CYTOFLUOROMETRIC ANALYSIS OF SMALL INTENSELY FLUORESCENT CELLS OF RAT ATRIUM AT STAGES OF POSTNATAL ONTOGENY

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The principal parameters of ontogeny of the pool of small intensely fluorescent (SIF) cells have been established mainly for sympathetic ganglia [7]. The pool of atrial SIF cells has been studied as a rule in adult life. Morphological characteristics of SIF cells in atrial tissues have been found to be similar to those in tissues of sympathetic ganglia. For instance, cells clustered together and solitary SIF cells have been described. Patterns of their relations with blood vessels and neurocytes have much in common [8]. Various similar features also have been found with respect to the basic principles of topography and response of SIF cells to acute and chronic lowering of the sympathetic mediator level in the tissue of the intramural ganglia of the heart and paravertebral ganglia [1-3]. In rat ontogeny SIF cells have been identified as the first aminergic structures of the atria. In the rabbit heart SIF cells are most numerous on the 29th day of pregnancy, and their number evidently does not subsequently fall [7]. It is important to obtain data on age changes both in the number of SIF cells constituting the pool and the quantity of mediator in them, for these properties may be linked with luminescence-microscopic visualization of the cells.

The aim of this investigation was to analyze the number of SIF cells in nerve ganglia in different zones of the atria and their content of paraform-induced fluorophores.

EXPERIMENTAL METHOD

Atrial SIF cells of rats aged 1, 7, 14, and 28 days and 30 months, 3 or 4 animals in each age group, served as the test object. Altogether 16 rats were used. Under pentobarbital anesthesia (40 mg/kg) perfusion was carried out with a solution containing 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3 [2]. The atria were incubated in the same solution for 6-24 h, oriented in the same direction, and frozen; sections were cut on a freezing microtome to a thickness of 15 μ and mounted in glycerol. Photographs of the sections were used to map the SIF cells on the basis of the results of subsequent luminescence-microscopic analysis. Five zones were distinguished in the atria, in which large nerve ganglia were identified: the region of the superior vena cava (SVC), the left atrium (LA), the atrial septum (AS), the oblique vein (OV) of LA, and the coronary

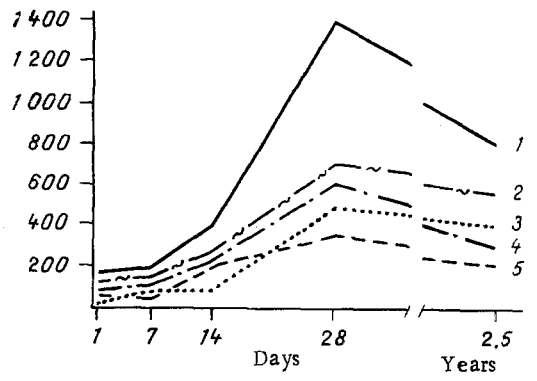

Fig. 1. Number of SIF cells in ganglia in regions LA (1), AS (2), OV (3), CS (4), and SVC (5) at different times of postnatal ontogeny. Abscissa, age of animals; ordinate, number of SIF cells.

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TABLE 1. Intensity of Fluorescence (in relative units) of SIF Cells in Regions of Atria at Different Times of Postnatal Ontogeny (M ± m)

Region of atrium	Time of postnatal development				
	1 day	1 week	2 weeks	4 weeks	30 months
SVC	85,6±3,5	40,5±1,5	41,8±0,8	80,4±2,5	96,4±2,9
AS	58,1±1,5	37,7±1,1	38,6±0,9	75,4±2,1	91,4±2,5
LA	89,7±3,0	42,3±0,9	37,5±0,8	93,7±2,8	99,7±2,3
OV	64,9±3,4	40,7±1,7	39,2±1,4	89,7±2,7	110,1±3,4
CS	54,6±1,5	42,3±1,2	27,5±1,3	75,2±1,7	112,4±2,9

sinus (CS). The intensity of fluorescence was measured on the LYUMAN I3 microscope-photometer, with FMÉL-2 photometric attachment, with a wavelength of exciting radiation of 405 nm, a "green" light-dividing plate, and cutoff filter with transmission maximum in the region of 495 nm [4]. The level of fluorescence was determined for 100-120 cells of each zone of the atria of each animal. The results were subjected to statistical anaysis on the SM 1403 computer.

EXPERIMENTAL RESULTS

The data given in Fig. 1 reflect changes in the number of SIF cells belonging to the intramural ganglia of the corresponding zones of the atria. In most cases SIF cells of different zones exhibit similar changes in this parameter at successive times of ontogeny. The distribution of the number of cells consists of a unimodal polygon of values with a maximum at 4 weeks. During the first 7 days of postnatal development the number of SIF cells in all zones of the atria did not change significantly. In the second week of development there was a marked increase in their number, and in the period from the 2nd through the 4th weeks, maximal values were reached. At a more advanced age all zones lost a certain number of SIF cells. The minimal range of age changes was observed in the ganglion in the region of SVC, the maximal range in the region of LA. SIF cells in LA, AS, and CS regions, accounting for the greater part of the pool, also determine the general pattern of distribution of the number of cells with age.

