RESPONSE OF THE LOCUS COERULEUS TO ASPHYXIA

T. I. Belova and M. A. Pal'tsev

UDC 616.831.371-02:616-001.8]-091

To discover the mechanisms of function of the locus coeruleus (LC), methods of light and electron microscopy were used to study the responses of neurons of this nucleus to experimental asphyxia. Besides specific neuronal effects (taking place either directly through axons of LC cells or indirectly through the brainstem reticular formation), LC was shown to have a humoral effect on the brain structures. Two possible mechanisms of transmission of catecholamines synthesized in the soma of the LC cells into the bloodstream are postulated: through the cell membrane and subsequently through the basement membrane of the capillary and cytoplasm of the endothelial cell, and with the participation of glial elements.

KEY WORDS: *locus coeruleus; catecholamines; asphyxia; pneumotaxic center; homeostasis.*

The question of the functional properties of the locus coeruleus (LC) is being widely debated at the present time, because the specific neurochemical features (high concentration of catecholamines, especially noradrenalin, in the soma of LC cells) [7-9, 10] and the extensive connections (the axons of LC cells send their branches to the cerebral and cerebellar cortex and to many of the nuclei of the brain stem and diencephalon) [4, 5, 6] suggest that this nucleus plays a part in the organization of the most important responses of the body.

LC lies in the region of the isthmus of the brainstem and it is a component of the "pneumotaxic center." It might thus be supposed that the regulation of gas homeostasis is the principal function of LC, and that an experimental change in the gas balance would be the most adequate load for LC in order to discover the pattern and mechanism of function of the neurons of that nucleus.

It was accordingly decided to study the specific features of the role of LC in the responce to asphyxia.

EXPERIMENTAL METHOD

Light and electron microscopy was used for the investigation. The ordinary neurohistological methods (Nissl, Einarson, Golgi, Boeke–Bielschowsky) and the method of semithin sections, specially adapted for local investigation of the small structures of the brain under the electron microscope [2] were used; for electron-microscopic study fixation in glutaraldehyde was followed by postfixation in 1% OsO_4 and embedding in Epon–Araldite. The experiments were carried out on adult male CC57w albino mice.

Asphyxia was induced by placing the animal in a closed container with a volume of 70 ml. The animal was killed immediately after developing severe asphyxia, of which the main sign was immobility of the animal. The composition of the blood gases was investigated by the Astrup method using electrochemical equipment from Radiometer (Denmark).

P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 7, pp. 106-109, July, 1977. Original article submitted May 12, 1976.

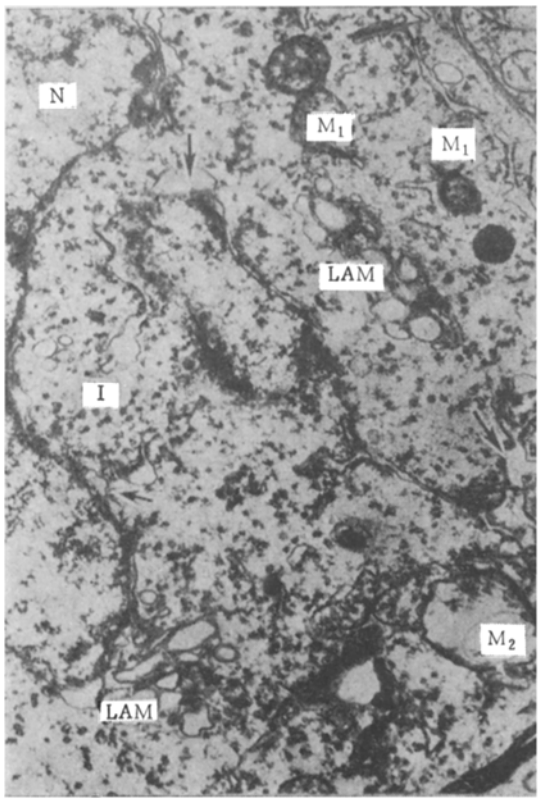

Fig. 1. Neuron of LC under conditions of asphyxia. Nucleus (N) as a deep invagination (I). Increase in relative content of vesicular component and vacuoles in lamellar complex (LAM). Swelling of mitochondria (M_1), sometimes with almost total reduction of cristae (M_2). Irregular widening of perinuclear space and cisterns of cytoplasmic reticulum (arrows). 12,000×.

