LITERATURE CITED

1. O. V. Volkova and S. B. Tarabrin, Arkh. Anat., No. 7, 5 (1975).
2. O. V. Volkova, The Neurodystrophic Process (Technical Aspects) [in Russian], Moscow (1978).
3. Yu. K. Eletskii, T. K. Dubovaya, A. V. Bykov, et al., Cytologic Mechanisms of Histogenesis [in Russian], Tashkent (1983), pp. 58-60.
4. Yu. K. Eletskii, S. G. Mamontov, T. V. Savchenko, and T. K. Dubovaya, Byull. Éksp. Biol. Med., No. 10, 482 (1984).
5. V. B. Zakharov, Byull. Éksp. Biol. Med., No. 5, 584 (1980).
6. A. G. Malenkov and G. A. Chuich, Intercellular Junctions and Tissue Reactions [in Russian], Moscow (1979).
7. E. A. Modyanova, O. A. Bocharova, and V. F. Ushakov, Byull. Éksp. Biol. Med., No. 4, 459 (1980).
8. L. A. Radkevich, Biofizika, 24, No. 5, 906 (1981).
9. V. P. Yamskova, Chalones: Their Importance and Role in Normal and Pathologial Processes [in Russian], Moscow (1981), pp. 60-67.

CYTOCHEMICAL STUDY OF LOCUS COERULEUS NEURONS AFTER GUANETHIDINE DESYMPATHIZATION IN RATS

G. Altan, V. N. Yarygin, and A. V. Grigor'eva

UDC 612.827.014.22].014.46.09]-08

KEY WORDS: locus coeruleus; desympathization; histones; template activity; noradrenalin fluorescence.

Convincing evidence has been obtained in the last decade that the principal noradrenergic brain formation, the locus coeruleus (LC), is involved in the regulation of the most important autonomic functions of the body (vascular tone, temperature regulation, motor activity, etc.) [11, 13].

From the structural point of view LC can evidently be regarded as a link in the chain of visceral afferent pathways maintaining the autonomic component of several important physiological acts [10], and also as an essential component of afferent synthesis in the homeostasis maintaining system [1].

Great similarity is observed between the noradrenergic system of the brain and the sympathetic division of the peripheral nervous system with respect to several morphological, biochemical, and physiological parameters. It has even been suggested that LC is the "cranial ganglion" of the sympathetic system [8].

One possible experimental approach to the problem of relations between peripheral and central noradrenergic structures is to study the response of the central component to structural and functional changes in the peripheral component. In particular, by recourse to a model of measured desympathization, it is possible to monitor the morphological and functional state of LC neurons under conditions of death of a certain number of sympathetic neurons, since guanethidine has no direct effect on brain cells [9].

The aim of this investigation was to study transcription and the histone component of chromatin, and also to undertake a quantitative microfluorometric estimation of the noradrenalin (NA) concentration in the cytoplasm of LC neurons of intact and partially desympathized adult rats.

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR O. S. Andrianov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 242-245, February, 18989. Original article submitted March 5, 1988.

 0007-4888/89/0002-0262$12.50

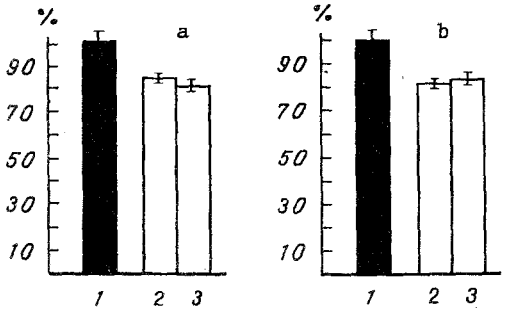

Fig. 1

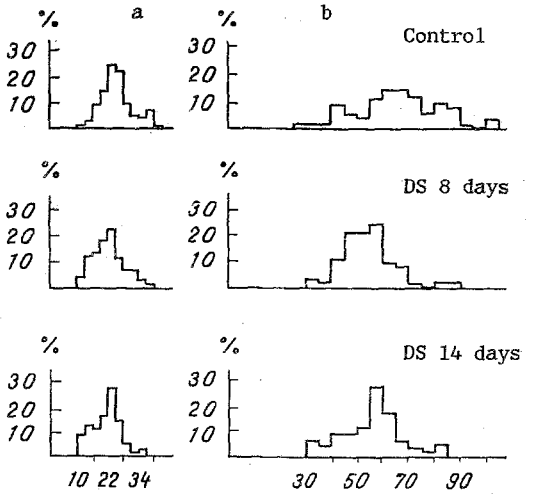

Fig. 2

Fig. 1. Changes in average level of labeling of nuclear structures of LC neurons in rats aged 4 months following desympathization (DS). a) Nucleolar labeling; b) extra nucleolar labeling. Abscissa, groups of animals: 1) control, 2) 8 days after DS, 3) 14 days after DS; ordinate, level of labeling (in % of control). Short vertical lines indicate mean error, p) significance of differences between control and DS ($p < 0.01$).

