AUTOMATED MORPHOMETRIC EVALUATION OF STRUCTURE OF NUCLEAR CHROMATIN OF LIVER CELLS AFTER VAGOTOMY

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Investigation of changes in the structure of cell chromatin is an important step toward our understanding of the fine mechanisms of development of various pathological processes [9, 10] and, in particular, of neurodystrophies. Much information has been published on morphological changes in various cytoplasmic organelles of cells in the course of the neurodystrophic process [2, 4]. However, against the background of the pronounced destructive changes in the cytoplasm, morphological changes in the nucleus are virtually impossible to discover by the use of light-optical microscopic methods. This is evidently due to the lack of informativeness and accuracy of the methods used and the poor quality of visual evaluation. Consequently, in order to discover possible changes in the nucleus, accurate quantitative methods of investigation are required, one such method being automated morphometry.

This paper describes a morphometric analysis of the structure of the interphase chromatin of hepatocytes in the composition of the liver, at different times after the operation of vagotomy.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 160-180 g at different times (1, 2, and 4 weeks) after bilateral subdiaphragmatic vagotomy and a control group of animals undergoing a mock operation. The investigation was conducted on squash preparations of the liver, stained with gallocyanin and chrome alum by Einarson's method. At each experimental point 200 nuclei were studied. The morphometric analysis was carried out on an automated television image analysis system (the IBAS-2, from Opton, West Germany), using a "Universal" microscope with Ultrafluar 100/1.25 objective, giving a total magnification of 2500×. The receiver was a television camera, with constant grid (scanning element) of 0.14 μ. The analysis was carried out in accordance with an original "Grancor" program [7], yielding about 200 parameters of chromatin structure. A standard IBAS-1 program was used for statistical analysis of the data.

EXPERIMENTAL RESULTS

To obtain the fullest information on possible changes in elements of the chromatin (granular and nongranular components) in the hepatocyte nuclei, 50 structural parameters were studied. Analysis of these parameters showed that the greatest structural changes in the supramolecular organization of chromatin of the hepatocyte nuclei of the denervated liver, compared with the control group, took place 1 week after vagotomy. At this time the following changes were found in the granular (optically denser) component of chromatin: a decrease in size of the granules (Fig. 1a) and in the area of the granular component according to integral optical density (OD; Fig. 1b) and reduction of contrast (Fig. 1c). These results are in good agreement with data on structural changes in chromatin during activation of the cells by xenobiotic genetic inducers [1, 5, 6] and by mitogens [1].

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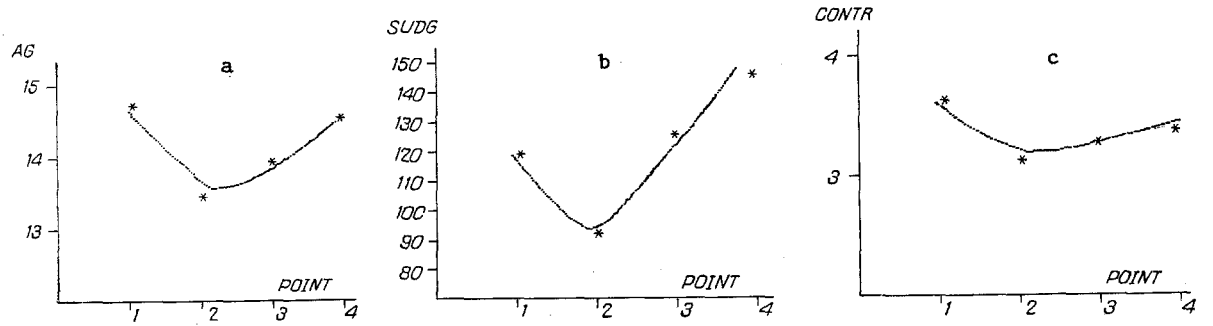

Fig. 1. Changes in parameters of supramolecular organization of nuclear chromatin of liver cells at various times after bilateral subdiaphragmatic vagotomy. Abscissa: 1) control, 2) 1 week, 3) 2 weeks, 4) 4 weeks after operation; a) change in size of chromatin granules (in conventional units); b) change in area of granular component (in conventional units); c) change in contrast (in conventional units).

