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AGE CHANGES IN NUMBER AND INTENSITY OF FLUORESCENCE OF SMALL INTENSIVELY FLUORESCENT CELLS OF RAT AUTONOMIC GANGLIA

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UDC 612.891/.893:612.65/.086

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KEY WORDS: small intensively fluorescent cells; autonomic ganglia; cytofluorometry; postnatal ontogeny

The general principles of the postnatal development of the extra-adrenal chromaffin tissue in rodents are considered to be well established. It has been shown that reduction of the chromaffin tissue with age has many exceptions, characterizing both its species-specificity and its differences in different organs [4]. The tissue of paraganglia is preserved and actually increased in volume many times in Fisher 344 rats between the ages of 3 and 33 months [7], whereas the organ of Zuckerkandl is destroyed during the first week of postnatal development [6]. Small intensively fluorescent (SIF) cells contained in autonomic ganglia are evidently the earliest adrenergic structures to be differentiated. In rat ontogeny concentrations of SIF cells in the superior cervical sympathetic ganglion are observed from the 13th day of embryonic development [8] and the number of SIF cells in ganglia of the rat fetal heart reaches a maximum at the 29th day of pregnancy [9]. After birth the number of SIF cells in the superior cervical ganglion increases until the 23rd day of life, and thereafter undergoes very little change [5]. In rats of the long-living Fisher 344 line no marked changes in the number of SIF cells in the superior cervical sympathetic ganglion are observed with age, whereas in the great pelvic ganglion their number increases [7]. A more detailed study of the SIF-cell pool of the ganglia at successive stages of postnatal ontogeny is therefore interesting.

The aim of this investigation was to analyze the number of SIF cells and their content of paraform-induced fluorophores in the lumbar ganglia (LG) of the sympathetic trunk and in the great pelvic ganglion (GPG) of rats at the times of formation of the sympathetic and parasympathetic innervation and in later life.

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TABLE 1. Intensity of Fluorescence and Number of Small Intensively Fluorescent Cells in Lumber Ganglia of Sympathetic Trunk and in Great Pelvic Ganglia of Rats at Stages of Postnatal Ontogeny

Parameter	Times of postnatal development				
	¹ day	7 days	14 days	28 days	26-30 months
Intensity of fluorescence, relative units LG GPG Number of SIF cells	77,3±1,5 91,1±1,6	45,9±1,0 84,9±2,6	$59,9\pm1,5$ $53,5\pm1,5$	$94,8\pm2,2 \\ 74,3\pm2,1$	$211,1\pm 5.6$ 258.7 ± 6.3
LG GPG	$363\pm25\ 3606\pm237$	308±42 4153±452	120 ± 42 5105 ± 460	$^{44\pm 6}_{4967\pm 329}$	44±6 8317±1142

EXPERIMENTAL METHOD

The SIF cells of LG and GPG of rats aged 1, 7, 14, and 28 days and 26-30 months, with 8-12 animals in each group, served as test object. Altogether 48 rats were used. Under pentobarbital anesthesia (40 mg/kg) the animals were perfused with a solution containing 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3 [1]. The ganglia were removed, incubated in the same solution for 6-24 h, and frozen; serial frozen sections were cut to a thickness of 15 μ and mounted in glycerol. The cells were counted in all sections of the series. The intensity of fluorescence was measured on the LYUMAM IZ microscope-photometer with FMEL-2 photometric attachment, with excitation wavelength of 405 nm, a "green" light-dividing plate, and cutoff interference filter with peak transmission in the 495 nm region [2]. The results were subjected to statistical analysis on the CM 1403 computer.

EXPERIMENTAL RESULTS

Mean values of the number of SIF cells in ganglia of rats of different ages and of the intensity of fluorescence, in relative units, for all times of testing are given in Table 1. LG of the sympathetic trunk were found to lose most of the SIF cells in the first 2 weeks after birth. This process continues for the next 2 weeks, after which the number of SIF cells stabilizes. The intensity of fluorescence of the SIF cells falls a little in the first week of the animal's life, then rises steadily with age. Both parameters analyzed changed in the same direction during the first week.

