CYTOFLUOROMETRIC STUDY OF SMALL INTENSELY FLUORESCENT CELLS IN THE RAT ATRIUM AFTER PHARMACOLOGIC DESYMPATHIZATION

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KEY WORDS: fluorescent cells; desympathization; guanethidine.

The response of small intensely fluorecent (SIF) cells to guanethidine-induced desympathization has been investigated only in the case of the cranial cervical sympathetic ganglion. Chronic injections of guanethidine, as electron-microscopic studies have shown, lead to consistent changes in the number and morphology of the dense-core vesicles in SIF cells forming clusters. A marked decrease in the number of vesicles, but with preservation of their ultrastructure, is observed in some SIF cells. Meanwhile, accumulation of granules or maintenance of their original number but with an increase in their diameter and displacement of the electron-dense content of the organelles into a peripheral position, have been observed in certain SIF cells. Cytofluorometric determination of the catecholamine content under the conditions described above showed a decrease in the mean level of fluorescence to 0.7 of the control value, but the intensity of fluorescence in some SIF cells was unchanged [5]. Luminescence analysis of cultures of cells from the cranial cervical ganglion showed that the effect of guanethidine on SIF cells depends on its dose during chronic administration. In high concentrations guanethidine increased the number of detectable SIF cells but slightly reduced the intensity of their fluorescence [4].

The object of this investigation was to analyze the content of catecholamines detected by the condensation with paraformaldehyde reaction in atrial SIF cells, both single and grouped in clusters, at different times after chronic administration of guanethidine.

EXPERIMENTAL METHOD

Male Wistar rats weighing 120-150 g were used. The experimental and control groups consisted of 3-5 animals. Altogether 19 rats were used. The animals were given daily injections of guanethidine in a dose of 60 mg/kg body weight in physiological saline (10 mg/ml) intraperitoneally for 6 weeks. Under pentobarbital (40 mg/kg) anesthesia, 1 day and 3 and 6 weeks after the end of the guanethidine injections thoracotomy was performed on the animals under pentobarbital anesthesia (40 mg/kg) and perfusion carried out through the left ventricle with solution containing 1% glutaraldehyde and 4% paraformaldehye in 0.1 M phosphate buffer, pH 7.4. The atria were incubated in perfusion medium for 24 h, after which film preparations were obtained by microdissection for fluorescence analysis. The choice of regions of the atria was determined by the results of investigation of the topography of intramural SIF cells [2]. The region of the orifice of the superior vena cava, the oblique vein of the left atrium, the orifices of the pulmonary veins and coronary sinus, and the atrial septum were studied. The intensity of fluorescence was noted on a cytofluorometer with an excitation wavelength of 405 nm and with ZhS-18 and ZhZS-19 cutoff filters [3]. The level of fluorescence was determined for 100-120 cells from each animal in the group. The "Mir-2" computer was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

SIF cells of clusters and single cells of the regions chosen for analysis differed somewhat in their initial level of fluorescence. The mean intensity of fluorescence of SIF cells in clusters in this series of experiments was higher (by 18%) than that of single cells.

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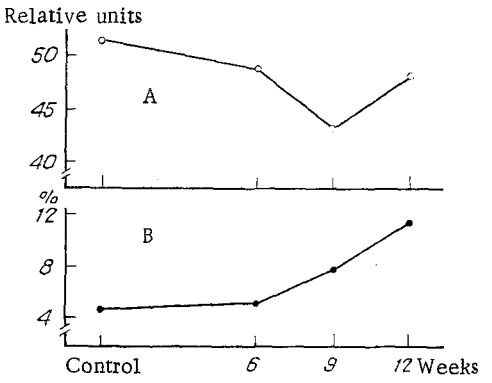

Fig. 1. Intensity of fluorescence of SIF cells (A) and number of SIF cells with processes (B) in rats' atria after desympathization. Abscissa, time of investigation (in weeks); ordinate: A) intensity of fluorescence (in relative units), B) number of SIF cells with processes (in %).

