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# RETICULAR NEURONS ACTIVITY OF THE BULBAR RESPIRATORY CENTER OF RATS IN DYNAMICS OF HYPOXIA

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The subject of the study is the involvement of reticular neurons (RN) of the bulbar respiratory center in respiration under conditions of hypobaric hypoxia. Such conditions were created in a laboratory altitude chamber and corresponded to a "lift" up to 8000 *m* altitude. At the beginning of such a "lift", at pressure corresponding to an altitude of 4000–5000 *m*, a decrease in the $P_{O_2}$ in the inspired air to 98–85 *mm* Hg resulted an increasing impulse activity of neurons. Within a phase of extreme hypoxia, at an "altitude" of 7500 to 8000 *m* ($P_{O_2}$= 64–58 *mm* Hg), considerable suppression of the activity of bulbar RN is observed. Impulse activity of RN in different discharge frequency at different stages of hypoxia has demonstrated considerable specificity.

***Keywords*:** hypoxia, bulbar respiratory center, reticular neurons, control of breathing.

**Introduction.** At the contemporary stage of scientific/technical progress, the intensity and significance of the effects of various negative environmental factors on the organism have increased significantly. Among such factors, oxygen insufficiency occupies a special position. Hypoxia frequently plays a central role in the pathogenesis of a number of diseases [1–3]. It is obvious that detection of changing mechanisms in the activity of neurons of the respiratory center (RC) under hypoxic conditions is an urgent and important medical/ biological problem.

Under the above-mentioned conditions, modulation of pulmonary ventilation intensity is one of the most important adaptation reactions. In this respect, we describe the examination results of impulse activity of reticular neurons (RN) of bulbar respiratory center in rats under conditions of increasing hypobaric hypoxia.

**Materials and Methods.** Experiments were carried out on albino male rats (body mass 200–230 *g*) anesthetized with a mixture of chloralose and nembutal (30 and 10 *mg*/*kg* respectively, i.p.). After partial removal of the cerebellum, an *obex* region of the medulla where respiratory neurons are concentrated was exposed. Impulse activity of single neurons was recorded extra cellularly using a routine technique; glass microelectrodes (tip diameter 1.5–2 *μm*, resistance 3–5 *MΩ*) filled with 2.0 *M* NaCl solution were used. Simultaneously the parameters of external respiration were recorded by means of a resistive carbon transducer.

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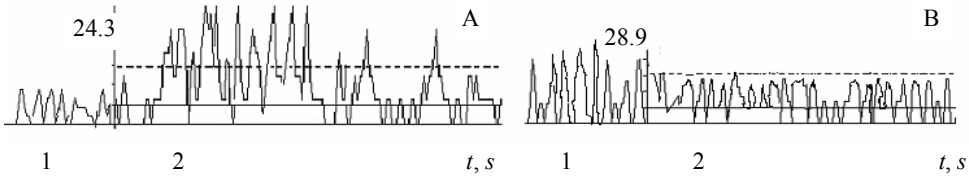
After its fixation in a stereotaxic device the animal was put in an altitude camera. Impulse activity of medullary neurons and characteristics of respiration were recorded in the dynamics of increasing hypobaric hypoxia at atmospheric pressures corresponding to the altitudes of 1000 *m* (according to the location of the laboratory in Yerevan with respect to the sea level), 4000–5000 *m*, 7500–8000 *m*. "Lifts" and "descents" of the animals were performed at a 15–20 *m*/*s* rate. After the experiment, rats were euthanized by injections of chloralose+nembutal in a triple dose.

Action potentials (APs) of the examined units were separated from the record by amplitude discrimination. The data obtained were analyzed using specially developed software [4, 5]. Peristimulus histograms (PSHs) of interspike intervals (ISIs) and graphs of the sliding frequency of APs were plotted. Based on values of the calculated means background frequency (BF) and SD (Standard deviation) of spiking of the neurons at one value of the atmospheric pressure or another, an M ± 2 SD range of the frequency was estimated, which allowed us to identify periods of significant activation or inhibition of the neurons with respect to the BF level at other pressure values (see Figure (A)). The significance of differences between the means was estimated using the Student's *t*-test ($p < 0.05$).




Graphs of the sliding frequency of spike activity generated by neurons of the bulbar respiratory center within different phases of hypoxia:

A. Normal conditions (1) and the first hypoxia phase, 4000–5000 *m* (2); the lower dotted line coincided with the abscissa.

B. Normal conditions (1) and the second hypoxia phase, 7500–8000 *m* (2).

Segments of the recorded background activity are shown before the ordinate axis. Mean frequency (M) and level ± 2 SD are shown by solid and dotted lines respectively.

