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HISTOMETRIC CHARACTERISTICS OF CAPILLARY–GLIA–NEURON RELATIONS OF THE CERVICO-THORACIC (STELLATE) GANGLIA OF CATS WITH EXPERIMENTAL ISOPROTERENOL NECROSIS OF THE MYOCARDIUM

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KEY WORDS: capillary–glia–neuron complex; cervico-thoracic ganglia; myocardium; experimental necrosis of the myocardium; electrocardiography; histometric analysis.

Investigations [4, 6] have shown that close morphological and functional correlation exists between the heart and the cervico-thoracic ganglia of the sympathetic trunk, which are the source of its postganglionic innervation. Morphological equivalents of irritation, in the form of coarsening of sensory nerve endings, hyperargyrophilia of the cytoplasma of the neurons, and pycnosis of their nuclei have been found in the cervico-thoracic (stellate) ganglia of persons dying from myocardial infarction [3, 9]. In previous investigations [5] the writer gave details of the architectonics of capillary–glia–neuron complexes in the normal cervico-thoracic ganglia. Since changes in the components of these complexes during the development of myocardial infarction have not been adequately studied, it was decided to examine this problem with the aim of shedding further light on the role of the neurovascular factor in the development of myocardial infarction.

EXPERIMENTAL METHOD

Experiments were carried out on 52 adult cats *(Felis domestica)*. Altogether 60 cervico-thoracic ganglia were studied under normal conditions and 44 at different times (3 h, 3 and 7 days, 2 weeks) after induction of experimental myocardial necrosis by three subcutaneous injections of isoproterenol (at intervals of 24 h) in a dose of 10 mg/kg body weight. The tests used to confirm the presence of foci of necrosis in the myocardium included electrocardiography and examination of sections from histological preparations of heart muscle stained with hematoxylin and eosin. The lower thoracic ganglia of the sympathetic trunk served as control objects. Six stellate ganglia taken at autopsy from persons dying from myocardial infarction also were investigated. Capillary–glia–neuron relations were investigated in microtome sections through the cervico-thoracic ganglia after staining with hematoxylin and eosin, by Van Gieson and Nissl's methods, and by gallocyanin and chrome alum. A suspension of Paris green in chloroform with ether was injected into their microcirculation. Histometric data on capillary–glia–neuron relations were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Under normal conditions cardiomyocytes stained with hematoxylin and eosin are cylindrical in shape, with a large, oval, weakly basophilic nucleus in the center of the sarcoplasm. Signs of hemostasis in the blood vessels and of myocytolysis [7], manifested as disappearance of cross striation, pallor of the sarcoplasm, and pycnosis of the nucleus and its displacement toward the periphery, were observed in the myocardium 3 h after the last injection of isopro-

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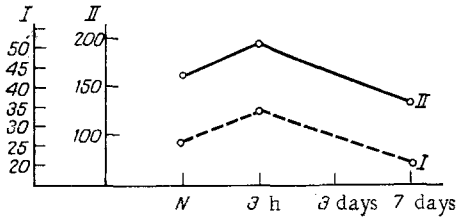

Fig. 1. Electrocardiographic data at different stages of experimental isoproterenol necrosis of the myocardium. Abscissa, time of experiment; ordinate, I) systolic index (in %), II) cardiac frequency.

TABLE 1. Dynamics of Histometric Indices of Capillary—Gliocyte—Neuron Complexes of Cervico-Thoracic Ganglia (in μ) under Normal Conditions and after Experimental Myocardial Necrosis (M ± m)

Time of experiment	Area of cross section of						Area of contact of capillaries with neurons	
	bodies of neurons		nuclei of neurons		nuclei of gliocytes			
	left side	right side	left side	right side	left side	right side	left side	right side
Normal	724,0±36,3	587,0±23,2	196,8±7,2	124,0±8,3	15,92±0,46	17,22±0,38	338,1±31,0	297,9±19,5
3 h	549,4±29,6*	535,0±34,2*	120,4±4,4*	123,3±4,8	28,1±0,6*	27,0±0,7*	349,2±22,5	337,3±29,1*
3 days	617,7±30,6*	574,2±25,1	117,4±3,9*	102,9±4,3*	22,7±0,5*	23,6±0,5*	337,8±28,1	310,4±29,9
7 "	524,2±30,3*	518,9±25,9*	71,1±4,2*	58,7±3,1*	19,2±0,5*	20,1±5,7	241,2±68,8*	376,8±48,9*
14 "	796,4±138,4*	753,8±36,3*	82,5±3,0*	106,9±5,2*	18,5±5,1	26,22	349,0±23,2	321,7±20,6*

*Differences between values are significant at the $p < 0.05$ level.

terenol. The muscle fibers showed different degrees of oxyphilia. Infiltration with round cells was accompanied by swelling of the wall of the large blood vessels. On the 7th day the degenerative changes in the myocardium described above had not progressed.