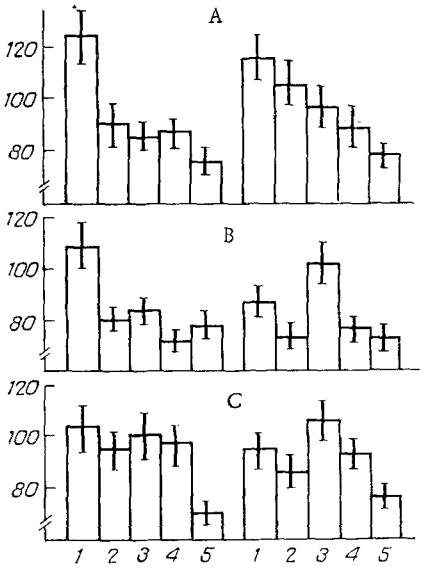

Fig. 2. Intensity of fluorescence of SIF cells (in % of initial level) of rats' atria 1 day (A), 3 weeks (B), and 6 weeks (C) after 6 weeks of guanethidine injections. 1) Superior vena cava; 2) oblique vein of left atrium; 3) coronary sinus; 4) orifices of pulmonary veins; 5) interatrial septum. Columns on left show SIF cells in clusters, on right — single SIF cells.

The level of fluorescence of atrial SIF cells fell during the 6-9 weeks after the beginning of the course of injections, but later it rose at approximately the same rate (Fig. 1A). However, separate analysis of this parameter for SIF cells in individual areas of the atria revealed definite differences.

Immediately after the end of guanethidine injections a significant increase in content of the fluorophore was observed compared with normal in cells organized into clusters in the region of the orifice of the superior vena cava, whereas in the remaining cells this parameter was reduced by 10-20%. After the next 3 weeks a further decrease in the content of fluorophore took place in SIF cells in clusters in the zones of the oblique vein of the left atrium, orifices of the pulmonary vein, and the interatrial septum. By the end of the 6th week after the last injection of guanethidine, the level of fluorescence of SIF cells in clusters in the region of the oblique vein of the left atrium, and the orifices of the pulmonary veins and coronary sinus were equalized and came close to the initial value. SIF cells

of the interatrial septum also continued to have a reduced level of fluorescence at this time (Fig. 2A).

By the end of guanethidine injections the intensity of fluorescence of the single cells was distributed along a declining curve from the orifice of the superior vena cava to the interatrial septum (Fig. 2B). A further fall in the level of fluorescence was recorded in single cells in all regions except the zone of the coronary sinus, where an increase in content of the fluorophore was observed compared with the previous time of observation. The ratio between the levels for individual zones determined during this period, remained the same for the next 3 weeks, during which SIF cells of all zones accumulated the fluorophore (Fig. 2B).

After desympathization, especially in the last 3 weeks of the experiment, an increase in the number of SIF cells with processes was observed in the atria (Fig. 1B).

The use of a single injection of guanethidine to produce a temporary fall in the level of sympathetic mediator in the heart leads to a moderate delayed reaction of part of the pool of atrial SIF cells [1]. The results of the present investigation, confirming the conclusion that there are regional differences in the reaction of the atrial SIF-cell pool to desympathization, at the same time indicate that the response of single SIF cells is similar in character to that of cells organized in clusters and located in the same zone of the heart.

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INTERMEDIATE FILAMENTS IN LUNG MACROPHAGES AND ENDOTHELIAL CELLS IN PATIENTS WITH CHRONIC ALCOHOLISM AND SUPPURATIVE DESTRUCTIVE LUNG DISEASES

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Intermediate filaments are thread-like structures in the cell cytoplasm whose mean diameter is about 10 nm. Together with actin microtubules and myosin filaments, the intermediate filaments belong to the principal filamentous system of eukaryote cells [2, 4]. Despite their ultrastructural similarity, these structures in epithelial, mesenchymal, and nerve cells consist of chemically heterogeneous subunits [3, 4].

This paper describes excessive accumulation of intermediate filaments in macrophages and endothelium of lung tissue in patients with chronic alcoholism and with suppurative destructive disease of the lungs.

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