The population of RN of RC which were examined in our experiment was divided into several groups; neurons with discharge frequency of 1–10 imp./*s* (I group), 11–30 imp./*s* (II group), 31–60 imp./*s* (III group).

**Results and Discussion.** Under initial conditions of atmospheric pressure, we recorded impulse activity of reticular neurons (RN) with frequency 1–10 imp./*s* 32 (31.6%) neurons, 11–30 imp./*s* 50 (49.5%) neurons and 19 (18.9%) neurons with frequency 31–60 imp./*s*. The neurons were most numerous within the group with frequency 11–30 imp./*s*.

Within the first phase of hypoxia, at a 4000–5000 "altitude" ($P_{O_2}$=98–85 *mm* Hg, moderate oxygen insufficiency) the number of neurons decreased. 23 neurons (29.4%) of the I group, 43 (55.1%) neurons of the II group and 12 (15.3%) neurons of the III group continued to satisfy this requirement (see Figure (B)). At this "altitude", breathing became somewhat more frequent and deeper under the influence of moderate hypoxia. Within the second phase of hypoxia, at "altitude" of 7500–8000 *m* ($P_{O_2}$= 64–58 *mm* Hg, severe hypoxia), the dramatic $P_{O_2}$ decrease resulted with a considerable drop in the number of "working" neurons of the bulbar RC.

Within this period only 17 (28.8%) neurons of the I group, 35 (59.3%) neurons of the II group and 7 (11.8%) neurons of the III group remained active. Analysis of the data on functioning of all neuron groups under the above mentioned conditions demonstrated somewhat higher resistance of neurons of the II group (11–30 imp./*s*) with respect to hypoxia, as compared to that of the I and the III neuron groups. At this stage of hypoxia breathing became slower under the influence of the above insufficiency; respiration acquired a superficial pattern, and it was entirely blocked in a part of animals. An increase or decrease in the duration of the respiratory cycle was realized mostly at the expense of the expiration duration.

After switching on the air supply to the altitude chamber, breathing movements gradually recovered.

Ten to twenty minutes after "descent" of the animals, initial indices of impulse activity of respiratory neurons recovered in most cases. It should be noted that, among all examined neurons, this process was most rapid in neurons of the II group. In most cells, this was observed 10–13 *min* after "descent"; the level of recovery of initial parameters was usually equal to 79%. Comparison of separate groups demonstrated a trend toward more complete recovery in neurons with intermediate frequency (11–30 imp./*s*). Within the initial hypoxia phase ("altitude" 4000–5000 *m*) the frequency of impulse activity of all functioning RN increased somewhat (Fig. A). When the "altitude" reached the maximum and the animal was subjected to dramatic oxygen deficiency, activity of the examined neurons was sharply suppressed (Fig. B). Modifications of the pattern of impulse activity were provided mostly according to the number of APs.

Oxygen insufficiency is one of the most prevalent and clinically significant stress-indusing factors, and a number of organisms are subjected to such effects during their life time. In the case of chronic respiratory and cardiovascular diseases, adaptational mechanisms are formed at different levels of organization (systemic, tissue, cellular and molecular). Modifications within the systems controlling respiration, in particular within the medullary RC, are the most important components of a complex process of adaptation. Detection of the regularities of changes in RC activity at a cellular level in the dynamics of hypoxia is of both theoretical and practical importance.

Impulse activity of single neurons is a rather informative phenomenon, because it is an adequate index of the functional state of the brain; each influence of the cell is finally reflected in the parameters of its background activity [8–10]. In our experiments we identified a greater number of neurons with a continuous discharge RN. The observed RN were somewhat 3–4 times numerous, than neurons with periodic activity: inspiratory and expiratory neurons. In the studies of activity of single RN, it should, however, always be remembered that these processes are not realized in an isolated manner in a separate nerve cell; these processes are not determined exclusively by its internal properties, but mostly result from interactions within the entire network of RN [8, 11, 12]. For our experimental conditions, when $P_{O_2}$ in the inspired air was 98–85 *mm* Hg (first phase of hypoxia, 4000–5000 *m*), hypoxic activation of nearly all functioning neurons of the bulbar RC is related, according to some authors [9, 10, 12], to both the direct action of hypoxia on the cellular membranes (which results in depolarization of the latter) and activation of neuronal networks in the brainstem reticular formation.

In our study a dissimilar resistivity of impulse activity generated by RN with respect to hypoxia was found. In particular, it was found that neurons of the II group (11–30 imp./*s*) are somewhat more resistant against hypoxic effects, than neurons of the I and the III groups (see Table). Probably, the process of recovery from oxygen deficiency in different types of neurons is realized unevenly, and this reflects the level of resistance or vulnerability of one type of neurons or another with respect to hypoxia [9].