The morphological data, indicating disturbance of myocardial nutrition, were confirmed by the results of electrocardiography. Tachycardia, shortening of the R-R interval, widening of the Q wave followed by an increase in its amplitude, and an increase in the systolic index were observed 3 h after the last injection of isoproterenol. On the 3rd-7th days the electrocardiographic indices gradually returned to normal (Fig. 1).

Depending on the area of cross section of the nerve cells (the product of the larger and smaller diameters of the neuron), three groups of neurons were distinguished in the cervico-thoracic ganglia: 1) with an area of 200-600 μ^2, 2) 601-1000 μ^2, 3) 1001-1400 μ^2. Normally the second group of neurons was predominant (51%). After 3 h of the experiment a shift was observed toward predominance (66%) of neurons of the first group in the left cervico-thoracic ganglia. Morphometry showed that the area of cross section of neurons in the right cervico-thoracic ganglia was smaller than that of neurons of the left ganglia under normal conditions and in experimental myocardial necrosis. At these early periods of the experiment pycnosis of nuclei of the neurons was observed, more marked in the cervico-thoracic ganglia on the left side, together with marked dilation of the capillaries (10.7-5.2-14.7-1.7 μ), compared with normal (6.6-0.8-7.2-0.46 μ). The number of gliocytes surrounding one nerve cell normally and after 3 h of the experiment did not exceed three cells, but the area of their cross section increased sharply (28.14 μ^2 compared with 15.92 μ^2 normally). Whereas under normal conditions the distance between capillary and neuron (with the surrounding gliocytes) in most cases (48%) was 5 μ, after 3 h of the experiment the percentage of neuron—glial assemblages located at a distance of 10 μ from the capillary increased to 32% compared with the normal 20%. The change in distance could be due to a decrease in size of the neurons. On the 3rd day of the experiment hyperchromatosis and pycnosis of the nuclei with karyorrhexis were observed in individual groups of neurons in the cervico-thoracic ganglia. Against the background of unchanged nerve cells, in some places neurons with swollen or pycnotic nuclei or nuclei in the form of a faint shadow could be sharply dis-