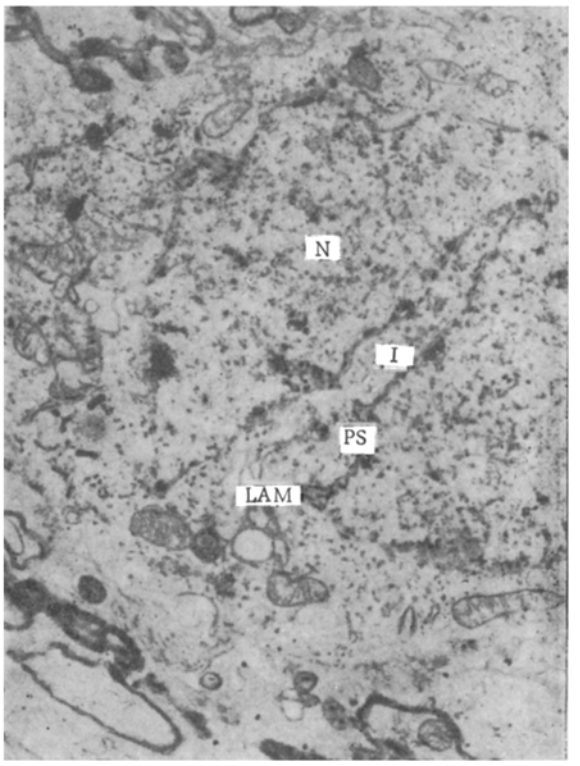

Fig. 2. Neuron of LC. Deep invagination (I) in nucleus (N). Elements of lamellar complex (LAM) and polysomes (PS) lie in the invagination. 3000×.

EXPERIMENTAL RESULTS

All the animals developed a severe degree of hypoxia, hypercapnia, and respiratory acidosis: pO_2 = 14.67 ± 2.29 mm Hg (control 43.09 ± 1.64 mm Hg; $P < 0.01$), pCO_2 = 83.98 ± 3.74 mm Hg (control 47.54 ± 0.54 mm Hg; $P < 0.05$); pH 6.6 ± 0.002 (control 7.2 ± 0.009; $P < 0.01$); true bicarbonate (AB) 15.75 ± 0.81 meq/liter (control 18.48 ± 0.49 meq/liter; $P < 0.01$); buffer base shift (BE) 20.71 ± 0.41 meq/liter (control 9.26 ± 0.444 meq/liter; $P < 0.001$).

Under the conditions of asphyxia the following structural changes were observed in the cells of LC and, by analogy with the results obtained previously by the Falck–Hillarp method on a similar type of model [1], were regarded as indicators of strengthening of the specific function of the LC cells, namely activation of catecholamine synthesis; weakening of the basophilia of all structures of the cell, tigrolysis, a spatial redistribution of the Nissl substance, pycnosis of the nuclei, an increase in the relative content of the vesicular component and of vacuoles in the lamellar complex, swelling of the mitochondria, the appearance of free ribosomes in the cytoplasm, a decrease in the number of granular vesicles in all structures of LC, and the appearance of vesicles containing a granule of lower electron density in the center (Fig. 1).

The increase in the functional activity of the LC cells presupposes activation of the reflex influence of this nucleus on other brain formations. The most extensive intracerebral connections, proved nowadays not only by the Falck–Hillarp histofluorescence method, but also autoradiographically, electron-microscopically, and also by the Fink–Heimer method, enable LC to exert its monosynaptic influence on all regions of the cerebral and cerebellar cortex and on many nuclei of the diencephalon and brainstem.