Fig. 2. Distribution of nuclear structures of LC neurons of normally developed and partially desympathized rats aged 4 months, depending on labeling level. a) Nucleolar labeling, b) extranucleolar labeling. Abscissa, labeling level (number of grains of reduced silver); ordinate, fraction of cells (in % of total number).

EXPERIMENTAL METHOD

Experiments were carried out on LC neurons on noninbred albino rats belonging to infantile (1 month) and young reproductive (4.5 months) age groups. Normally developed and partially desympathized animals were used: the latter were given daily subcutaneous injections for 3, 8, or 14 days after birth of a solution of guanethidine (isobarin, from "Pliva," Yugoslavia) in a dose of 15 mg/kg. Noradrenergic LC neurons were detected by a modified histofluorescence method [2]. Sections were examined in the luminescence microscope with wavelength of excitation of 405 nm, using ZhS-18 and ZhZS-19 cutoff filters. Transcription was analyzed by an autoradiographic method of demonstration of endogenous RNA-polymerase activity in fixed neurons in sections [13]. Template activity of nuclear structures was estimated as the number of grains of reduced silver above the nucleolus and nucleoplasm. The structure of histones in nuclei of LC neurons was assessed by staining them with ammoniacal silver (AS) by the method of Black and Ansley [9]. The type of nucleus was distinguished according to the absence or presence of brown inclusions, reflecting the location of reactive guanidine groups of arginine-rich histones, and the number of these inclusions. The significance of differences was estimated by Student's test.

EXPERIMENTAL RESULTS

According to the available data, two types of neurons can be distinguished in the rat LC: cells of type 1 are characterized by their distinctive morphology: uneven outlines of the cytoplasm because of numerous vacuoles, nucleus often irregular in shape, with invaginations of the nuclear membrane in semithin sections; cells of type 2 are similar to cells of the central gray matter, and most of their area is occupied by the nucleus. Virtually all LC neurons have been shown to synthesize NA [1].

In sections stained by AS the cell nuclei of LC of adult rats have a characteristic color, so that they can be clearly distinguished from cells of other types and, in particular, from those of the mesencephalic nucleus of the trigeminal nerve, lying close by them, and also from undifferentiated LC cells of week-old animals. The latter are a homogeneous bright yellow color, whereas in the overwhelming majority of differentiated LC neurons (4 months), evidently belonging to type 1, about 200 evenly distributed brown grains, intensity of staining of which

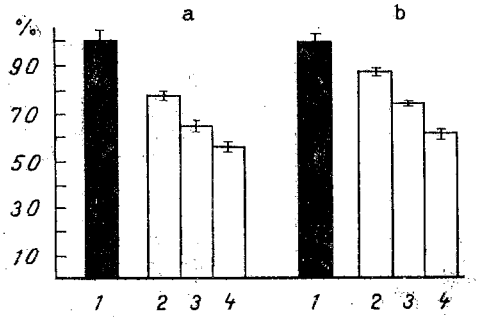

Fig. 3. Changes in mean level of fluorescence of NA in LC neurons of rats after desympathization. Animals aged: a) 1 month, b) 4 months. Abscissa, groups of animals: 1) control, 2) DS 3 days, 3) DS 8 days, 4) DS 14 days; ordinate, level of fluorescence (in % of control). Short vertical lines indicate mean error. p) Significance of differences between control and DS ($p < 0.01$).

varies from nucleus to nucleus from pale to quite dark, and also a bright yellow or orange nucleolus, are visible against the yellow background. The nuclei of neurons belonging to type 2 are smaller, less regular in shape, and they stain differently: besides grains of varied size, they also contain on average three to five brown masses. Grigor'eva and Yarygin [3, 4] showed that the character of staining of histones by AS in nuclei of different types of nerve cells is determined by the morphological and functional type to which they belong; unlike template activity it demonstrates the stability of differentiation after its completion, and varies with a change in the spectrum of protein synthesized [7], i.e., it is evidently connected with the spectrum of genes functioning (unblocked) in cells of the given type. In the present experiments desympathization, even maximal, did not cause changes in the type of staining of the nuclei with AS in LC, evidence of the absence of significant structural conformational changes in the chromatin in these neurons, which would have indicated possible reprogramming of the gene. Meanwhile the fraction of nuclei with dark brown granularity was significantly increased in the desympathized neurons (up to 65% on average compared with 45% in the control). Since the intensity of the brown color for objects stained simultaneously depends on the number of reactive guanidine groups of arginine-rich histones, in the composition of the nucleosomal cortex, the changes which we observed following desympathization evidently reflect changes in the relative percentages of neurons in LC differing in the physicochemical state of the cortical histones.