*Dynamic changes in the pattern of activity of reticular neurons of bulbar respiratory center under conditions of hypobaric hypoxia*

| Indices | Initial state (norm) | Altitude, *m* | | Descent |
|---|---|---|---|---|
| | | 4000–5000 | 7500–8000 | |
| 1–10 imp./*s* | | | | |
| Mean frequency, $s^{-1}$ | 6.7 ± 0.5 | 10.2 ± 0.8 ** | 3.8 ± 0.2 * | 7.9 ± 0.5 * |
| 11–30 imp./*s* | | | | |
| Mean frequency, $s^{-1}$ | 16.3 ± 1.1 | 21.1 ± 1.8 ** | 11.8 ± 0.9 * | 17.1 ± 1.3 * |
| 31–60 imp./*s* | | | | |
| Mean frequency, $s^{-1}$ | 50.7 ± 4.1 | 60.8 ± 4.8 * | 34.4 ± 2.1 * | 51.3 ± 3.9 * |

Means ± s.d. are shown. Asterisks show cases of significant differences from the initial indices with: * – $p < 0.05$; ** – $p < 0.01$.

Within the first stage of hypoxia effect, respiratory movements became more profound and more frequent; this was directed towards compensation of the oxygen deficiency and preservation of gas homeostasis in the organism. The inspiration phase of respiration is found to be more stable at increasing hypoxia. This is probably related to the weaker action of the mechanism of network self-excitation in the expiratory population of RN as compared with the inspiratory population [13]. Some authors mentioned that hypoxic stimulation of respiration is accompanied by a decrease in the duration of reflex vagal inhibition of inspiration. The effects coming from carotid chemoreceptors and facilitating inspiratory activity weaken the inhibitory effects from stretch receptors of the lungs [14, 15]. Such type of interaction can be one of the factors to determine shortening of the inspiration phase under conditions of developing hypoxia.

Within the phase of intense hypoxia (7500–8000 *m*, where $P_{O_2}$=64–58 *mm* Hg), we observed dramatic suppression and sometimes even complete inhibition of impulse activity of all groups of RC reticular neurons. Such modifications are explained by some authors as a result of sharp increase (sometimes by two orders of magnitude) in gamma-aminobutyric acid (GABA) amount in the brain, development of disorders in structural organization of cell membranes, and disorders in the functioning of K/Na pump [6, 16–18].

Within the integral nervous system, where each neuron possesses a number of synaptic connections with different afferent sources and other cells, generation of BA results, first of all, from synaptic interactions and is related to active processes requiring certain expenditures of energy and oxygen. Exhaustion of the stores of macroergic compounds in tissues is the final result of each type of hypoxia. Hypoxic effects of the brain lead to changes in the chemical composition

of the interstitial fluid, cerebrospinal fluid, and blood in the structures of the medulla, and this significantly influences metabolic processes in RN [7].

As was found, GABA provides clear neuroprotective effects on the cellular level under hypoxia conditions. The intensified release of GABA in this case results suppression of the activity of neurons with high frequency and, consequently, preservation of the stores of macroergic compounds in these cells. Such events increase the level of cell survival under conditions of hypoxia-induced energy deficiency [18].

At "altitudes" of 7000–8000 *m*, against the background of strong hypoxic inhibition of activity of RN, it was sometimes difficult to estimate the correlation between electrical activity of examined RN and integral external effect of their activity, i.e., breathing. The latter process (breathing) is an integral index of activity of the respiration-controlling neuronal system and is based on the functional activity of not a single neuron but of a great number of neurons in the brainstem RC [19].

After "descent" of the animals and their return to normal conditions of oxygen supply, the recovery of characteristics of impulse activity generated of RN developed in an uneven manner. The process of recovery was noticeably slower in neurons of the III group.

Such specificity of neurons is determined, to a definite extent, by their dimensions and peculiarities of organization of their membranes. The specific properties of membranes of these neurons are probably related to their definite positions in the respiratory generator network and with definite roles played by these units in the integral system of RN [6, 7].

The main mechanism providing different resistivity of neurons with respect to oxygen insufficiency is probably related to a dissimilar intensity of decreases in the content of calcium connected with the membranes of intracellular organelles [9, 20, 21]. According to other authors [22, 23], this peculiarity is explained by dissimilar levels of inhibition of separate groups by GABA, which is the main inhibitory transmitter within the zone of action of hypoxia.

**Conclusion.** Our findings allow us to believe that stable control of the respiratory function under conditions of hypoxia is provided, to a considerable extent, by a certain functional heterogeneity of neuronal organization of the bulbar RC. The complex mosaic structure and interrelations between these neurons provide some physiologycal assurance for the maintenance of gas homeostasis in the organism under extreme conditions of oxygen deficiency.

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