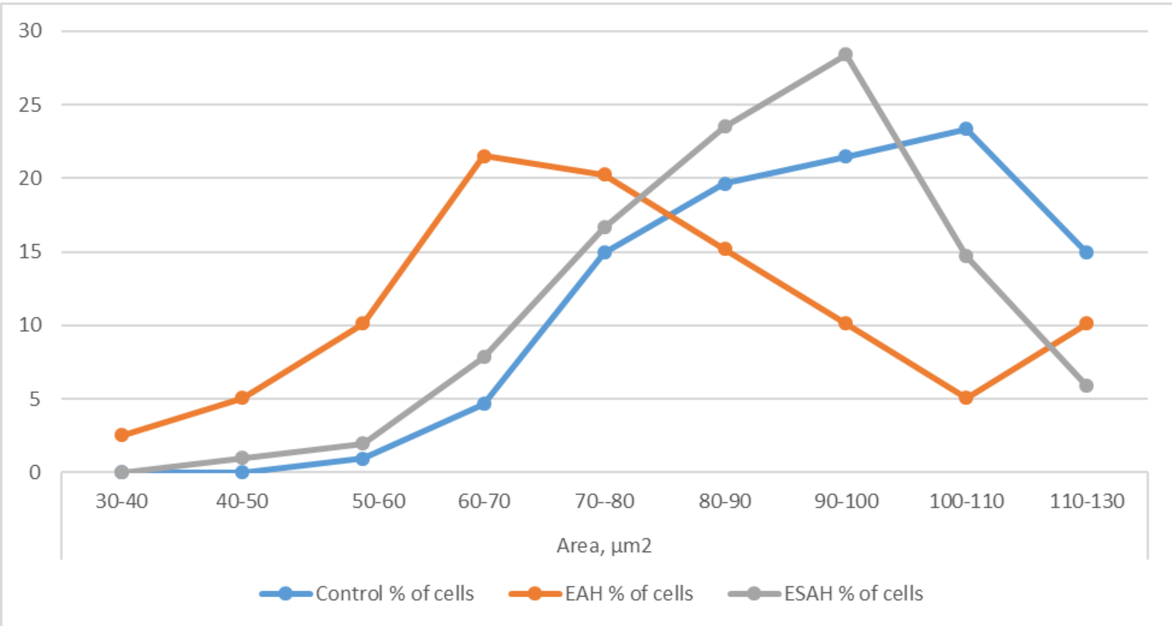

Figure - 1 Distribution of the DMN neurons` nuclei area in the studied groups

As one can see from Figure 1, in the rats of the control group, the maximum number of the neurons had nuclei area 100-110 μm^2. Moreover, neurons with area index more than 130 μm^2 and less than 60 μm^2 were almost never met in control group. At the same time, the curves which shows the distribution of DMN neurons` nuclei by their area in the groups with experimental AH are shifted to the left – towards smaller sizes. Thus, neurons with very small nuclei area (30–40 μm^2) were observed in the DMN of the rats with EAH, that is not typical for control animals and ESAH rats (see Fig. 1).

An analysis of the NTS neurons` nuclei distribution by their area in the animals of the control group showed that the NTS structure is represented by the neurons with a wide range in the area indices of their nuclei (from 40 to 110 μm^2). The maximum number of neurons had a nuclear area in the range of 60-70 μm^2. (see Fig. 2). At the same time in EAH rats the distribution curve shifts significantly to the left side, that characterizes the predominance of neurons with very small nuclei (40-50 μm^2) in the NTS structure; neurons with nuclei larger than 90 μm^2 were completely absent observed in EAH rats, that is not typical for the control and ESAH group. The distribution curve of neurons` nuclei area in ESAH rats is characterized by a wider variability in the size of neurons` nuclei than in the control group, although the largest number of neurons had a nuclear area 60-70 μm^2 as it was in the control group (see Fig. 2).

