CYTOFLUOROMETRIC STUDY OF SMALL HIGHLY FLUORESCENT CELLS IN RAT AURICLES AFTER A SINGLE INJECTION OF GUANETHIDINE

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Small highly fluorescent cells (SHF cells) in the tissue of the mammalian auricles have been found close to vessels and nerves in zones of distribution of autonomic intramural ganglia [7]. Two types of SHF cells are distinguished: type I) single cells with well-marked outgrowths; type II) round cells, grouped in clusters. According to Bjorklund et al. [3], SHF cells in rat auricles contain dopamine. Type I SHF cells have been classed by some workers as interneurons, whereas type II cells are generally regarded as performing an endocrine function. Activity of type I cells may evidently be regulated by the parasympathetic innervation, for cholinergic synapses have been found on some of them [6]. Type II cells may probably be under the influence of the sympathetic nervous system. Investigations by Jacobowitz [4] have demonstrated changes in fluorescence of auricular SHF cells by Falck's method after injection of reserpine. It is interesting to study how parameters characterizing catecholamine metabolism in SHF cells depend on the sympathetic mediator level in the heart. The absence of any marked effect of the cytotoxic action of guanethidine on SHF cells and the sharp fall in the noradrenalin (NA) level in the heart after a single injection of guanethidine indicate that this is a suitable model for use when assessing how SHF cell function depends on the sympathetic innervation.

In the investigation described below the reaction of auricular SHF cells, both single and in clusters, to a fall in the NA level in the auricles due to a single injection of guanethidine was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Guanethidine was injected into the animals in a dose of 60 mg/kg body weight in physiological saline (10 mg/ml). Guanethidine was obtained from the preparation "Isobarin" (from "Pliva," Yugoslavia), by extraction with physiological saline at 80°C. Each experimental and control group consisted of three or four animals. The total number of rats used was 14. Under pentobarbital anesthesia (40 mg/kg) thoracotomy was performed 3, 14, and 72 h after injection of the preparation, and the heart was perfused through the left ventricle with 50 ml of a solution containing 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 [5]. The zones of the orifice of the superior vena cava, the atrial septum, the oblique vein of the left auricle, and the coronary sinus were distinguished in the auricles under the binocular microscope. Film preparations for fluorescence analysis were obtained by microdissection. After incubation for 24 h in the perfusion fluid the preparations were transferred to slides and mounted in glycerol. The intensity of fluorescence was measured on a cytofluorometer, the action of which was based on the method of superposition of masks. An image intensifier, based on an image converter, was incorporated into the design of the instrument. The outlet window of the image converter was used as the screen. The intensity of fluorescence was measured after tracing around the outline of the cell and shading in the background on the screen of the image converter [1]. The wavelength of excitation was 405 nm, and cut-off filters ZhS-18 and ZhZS-19 were used. The intensity of fluorescence was measured for 50-60

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TABLE 1. Level of Fluorescence (in relative units) of Auricular SHF Cells under Normal Conditions and after a Single Injection of Guanethidine (M ± m)

Type of SHF cells	Control	Time after injection, h		
		3	14	72
I	51,3±7,4	44,6±4,7	62,1±6,8	57,5±5,4
II	51,1±1,6	49,2±1,3	53,2±1,6	50.6±1,5

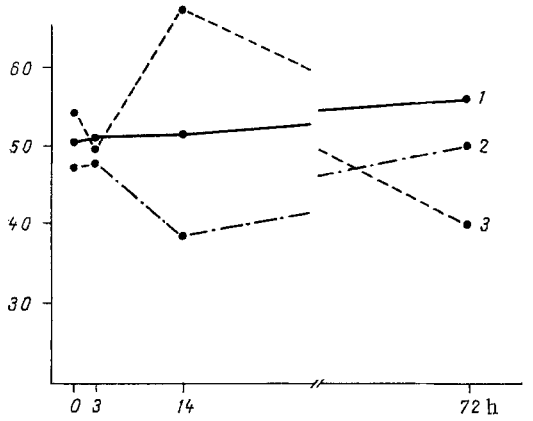

Fig. 1. Intensity of fluorescence of SHF cells of clusters in oblique vein of left auricle and coronary sinus (1), superior vena cava (2), and atrial septum (3) under normal conditions and after a single injection of guanethidine. Abscissa, time after injection (in h); ordinate, intensity of fluorescence (in relative units).

cells from each individual in the group. The results were subjected to statistical analysis on the MIR-2 computer.

