PHYSIOLOGY

Vagal Stimulation Modifies Parameters of Heterochromatin in the Nuclei of Vagosolitary Complex Neurons of Medulla Oblongata in Rats

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New data were obtained on modification of heterochromatin parameters in the nuclei of medulla oblongata neurons in Wistar rats after stimulation of the vagus nerve: decrease in the area of heterochromatin regions and redistribution of chromocenters within the neuronal nuclear system. It was concluded that realization of the viscero-visceral reflex is associated with rearrangement of chromatin in neurons involved in transmission of the corresponding information.

Key Words: *heterochromatin; vagus nerve; medulla oblongata; vagosolitary complex*

Function of visceral organs is regulated via visceral reflexes induced by activation of receptors of different modality. These reflexes are usually studied by neurophysiological methods [1]. On the other hand, investigation of molecular and genetic mechanisms underlying functional activity of the neurons representing the central link of these reflexes is also important.

This problem is little studied. There are data on changes in c-Fos immunoreactivity in hypothalamic and brainstem neurons involved in the respiratory reflexes [5]. An important way of regulation of gene expression is related to modification of the state (condensed/decondensed) of chromatin in the nucleus [4]. Epigenetic modifications of the genome, specifically, heterochromatin regions affecting the basic processes in cell nucleus, gene expression, and spatial structure of the nucleus are now intensively studied. However, the role of heterochromatin structure and its dynamic changes in the function of neurons during various physiological states of the organism remains unclear. Our aim was to study parameters of heterochromatin in the nuclei of neurons from vagosolitary complex of medullar oblongata during stimulation of the central end of cervical portion of the vagus nerve (VN), i.e. under conditions simulating induction of the vago-vagal reflex.

MATERIALS AND METHODS

Acute experiments were carried out on 4-month-old male Wistar rats (n=30) under urethane narcosis (1.5 g/kg). The animals were divided into three groups. Group 1 (n=8) comprised control rats (no manipulations were performed). Group 2 rats (n=7) were sham-operated animals (surgery without nerve stimulation). Croup 3 (n=8) comprised experimental rats, in which left VN was exposed on the neck, cut, and its central end was stimulated. Surgery and electrical stimulation of VN was described elsewhere [1].

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Stimulation of VN in experimental rats (group 3) was performed with rectangular pulses (10-20 V, 0.5 msec, and 10 Hz) in the following regimen: five 2-min stimulation periods were alternated with 2-min resting periods. The rats were decapitated, and the dorsal surface of the medulla oblongata was opened. Tissue was sampled with Folkman curette in the region corresponding to the vagosolitary complex (nucleus tractus solitarius and vagus dorsal motor nucleus) according to the rat brain stereotaxic atlas [7]. Previous studies showed that neurons in these structures change the firing rate during activation of the afferent link of vago-vagal reflex arch [1]. The tissue specimen (2-4 mm^3) was placed into physiological saline. The cells were suspended with Pasteur pipettes and fixed in three portions of methanol-glacial acetic acid mixture (3:1) with intermediary resuspension and centrifugation. The preparations were dried by the routine technique. Heterochromatin was detected in the nuclei of vagosolitary complex neurons by differential C-staining by the method of Sumner using Giemsa dye [8]. In each group of rats, 150 neuronal nuclei were analyzed (standardized by area). Structural parameters of condensed chromatin, the number and area of chromocenters (heterochromatin sites in the chromosomal complex retaining helical structure during the interphase and, therefore, stained more intensively than euchromatin regions), and spatial distribution of heterochromatin within the nuclei were evaluated.

The data were processed statistically using computer-assisted image analysis system [2]. To evaluate the changes in spatial localization of chromocenters, the nuclei were divided into four quadrants. The axes intersection point corresponded to the reference center of the nucleus.

RESULTS

Sham operation (without vagal stimulation) led to a pronounced decrease in the number of chromocenters without changes in heterochromatin area (Fig. 1). Vagal stimulation increased the number of chromocenters and decreased the area of heterochromatin in comparison with sham-operated group (Fig. 1). Probably, the decrease in the number of chromocenters resulted from their aggregation, while opposite change reflected disaggregation of these structures.

In addition, vagal stimulation modified the spatial distribution of heterochromatin (reference coordinate system used for the analysis of distribution of chromocenters in neuronal nuclei was proposed by K. N. Dudkin) [2]. By contrast to control and sham-operated groups, the conventional center of gravity for the chromocenters in experimental rats was shifted towards the left region of quadrant 4 (Fig. 2). The processes of