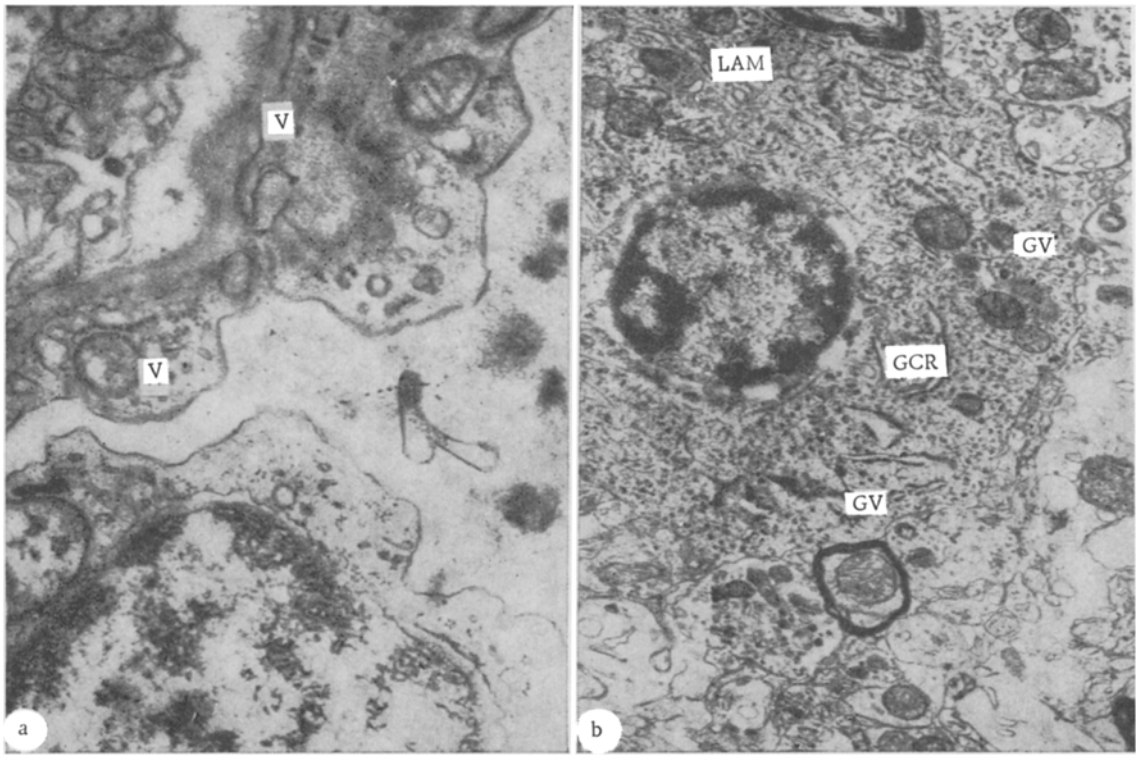

Fig. 3. Vesicles of granular type in structural elements of LC: a) Pinocytotic vesicles with electron-dense central granule in endothelium of capillary (V). 35,000×; b) pinocytotic vesicles of granular type (GV) in cytoplasm of oligodendrogliocyte; GCR) cytoplasmic reticulum of granular type; LAM) lamellar complex. 2800×.

Our study of the neuronal organization of LC also showed that it can influence brain structures through the reticular formation of the brainstem. A giant reticular neuron, the processes of which penetrate both into LC and into adjacent structures, has been shown to be present usually near the ventral borders of LC.

The results of these investigations indicate that catecholamines synthesized in the soma of the LC cells perform their function not only specifically by the neuronal route. Features characteristic of the hypothalamic neurosecretory nuclei (an abundant blood supply, facetization of the nuclei, invaginations in the nuclear membrane, vacuolation of the cytoplasm, intensive development of the lamellar complex, an eccentric position of the nucleoli, and the presence of free ribosomes) were discovered in LC (Fig. 2). The presence of these distinguishing features and the fact that most of them were more marked during asphyxia leads to the conclusion that, besides its neural influence, LC also has a neurosecretory action on brain structures.