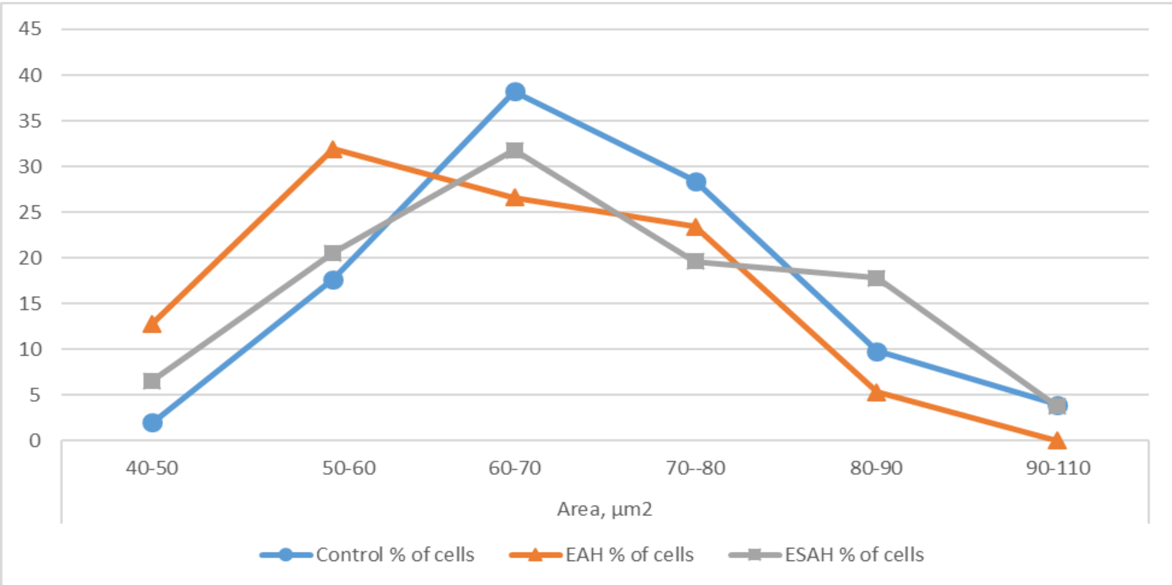

Figure - 2 Distribution of the NTS neurons` nuclei area in the studied groups

Discussion. High-precision morphometric and densitometric parameters were obtained with a semiautomatic method of DMN and NTS neurons microphotographs processing. It gave the possibility to evaluate their histochemical characteristics in a quantitative reaction on NA with gallocyanine-chromium alum, that characterizes neurons synthetic function indirectly, without using electrophysiological methods.

Morphometric studies were widely popular among the scientists at 1980-th when it was reliably established that the size of the nucleus in non-replicating cells which include brain neurons is closely related to NA content and the level of cellular activity [16].

Our study showed that the morphometric and densitometric indices of the DMN neurons` nuclei in experimental rats with different models of AH evidence a decrease of its functional activity. Thus, in EAH animals a significant decrease of nucleus area along with the increase of NA content and, accordingly, an increase in their concentration, was regarded as a transition of the neuron to functionally inactive state. It can be explained probably by the transition of the genetic material to a condensed functionally inactive state (heterochromatin). On the other hand, the number of researchers have shown that there is a decrease in the number of cardiac-projected DMN neurons and remodeling of the remaining projections in the rats of SHR line older than 12 weeks. Possibly, the established changes in the karyometric and densitometric indices of DMN neurons` nuclei indicate the ongoing process of neuroapoptosis in this structure in EAH rats [17]. In contrast, ESAH animals showed a simultaneous significant decrease in all morpho-densitometric characteristics of the neurons compared with the control. This fact can be accounted for a decrease in the functional activity of DMN that probably occurs under the influence of certain pathogenetic factors of the ESAH development (increase of blood circulating volume, medicinal hypercorticism, hypernatremia due to the prednisolone mineralocorticoid activity) [18]. In addition, water-electrolyte and endocrine disorders may affect other brain structures which are functionally connected the with the DMN (hypothalamic PVN, area postrema)[19]. The revealed decrease of DMN morpho-functional state in the rats with ESAH can result from the inhibitory projections activation of the above named structures, or it can be an element of DMN long-term AH compensation derangement.

Summarizing our data for DMN in two different AH models, we can conclude that a decrease of the efferent vagal center functional activity is observed in both of them.

The results of a morpho-densitometric study of NTS neurons` nuclei showed that it is difficult to interpret them definitely in the animals with AH due to the fact that this nucleus is heterogeneous in relation to the regulation of BP. Today NTS includes about 10 subnuclei, among them commissural (c-NTS) and intermediate (i-NTS) zones are considered to be important for BP regulation. Their role in BP regulation is multidirectional. It was shown that c-NTS is tonic active in SHR, and its destruction leads to a disastrous decrease in BP. At the same time, the destruction or inhibition of i-NTS leads to chronic prolonged hypertension [20, 21]. Due to the fact that it was not possible to divide NTS into these areas in our study the averaged data are obtained.

In general, the obtained parameters of the karyo- and densitometric characteristics of NST neurons in hypertensive rats evidence an alteration in their morphofunctional state. But it is extremely difficult to characterize the direction of these changes using the morphodensitometric research method only.

Conclusions: The following conclusions can be made as a research result:

1. Morpho-densitometric changes occur in NTS and DMN in arterial hypertension, regardless of its pathogenesis.

2. The most distinct changes of the studied indices of neurons` nuclei were observed in DMN of the rats with EAH. Although only the neurons` nuclei area significantly changed in the NTS. The distribution of neurons` nuclei by their area in DMN and NTS in SHR rats showed a downward trend.

3. Significant decrease in all morpho-densitometric characteristics of the neurons` nuclei is observed in the DMN in the rats with an ESAH. The distribution of neurons` nuclei by their area showed a tendency to decrease. In the nuclei of NST neurons, only the content and concentration of NA were reliably low in comparison with control animals. The distribution curve of neurons by the area of their nuclei in ESAH rats is characterized by wider variability of size than in the control group.

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