The table of values of intensity of fluorescence determined in the SIF cells at the same time demonstrates the common character of the changes in the postnatal period of development for all the zones distinguished. Some decrease in the content of the fluorophore was observed in the first week, during the next 7 days the level of fluorescence did not change statistically significantly, and later there was a marked rise until the 4th week and a small increase until the age of 30 months. However, despite the similarity of the general time course of the intensity of fluorescence, its initial level and the values reached in the adult state were characteristic of SIF cells of different regions. The minimal range of values of the mediator concentration corresponded to regions SVC and AS.

The time of determination of the number of SIF cells and the quantity of fluorophore in them were chosen in consideration of the known data on detection of SIF cells in ontogeny, and the direction of the change in their number under experimental influences. The most marked increase in the number of SIF cells following exposure to pharmacologic agents takes place in the period of 1-2 weeks [6]. The results of the present investigation indicate that this time interval is the basis for the increase in number of atrial SIF cells during normal ontogeny also. It is difficult to judge from the results described the causes of the age changes in the number of SIF cells, for the possibility of their postnatal proliferation and degeneration cannot be ruled out. An attempt to asssess the influence of the time course of the fluorophore concentration in the SIF cells on their enumeration in the atria as a whole was unsuccessful: the coefficient of correlation between these parameters did not exceed 0.3 at all times.

An increase in the number of SIF cells from 200 in newborn rats to 600 by the age of 23 days has been observed in the cranial cervical sympathetic ganglion [5]. The general principle of the age dynamics of the number of SIF cells and their mediator content recorded in that investigation may be evidence that the basic stages of formation of the SIF cell pool are linked with periods of formation of innervation relations in the ganglion.

LITERATURE CITED

1. L. A. Knyazeva and A. S. Pylaev, Physiology of Autonomic Ganglia [in Russian], Kiev (1981), p. 19.
2. L. A. Knyazeva, D. A. Kozlov, P. V. Sutyagin, et al., Byull. Éksp. Biol. Med., No. 7, 108 (1981).
3. L. A. Knyazeva, V. N. Yarygin, and A. S. Pylaev, Byull. Éksp. Biol. Med., No. 12, 90 (1982).
4. A. S. Pylaev, B. V. Turovetskii, and L. A. Knyazeva, Byull. Éksp. Biol. Med., No. 7, 114 (1985).
5. O. Eränkö and S. Soinila, J. Neurocytol., 10, 1 (1981).
6. H. Pärvärinta, S. Soinila, and O. Eränkö, Int. J. Dev. Neurosci., 2, 535 (1984).
7. J. Taxi, Int. Rev. Cytol., 57, 283 (1979).
8. E. van der Zypen, Cell Tissue Res., 151, 201 (1974).

SKELETAL MUSCLE LESIONS IN RATS WITH ACUTE ALCOHOL INTOXICATION

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Skeletal muscle damage is found in 20-60% of cases of alcohol intoxication [3, 5] and it is a special and significant component of alcohol disease. Experimental studies of the skeletal muscle, however, could not be found in the literature. It was accordingly decided to study lesions of the gastrocnemius muscles of rats with acute alcohol intoxidation.

EXPERIMENTAL METHOD

Experiments were carried out on 40 female Wistar rats weighing 200-230 g, divided into three groups. Animals of groups 1 and 2 (18 and 14 rats, respectively) were given a single dose of 1.2 ml/100 g body weight of 50° ethanol by gastric tube. Rats of group 2 were kept on a starvation diet for 48 h before the beginning of the experiment. In group 3 (control) eight rats were kept under similar conditions. The animals were killed by decapitation under ether anesthesia 24 h after the beginning of the experiment. Besides histological stains, histochemical and enzyme-histochemical methods were used to study glycogen, lipids, ribonucleoproteins, and oxidation-reduction and hydrolytic enzymes. Activity of oxidation-reduction enzymes and concentrations of lipids were determined by measuring the optical density of the reaction product with the LYUMAN-I-2 cytophotometer. Cytophotometric parameters of myocyte metabolism (in optical density units) are given in Table 1.

EXPERIMENTAL RESULTS

In response to administration of alcohol 12 rats of group 1 become apathetic and lethargic, and six became restless and excited. Toward the end of the first hour of the experiment all the animals fell asleep. In group 2, after starvation for 48 h, the animals sat still, in close contact with each other. After administration of alcohol and food for 2 h they were active. Examination 24 h after alcohol administration revealed a fixed posture, untidiness of the hair, and rapid respiration in 13 rats of group 1 and seven of group 2; the remaining animals moved actively around the cage. At autopsy moderate congestion of the internal organs was found, with petechial hemorrhages beneath the pleura and on the gastric mucosa. The liver was yellowish brown in color, sometimes with a nutmeg pattern at the edge. The skeletal muscle was pale brown in color and of the ordinary consistency.

Microscopic investigations of the skeletal muscles showed that most muscle fibers were polygonal in shape and the rest were round. Cross-striation in most fibers was well-marked. The nuclei were rod-shaped and distributed, 7-10 in number (normally 8-12), beneath the sar-

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