# RESPONSE OF MEDULLARY AREA POSTREMA CELLS TO EXPERIMENTAL ALLOXAN DIABETES

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UDC 616.379-008.64-092.9-07:616.831.83-091.8

KEY WORDS: regulation of carbohydrate metabolism; bulbar autonomic centers; area postrema; hemoreception.

An essential role in the central regulation of carbohydrate metabolism is played by the autonomic centers of the medulla and, in particular, the dorsal nucleus of the vagus [1, 2]. Meanwhile structures supplying them with feedback from the internal medium of the body are of great importance for the activity of these centers. Besides sensory nerve pathways, these structures also conjecturally include the periventricular organ of the medulla, which has been called the area postrema (AP). Among its particular features are direct contact with the CSF of the cerebral cisterns, high permeability of the bloodbrain barrier, and also ability to respond to fluctuations in the levels of some humoral agents. It has been postulated [2] that AP may be involved in the regulation of carbohydrate metabolism due to the ability of its cells to react to a change in insulin and glucose levels in biological fluids.

To test this hypothesis, a karyometric study was undertaken of the response of AP cells and morphological changes in the organ under conditions of disturbed carbohydrate metabolism, created experimentally in the form of alloxan diabetes.

## EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 170-180 g. Control (n = 15) and experimental (n = 15) animals were kept under identical conditions. Experimental diabetes was induced by a single intraperitoneal injection of alloxan in a dose of 26 gm/100 g body weight. The animals were decapitated 30 days after injection of alloxan. The glucose concentrations in the urine and blood were determined by the glucose oxidase method, and the insulin level by radioimmunoassay.* At the time of sacrifice these parameters in animals with diabetes were 2.0%, 30.20 ± 0.53 mM, and 7.64 ± 0.9 μU/ml respectively, and in the control 0%, 6.05 ± 0.43 mM, and 24.15 ± 1.56 μU/ml. The medulla was fixed in 10% buffered neutral formalin, Carnoy's fluid, and "Susa" mixture. Celloidin-paraffin sections were stained with hematoxylin and eosin, with gallocyanin by Einarson's method, and by the PAS method after Hotchkiss (Fig. 1). The areas of the cell nuclei were measured by means of a "Vidiomat-1" television image anlayzer, and the results were subjected to statistical analysis by "Pidipi-12/20" computer.

## EXPERIMENTAL RESULTS

The experiments showed that AP in rats consists of two symmetrical cylindrical formations on the lateral surfaces of the caudal constriction of the rhomboid fossa, which merge more caudally into a single structure forming the roof of the cerebrospinal canal. The anterior surface of AP, facing the cavity of the fourth ventricle, is covered by flattened ependymal cells. The nuclei of the cells are round or oval in shape, and their nuclear membrane is often folded. The ependyma of AP is almost without cilia, and for that reason it has been called aciliate [5].

*The author is grateful to Doctor of Medical Science L. K. Stareseltseva for help with the biochemical tests.

Laboratory of Experimental Morphology, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 753-755, December, 1984. Original article submitted March 16, 1984.

0007-4888/84/9812-1751$08.50  

TABLE 1. Areas of Nuclei of Cells in AP of Control and Diabetic Animals (in $\mu^2$, $\bar{x} \pm m$)

| Region studied | Control | Expt. | $t$ | P |
|---|---|---|---|---|
| Ependymal cells: | | | | |
| AP | 25,5±0,4 | 34,8±0,5 | 15,04 | <0,001 |
| ASP | 22,0±0,5 | 36,6±0,5 | 19,59 | <0,001 |
| Glia-like cells: | | | | |
| Central parts of AP | 31,9±0,4 | 37,8±0,3 | 11,74 | <0,001 |
| Marginal zone of AP | 37,7±0,4 | 20,2±0,4 | 32,99 | <0,001 |
| Nerve cells of AP | 38,1±0,4 | 61,4±0,4 | 45,74 | <0,001 |

Legend. Number of nuclei measured was 1000 in each case. Distributions of areas of nuclei of all types of cells corresponded to normal ($x^2 < 12.02$, $P > 0.1$ compared with normal distribution).