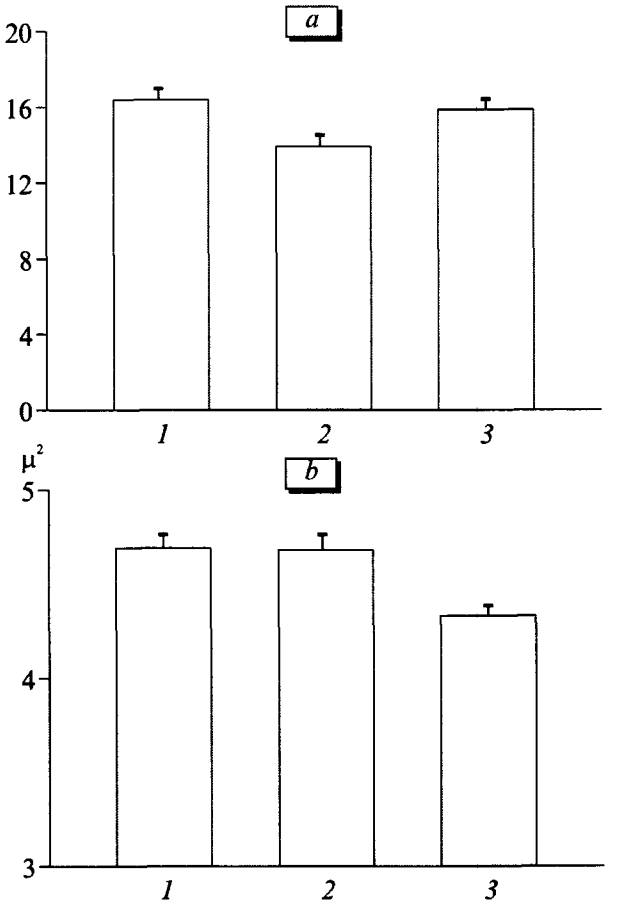

Fig. 1. Effect of vagal stimulation on parameters of heterochromatin in neuronal nuclei of vagosolitary complex. *a*) number of chromocenters, *b*) area of chromocenters. Here and in Fig. 2: *1*) control, *2*) sham-operated, and *3*) experimental groups.

aggregation/disaggregation of chromocenters, changes in the area of heterochromatin and its position within the nucleus reflected alterations in chromocenter organization in neuronal nuclei, which can be related to coordinated activation/inactivation of certain sites in the genome.

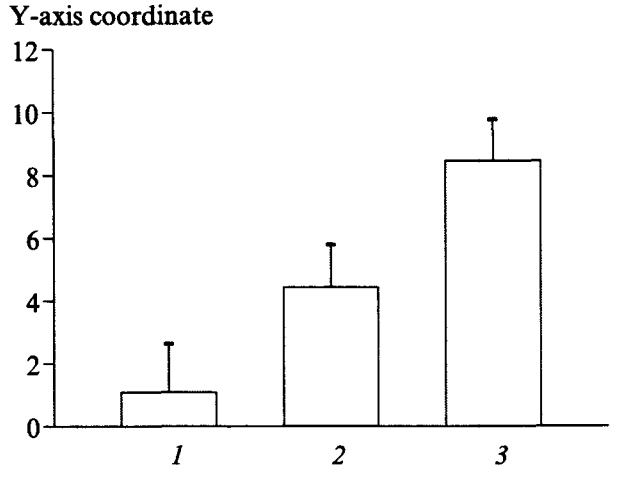

Fig. 2. Effect of vagal stimulation on distribution along Y-axis of the chromocenters in neuronal nuclei of vagosolitary complex.

These data agree with established facts on the redistribution of heterochromatin in the nucleus tractus solitarius after long-term pharmacological activation [6], and also with changes in the area of heterochromatin regions in neurons of the sensorimotor cortex, hippocampus (CA3 field), and midbrain after emotional and painful stress in dependence on genetically determined excitability of the nervous system [3].

Thus, it can be concluded that not only the long-term systemic stimulation of the nervous structures provokes changes in the function of genome, but also short-term afferent stimuli can modify the parameters of heterochromatin in neuronal nuclei of the vagosolitary complex, which probably indicates participation of neuronal genome in realization of visceral reflexes.

REFERENCES

1. V. A. Bagaev, A. D. Nozdrachev, S. S. Panteleev, *Vago-vagal Reflex Arch* [in Russian], St. Petersburg (1997).
2. K. N. Dudkin, S. V. Mironov, N. A. Dyuzhikova, N. G. Lopatina, *Tsitologiya*, **41**, Nos. 3-4, 237-249 (1999).
3. N. A. Dyuzhikova, N. E. Sokolova, A. I. Vaido, *et al.*, *Zh. Vyssh. Nervn. Deyat.*, **51**, No. 4, 511-513 (2001).
4. L. I. Patrushev, *Gene Expression* [in Russian], Moscow (2000).
5. P. Berquin, L. Bodineau, F. Gros, and N. Larnicol, *Brain Res.*, **857**, 30-40 (2000).
6. R. K. Chan, C. A. Peto, P. E. Sawchenko, *J. Comp. Neurol.*, **422**, No. 3, 338-351 (2000).
7. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, New York (1998).
8. A. T. Sumner, *Exp. Cell. Res.*, **75**, 304-306 (1972).