The results obtained by analysis of the structure of hepatocyte chromatin 2 and 4 weeks after vagotomy revealed a tendency toward normalization of the parameters studied. It must be particularly emphasized that 4 weeks after denervation the area of the granular component was increased by 20% ($p \leqslant 0.05$), the maximal OD of the granules was increased by 21% ($p \leqslant 0.05$), and the minimal OD of the nongranular component was increased by 43% ($p \leq 0.01$) evidence of possible inactivation of the chromatin of the hepatocyte nuclei at this experimental point.

Analysis of the parameters also revealed conflicting changes in the granular and nongranular components 1 week after vagotomy. For instance, with a decrease in area of the granular component the area of the nongranular component did not increase. This disparity can be explained by the different staining properties of the chromatin components studied, due to changes in its physicochemical properties in the cell in different functional states [8].

This nonuniformity of the parameters of individual components of chromatin, assessed quantitatively, showed that changes in the granular component were more informative than changes in the nongranular component (difference 15-20%). This was confirmed by results showing that the granular component was more sensitive, with respect to parameters of its supramolecular organization, to the functional state of the cell [3, 6, 11]. To assess the functional significance of changes in structure of the hepatocyte chromatin of the control and experimental animals, correlation analysis was carried out, to compare the morphometric parameters with the level of template activity of the chromatin, determined by a histoautoradiographic method in situ [12] in an equivalent experimental situation. The highest level of transcription activity of the chromatin (230%) was determined 1 week after the operation.

As a result of correlation analysis, a group of morphometric parameters of chromatin structure correlating most strongly with the level of chromatin template activity at different times after vagotomy was discovered. For instance, the coefficient of correlation between size of granules and level of template activity of chromatin was 0.97 ($p \leqslant 0.001$), whereas between contrast and template activity it was 0.93 ($p \leqslant 0.001$). Thus with elevation of the level of template activity, the contribution of the granular component decreased, confirming the above conclusion on activation of the hepatocyte nuclei 1 week after vagotomy, and obtained on the basis of morphometric parameters of chromatin structure.

Consequently, it can be concluded from the results of this investigation that disturbance of the vagal innervation of the liver leads to changes in the morphological and functional organization of the chromatin of the parenchymal cells; in the early stages (after 1 week, moreover) activation of hepatocyte chromatin takes place, whereas in the later stages (after 2 and 4 weeks) the morphometric parameters of chromatin structure approach the control level.

LITERATURE CITED

1. N. N. Butusova, A. I. Shchegolev, and A. V. Zhukotskii, Manuscript Lodged with the All-Union Institute of Scientific and Technical Information, No. 7223-V8 (1986).

2. O. V. Volkova, The Neurodystrophic Process [in Russian], Moscow (1978).
3. V. G. Grif, T. V. Aleksandrov, and E. M. Valovich, Tsitologiya, No. 3, 295 (1987).
4. Yu. K. Eletskii, Cytological Mechanisms of Histogenesis [in Russian], Moscow (1979), pp. 108-119.
5. A. V. Zhukotskii, M. V. Kormilets, I. G. Solov'eva, et al., Manuscript lodged with the All-Union Institute of Scientific and Technical Information, No. 5921 (1983).
6. A. V. Zhukotskii, A. I. Shchegolev, N. N. Butusova, and É. M. Kogan, Manuscript lodged with the All-Union Institute of Scientific and Technical Information, No. 6591 (1984).
7. A. V. Zhukotskii, N. N. Butusova, A. I. Shchegolev, and É. M. Kogan, Automated Cytologic Diagnosis [in Russian], Pushchino (1985), pp. 78-87.
8. A. V. Zhukotskii, A. I. Shchegolev, N. N. Butusova, and É. M. Kogan, Byull. Éksp. Biol. Med., No. 6, 726 (1985).
9. D. M. Spitkovskii, Vest. Akad. Med. Nauk SSSR, No. 1, 29 (1973).
10. A. Chorst, Molecular Principles of the Pathogenesis of Diseases [Russian translation], Moscow (1982).
11. A. V. Zhukotskii (A. V. Zhukotsky), N. N. Butusova, Kh. M. Umudov, and É. M. Kogan, Biomat. Med. Devices Artific. Organs, 15, No. 1, 137 (1987).
12. J. Moore, Exp. Cell Res., 11, 317 (1978).