The study of transcription of LC neurons in rats aged 4 months showed that desymphatization leads to a significant fall of the average template activity of both nucleolar and extranucleolar chromatin (Fig. 1); moreover, a tendency toward dose dependence is evident. Analysis of the character of distribution of LC neurons depending on template activity for extranucleolar chromatin showed (Fig. 2) that in adult intact rats discrete classes of activity within the regions of 30-40, 40-53, 53-75, and 75-100 conventional units can be clearly distinguished. The fall of the average values of extranucleolar labeling after desympathization was due to redistribution of the cells among these classes, namely, a decrease in the fraction of intensively labeled nuclei accompanied by a simultaneous increase in the fraction of weakly labeled nuclei. The above conclusion is valid also for the nucleolus. The effect of desympathization was similar in rats aged 1 month.

The existence of discrete classes, detectable by analysis of the distribution of the cells by template activity, has been found for Purkinje cells of the rat cerebellar cortex [5]. This fact is of interest on its own account, for it indicates the quantal nature of the structural and functional changes in chromatin and is worthy of special investigation. Similar results were obtained previously by Nemirovskii and co-workers [6], who used the content of fractions of nuclear DNA, highly sensitive to depolymzerization, as an indicator of chromatin activity.

Fluorometric analysis of the fluorescence of NA in the cytoplasma of the cells studied showed that, as in the case of template activity, injection of guanethidine caused a decrease

in the intensity of fluorescence, evidence of a fall in NA concentration, proportional to the degree of desympathization. After 4 months, in all experimental groups a significant decrease was observed in the changes in the parameter observed, caused by desympathization (Fig. 3).

Similarity of the trend in changes in template activity and NA content in the desympathized animals suggests that reduction of the latter may be due to depression of the transcription function of the genetic apparatus. Death of some sympathetic nerve cells at the periphery thus is reflected in the level of specific functioning of central NA neurons of LC, leading to a fall in the corresponding parameters both in the nucleus and in the cytoplasm. The absence of change in the type of nuclear staining of neurons with AS indicates the quantitative character, i.e., not connected with a change in spectrum of transcribed genes, of the structural changes in chromatin.

LITERATURE CITED

1. T. I. Belova, E. L. Golubeva, and K. V. Sudakov, Homeostatic Functions of the Locus Coeruleus [in Russian], Moscow (1980).
2. I. V. Viktorov and N. A. Shashkova, Byull. Éksp. Biol. Med., No. 12, 729 (1984).
3. A. V. Grigor'eva and V. N. Yarygin, Tsitologiya, 27, No. 2, 186 (1985).
4. A. V. Grigor'eva and V. N. Yarygin, Tsitologiya, 28, No. 8, 837 (1986).
5. A. V. Grigor'eva, Byull. Éksp. Biol. Med., 103, No. 3, 358 (1987).
6. L. E. Nemirovskii, N. M. Vakhtel', I. G. Suvorova, and É. M. Kogan, Current Problems in Experimental and Clinical Medicine [in Russian], Part 3, Moscow (1979), p. 34.
7. D. G. Amaral and H. M. Sinnamon, Prog. Neurobiol., 9, No. 3, 147 (1977).
8. P. U. Angeletti and R. Levi-Montalcini, Proc. Nat. Acad. Sci. USA, 69, 86 (1972).
9. M. M. Black and H. R. Ansley, Science, 143, 693 (1964).
10. P. Hinckel and W. T. Perschel, Can. J. Physiol. Pharmacol., 65, 1281 (1987).
11. M. Elam and P. Thoren, Brain Res., 375, No. 1, 117 (1986).
12. G. P. M. Moore, Exp. Cell Res., 111, 317 (1978).
13. A. F. Sved and G. Felsten, Brain Res., 414, 119 (1987).

IMMUNOHISTOCHEMICAL STUDY OF INSULIN-SENSITIVE CELLS OF THE MEDIAN EMINENCE OF THE HYPOTHALAMUS

I. I. Babichenko

UDC 612.826.4.014.467:615.357.37:577.175.722

KEY WORDS: insulin; insulin-like growth factor; receptors; hypothalamus.

There is much experimental evidence that insulin affects physiological processes of the brain and of the body as a whole [7, 9]. Nevertheless, information on the distribution of insulin receptors in the CNS is contradictory [4, 13]. Besides insulin, the attention of research workers is currently drawn to a group of peptides known as insulin-like growth factors (ILGF), which can also activate growth processes in various tissues of the body. In turn, injection of ILGF-1 into the cerebrospinal fluid causes a decrease in the secretion of somatotrophic hormone in the adenohypophysis [12]. Considering the ability of these peptides to induce DNA synthesis in a culture of fibroblasts [10], it has been suggested that specific receptors exist to ILGF-1. Such receptors have now been discovered not only in actively proliferating cells, but also in brain homogenates from adult animals [6], the formation of which is largely complete during the prenatal period of development. On the basis of investigations [2] showing that the distribution of receptors for ILGF-1 in the brain is limited to the outer zone of the median eminence of the hypothalamus, it can be postulated that this peptide has a regulatory function in the CNS.

Central Research Laboratory, Medical Faculty, P. Lumumba Peoples' Friendship University, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR K. V. Sudakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 245-247, February, 1989. Original article submitted May 3, 1988.