The ultrastructural features of LC neurons in animals in a state of deep asphyxia suggest two possible mechanisms whereby catecholamines are transmitted from LC cells into the bloodstream. Direct contact demonstrated in these experiments between the cell membrane of LC neurons and the basement membrane of the capillaries, and also the presence of vesicles with an electron-dense core in the capillary endothelium (Fig. 3a), are evidence that catecholamines can be transported in the dissolved form across the cell membrane and then by diffusion or by active pinocytosis through the basement membrane and cytoplasm of the endothelial cell, with their subsequent entry into the bloodstream. This mechanism is similar to that observed in the adrenal medulla [3].

Another possible route for liberation of neurosecretion is specific for the brain (and perhaps for LC), and takes place with the participation of glial elements (Fig. 3b). The presence of single granular vesicles in the cytoplasm of all glial cells of LC indicates a possible role of the glial cells in the accumulation and transport of catecholamines into the bloodstream.

By the use of asphyxia as an adequate load for LC it was thus possible to discover the different ways whereby catecholamines synthesized in the neurons of this nucleus exert their effect.

It can be postulated that LC participates in the organization of defensive responses of the body in two ways: by its specifically neural action on brain structures (directly and indirectly through the reticular formation of the brainstem) and humorally. The latter method is associated with changes in the metabolism of the neuron and glial cell.

LITERATURE CITED

1. T. I. Belova and L. S. Bunkina, Byull. Éksp. Biol. Med., No. 12, 93 (1970).
2. M. A. Pal'tsev and G. A. Kosolapov, Arkh. Patol., No. 8, 83 (1974).
3. É. Kh. Priimak, Tsitologiya, No. 2, 169 (1967).
4. N. E. Anden, A. Dahlstrom, K. Fuxe, et al., Acta Physiol. Scand., 67, 313 (1966).
5. A. Dahlstrom and K. Fuxe, Acta Physiol. Scand., 62, 1 (1964).
6. K. Fuxe, T. Hokfelt, O. Nillson, et al., Anat. Rec., 155, 33 (1966).
7. G. Lynch, R. L. Smith, and R. Robertson, Exp. Brain Res., 17, 221 (1973).
8. M. Segal, V. Pickel, and F. Bloom, Life Sci., 13, 817 (1973).
9. V. Ungerstedt, Acta Physiol. Scand., Suppl. No. 367, 1 (1971).

CHANGES IN THE ULTRASTRUCTURE OF THE RAT MYOCARDIUM FOLLOWING ADAPTATION FOR 12 MONTHS TO HIGH ALTITUDES

B. Zhaparov and Academician M. M. Mirrakhimov*

UDC 612.172.6-06:612.275.1

The myocardium of the right and left ventricles of rats during adaptation for 12 months to high altitudes (3200 m above sea level) was studied. During the animals' long stay in the mountains hypertrophy mainly of the right and partly of the left ventricles developed. Hyperplasia and hypertrophy of individual organelles, especially mitochondria, were found in most cardiomyocytes of both ventricles. In animals adapted to high altitudes succinate dehydrogenase activity in the mitochondria was higher than in the control. The results are evidence of intensification of intracellular metabolism, reflecting compensatory and adaptive responses of the organs.

KEY WORDS: *high mountain altitude; hypertrophy of the myocardium, mitochondria; succinate dehydrogenase.*

During a prolonged stay in the mountains hypertrophy of the heart muscle develops in animals and man on account of pulmonary hypertension [6, 8]. An increase in the intensity of function of the intracellular structures is also found, which may amount to an increase in the number of mitochondria and other organelles [1, 2, 5]. During adaptation to high-altitude hypoxia the hypertrophy of the right ventricle caused by pulmonary hypertension leads to an increase in its mass by 40-80% within the course of only 7-10 days [4]. Hyperplasia of the ultrastructures arises in the cells with the need for stimulation of their working activities. Under these circumstances the dimensions of the cell and organ as a whole are increased [7]. Individual studies of changes in the ultrastructure of the myocardium of intact rats from the age aspect have been published [3, 11]. Recently investigations

*Academy of Sciences of the Kirghiz SSR.

Department of Electron Microscopy, Central Research Labroatory, and Problem Laboratory, Kirghiz Medical Institute.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 7, pp. 109-112, July, 1977. Original article submitted January 14, 1977.