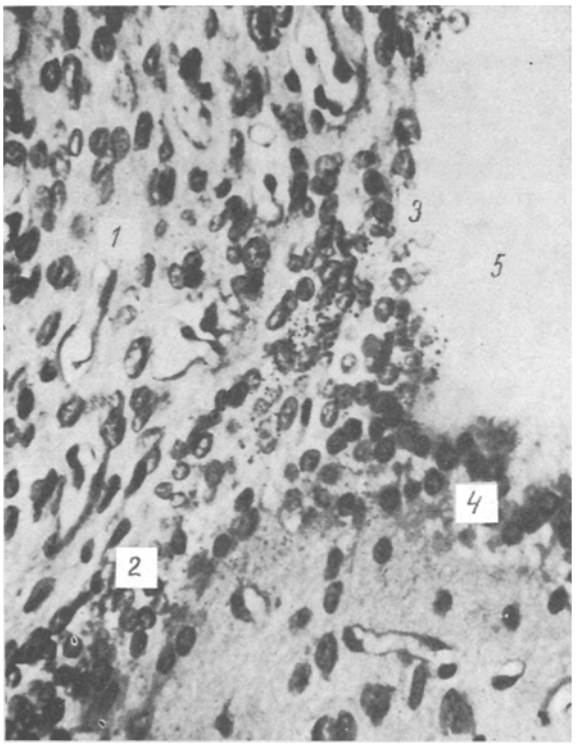


Fig. 1. Area postrema (AP) and area subpostrema (ASP) of rat with alloxan diabetes: 1) tissue of AP: nuclei of nerve and glial-like cells can be seen with blood capillaries; 2) marginal zone of AP; densely packed glia-like cells; 3) ependyma of AP, dark granules of PAS-positive secretion are visible; 4) ependyma of ASP; surface of ependyma covered by stratified deposits of amorphous secretion; 5) cavity of fourth ventricle. Stained by PAS reaction and counterstained with gallocyanin by Einarson's method. Objective 60, ocular 4.

Below the ventricular surface of AP nuclei of ependymal cells are arranged in several rows. Many cells have no cilia, but ciliated ependymal cells also are found. PAS-positive secretion can be seen in some cells and stratified deposits of secretion also are visible on the surface of cells in the cavity of the fourth ventricle. The subependymal tissue also

gives a bright PAS-positive reaction. This region, at the suggestion of several investigators [3], has been called the area subpostrema (ASP).

The tissue of AP consists of small, closely packed cells and a loosely structured neuropil and many blood capillaries pass through it. The parenchyma of the organ contains glial cells, small neurons, and cells which have been called glia-like [6, 7]. The glia-like cells have oval nuclei, a little larger than the nuclei of astrocytes, and irregularly shaped bodies which give off several processes; one of these as a rule, longer than the rest, endows the cell with polarity, and sometimes it can be traced as far as a vessel wall. Neurons of AP have a large pale nucleus with distinct nucleolus; the nuclear membrane often forms folds. The cytoplasm of the neurons is scanty and does not contain distinct Nissl's bodies, although it exhibits weak basophilia. At the periphery of AP the cell nuclei and fibrous structures of the neuropil are more densely packed than in the main part of the parenchyma. This region is described in the literature as the limiting or marginal zone [4].

Considering the topographic, morphological, and histochemical features of AP, we distinguished several types of cells in it, and subjected them to karyometric investigation in intact and experimental animals; the results are given in Table 1.

As Table 1 shows, ependymal cells of AP and ASP, and also nerve and glia-like cells of the central region reacted to alloxan diabetes by a significant increase in area of the nuclei, by 36.47 ± 2.44, 66.36 ± 3.21, 61.15 ± 1.48, and 18.50 ± 1.57% respectively, whereas the glia-like cells of the marginal zone responded with a decrease in area of the nuclei by 46.42 ± 1.5% compared with the control.

Karyometric data were supplemented by a study of the structure of AP of the animals with diabetes. The nuclei of the ependymal cells of AP of the experimental animals contained many chromatin granules, and sometimes flattened dark nuclei resembling pycnotic nuclei could be observed. The quantity of glycoprotein secretion in the ependymal cells of ASP was increased. The secretion formed stratified deposits on the surface of the ependyma, much more conspicuous than in the control animals. Glia-like cells of the central regions and marginal zone of AP had nuclei with paler karyoplasm than in the control and with many clearly stained chromatin granules. Moderate basophilia was observed in the cytoplasm of the nerve cells of AP, and some neurons contained brightly stained granules of Nissl's substance. Numerous small granules giving a bright PAS-positive reaction were found in the parenchyma of AP in diabetic animals, and their number increased in the marginal zone of this organ.

These results are evidence of the sensitivity of the different cells of AP to changes in carbohydrate homeostasis arising in the experimental model of alloxan diabetes. They confirm the previous hypothesis that AP is involved in the bulbar nervous mechanisms regulating carbohydrate metabolism. In these mechanisms AP perhaps plays the role of chemoreceptor for circulating insulin, a view which is supported also by recently discovered insulin receptors in the dendrites of nerve cells of AP [8].

## LITERATURE CITED


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