EXPERIMENTAL RESULTS

The character of the general reaction of the SHF cell pool of the clusters in the auricles was estimated by averaging the fluorometric readings under normal conditions and after injection of guanethidine. The intensity of fluorescence of the cell clusters remained virtually unchanged and differences between means at all times of the experiments were not statistically significant (Table 1). The level of fluorescence of single SHF cells was 122% of the control 14 h after injection, and it remained at the 114% level until 72 h after the injection, but the wide scatter of the means makes these differences not significant (Table 1).

Further analysis revealed special features of the response of SHF cells in different zones of the auricles. The most labile with respect to the parameter studied were the SHF cells of clusters located in the atrial septum and the wall of the superior vena cava. Highest intensities of fluorescence, reaching 123% of the control ($P < 0.05$) were recorded in SHF cells of the septum toward 14 h after injection of guanethidine, and this was followed by a progressive fall to the level of 71% ($P < 0.05$) of the original value (Fig. 1). The dynamics of fluorescence of SHF cells of clusters in the superior vena cava was opposite in character: a minimum of fluorescence by 14 h and a return to values a little above the original level by 72 h after the injection. The intensity of fluorescence of the SHF cells in the region of the oblique vein of the left auricle and the coronary sinus was virtually unchanged at all times of the experiment (Fig. 1).

The over-all response of the auricular SHF cells is thus characterized by a narrow range of changes and by mildness of the aftereffects of guanethidine injection.

The times of determination of the aftereffects were chosen on the basis of results of measurement of NA levels in the rat heart [2]. During the first 3 h after a single injection of guanethidine a sharp fall was observed in the NA content in the heart. The minimal value of the NA level was 14 h after injection, rising ro 40% of the control after 72 h.

Changes in fluorescence observed in this investigation and corresponding to the above-mentioned times allow correlation between the functional state of the SHF cells and fluctuations in the NA concentration in the heart tissue. The direction of changes in the intensity of fluorescence reflecting changes in the catecholamine level in the SHF cells in the

individual zones — superior vena cava, atrial septum, oblique vein of the left auricle, and coronary sinus — evidently indicates a regional principle of organization of the SHF pool in auricular tissue.

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INVESTIGATION OF TRANSCRIPTION IN RAT SYMPATHETIC NEURONS AT DIFFERENT STAGES OF POSTNATAL DEVELOPMENT

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Morphological and functional changes arising in nerve cells in the course of life in connection with events such as differentiation, maturation, variations in functional load, and aging are closely linked with structural changes in the protein-synthesizing apparatus of the cell, leading to changes in the volume and spectrum of proteins synthesized [9, 13]. The concrete mechanisms of these structural changes have received little study, although there is no doubt that some of them at least are responsible for changes at the transcription level.

The aim of the present investigation was an autoradiographic analysis of transcription in the nuclei of sympathetic nerve cells of rats at different stages of postnatal development.

EXPERIMENTAL METHOD

Neurons from the cranial cervical ganglion of rats aged 1, 6, 13, and 25 days and 1, 5, 9, and 30 months (3-5 animals in each age group, 50 cells from each animal) were used as the test object.

To assess the state of transcription in the test neurons an autoradiographic method was used to demonstrate activity of endogenous RNA polymerases in the fixed cells [10]. For this purpose the sections through the cranial cervical ganglion 8 μm thick, cut on a freezing microtome at −20°C, were air-dried and fixed in alcohol—acetone (1:1 v/v) for 5 min at 4°C. The preparations were kept until required at −20°C. To each section 0.02 ml of an incubation mixture of the following composition (in μM) was applied: Tris-HCl buffer (pH 7.9) 100, sucrose 150, ammonium sulfate 80, 2-mercaptoethanol 12, ^{3}H-ATP (specific activity 27 Ci/mmole, from Radiochemical Centre, Amersham, England) 0.02, unlabeled triphosphates 0.6 of each, $MgCl_2$ 8, $MnCl_2$ 2. After application of the mixture the sections were incubated at 37°C for 30 min. The reaction was stopped by washing the preparations thoroughly in distilled water and they were then fixed for 30 min with ethanol—acetic acid (3:1 v/v). Unincorporated triphosphates were removed with 5% TCA (15 min at 4°C), after which the sections were rinsed for 30-60 min in tap water. The sections were dried, coated with type M emulsion, and exposed for 10 days.

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