The time course of the number of SIF cells in GPG is complex. The greatest increase in their number is observed in the first 2 weeks after birth. The level reached is maintained for the next 2 weeks, after which there is a further increase in the number of SIF cells in the ganglion. This increase is due mainly to growth of large concentrations of SIF cells. Changes in the number and intensity of fluorescence in the late age period in the case of GPG are in the same direction. The intensity of fluorescence of the SIF cells of GPG falls significantly in the period from the 7th to the 14th day of life, then regains its initial level in the 4th week of development, and exceeds the initial level considerably in later life. Determination of the number of SIF cells in sections through the autonomic ganglia may be affected by their fluorophore content. However, the contribution of this dependence is evidently very small, for the fall in the level of fluorescence of GPG in the 2nd week of development is accompanied by a definite increase in the number of SIF cells. The most marked decrease in the number of SIF cells in ganglia of the sympathetic trunk takes place at the beginning of the increase in their fluorophore content.

The results of this investigation are in agreement with those obtained by workers who observed an increase in the number and in the intensity of fluorescence of the SIF cells of GPG with age [3, 7]. Analysis of this effect must include analysis of the proliferative potential of chromaffin tissue cells. It is now considered that SIF cells and nerve cells of autonomic ganglia arise from common precursor cells, which are derivatives of the neural crest. Preservation of sympathetoblasts in the adult animal has not been demonstrated, but mitosis of an SIF cell in the autonomic ganglion of an adult guinea pig has been reported as a rare finding [10]. The opposite direction of postnatal changes in the number of SIF cells in different ganglia may be due to local factors stimulating their proliferative activity. The increase observed in the content of paraform-induced fluorophores in SIF cells of the autonomic ganglia with age is a phenomenon that is evidently characteristic of all SIF cells, irrespective of their location, whereas variations in the number of SIF cells form a picture that is characteristic of each type of ganglion.

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EFFECT OF IRRADIATION ON DIMENSIONS OF RAT CARDIOMYOCYTE MITOCHONDRIA

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UDC 612.172.014.21.014.482

KEY WORDS: mitochondria; function; morphometry; irradiation; laws of distribution; Pearson's and Kolmogorov's criteria

Correlation between the redistribution of dimensions of mitochondria and the biochemical parameters of their function [2, 5, 6] has been demonstrated by morphometric analysis of isolated liver mitochondria [7-11]. The time course of the changes in their size enables empirical data to be approximated by a theoretical gamma law of distribution [3]. It has been concluded from investigations that the parameters of this law of distribution, characterizing changes in the size of isolated liver mitochondria from irradiated control animals, are basic informative parameters of the state of the mitochondria, and it has now become possible to judge the functions of these organelles objectively by a study of electron-microscopic images.

The aim of this investigation was to study the particular features of rat cardiomyocyte mitochondria on the basis of changes in their size in response to external factors.

EXPERIMENTAL METHOD

Analysis was made of 16 series of experiments in which the dimensions of cardiomyocyte mitochondria were determined in 42 noninbred male albino rats weighing from 150 to 250 g. The first series of experiments constituted the control, namely cardiomyocyte mitochondria from intact, nonirradiated animals. In 13 series single irradiation in various doses was used: three series of experiments with a dose of 6 Gy, three series with a dose of 9 Gy, and seven series with a dose of 20 Gy. In three series of experiments, with a single dose of irradiation of 6 Gy, random samples of cardiac mitochondria were studied 2 and 24 h and 5 days after irradiation. In three series of experiments with irradiation in a dose of 9 Gy, cardiac mitochondria were studied after the same time intervals (2 and 24 h and 5 days). In seven series of experiments with irradiation in a dose of 20 Gy, cardiomyocyte mitochondria were analyzed after the following time intervals: 2 h, 1, 5, and 10 days, and 1, 6, and 12 months. The rats were irradiated on the TKhN-250 x-ray therapy apparatus, by means of a specially devised method of focusing the beam on the heart region [4]. Specimens of hearts for study were prepared for examination in the electron microscope by known methods, with fixation in buffered

Department of Pathological Anatomy, Central Research Laboratory, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Ekpserimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 751-754, December, 1988. Original article submitted January 13, 1988.