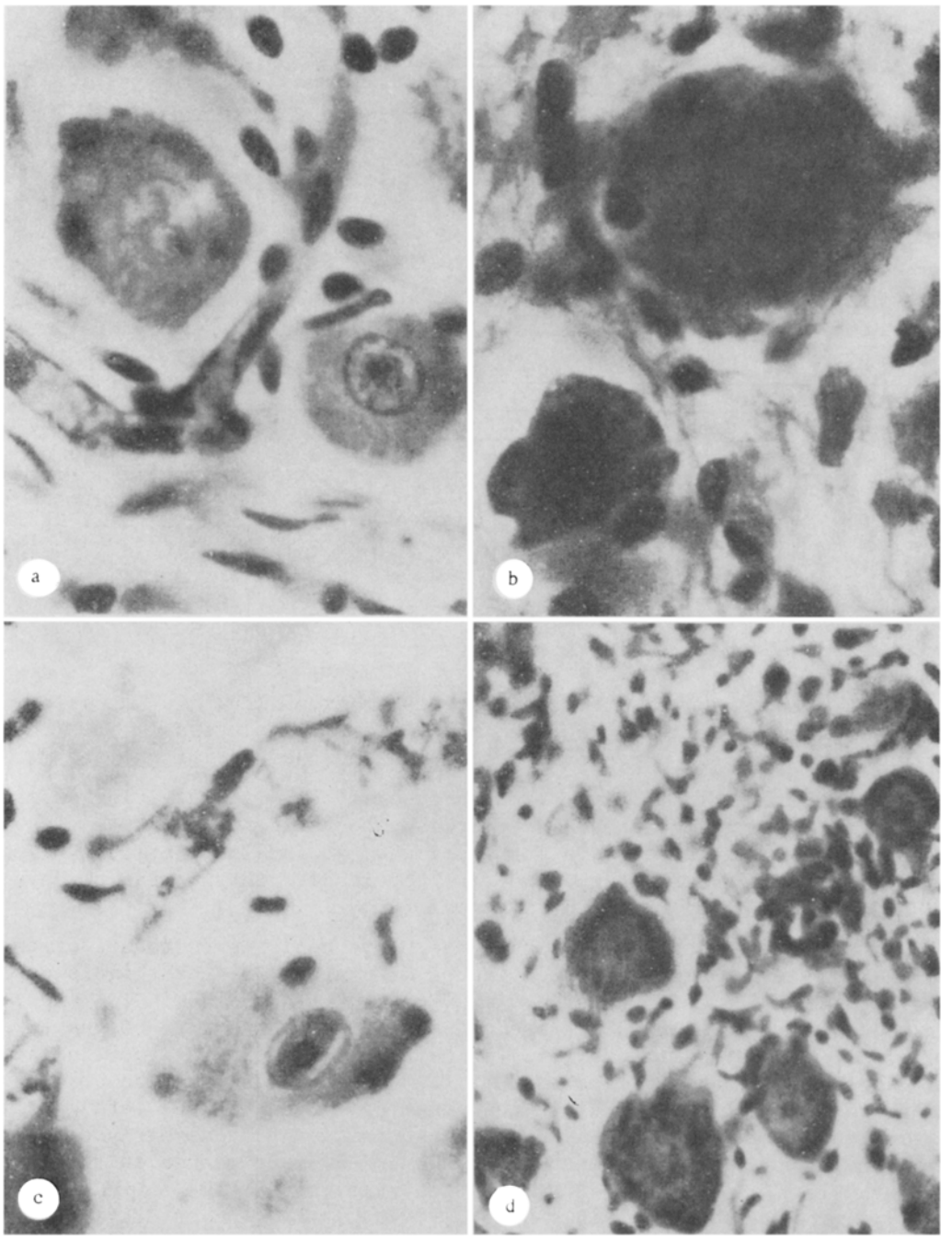

Fig. 2. Changes in structural organization of cervico-thoracic ganglia of a cat with experimental isoproterenol necrosis of the myocardium. Gallocyanin and chrome alum. a) Capillary—glia—neuron complex in normal cervico-thoracic ganglion; b) deformation of neuron bodies and neuronophagy on 3rd day of experiment; c) separation of nuclear substance from nuclear membrane (7th day); d) marked satellite infiltration on 7th day. Magnification: a, b) 900, c) 400, d) 80. Staining: a, c, d) gallocyanin and chrome alum. b) hematoxylin and eosin.

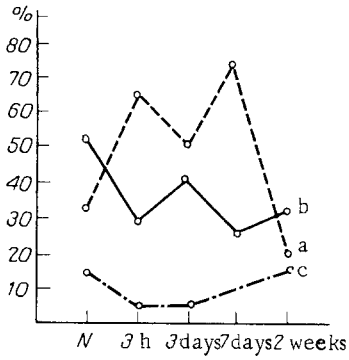

Fig. 3. Relative percentages of different groups of neurons in left cervico-thoracic ganglia in experimental isoproterenol necrosis of the myocardium: a) 1st group, b) 2nd group, c) 3rd group.

tinguished. On the 7th day pycnosis of the nuclei of the neurons and hyperchromatosis were observed, the substance of the nucleus was separated from the nuclear membrane, and satellite infiltration was marked. Deformation of some of the bodies of the neurons and neuronophagy could be seen (Fig. 2), in the form of deep penetration of the cytoplasm of the neuron by a glial cell. In the course of pycnosis and deformation of the cell body, it gradually was moved toward one pole of the connective-tissue capsule, which was greatly widened. Nuclei of satellites, with well-marked basophilia, were swollen. Their area of cross section increased on the 3rd and 7th days of the experiment to 22.68-19.22 μ^2 (compared with the normal 15.92 μ^2). At these same times, neurons of the first group with an area of cross section of 200-600 μ^2 predominated numerically. They accounted for 52% on the 3rd day and 75% of the total on the 7th day (Fig. 3). The number of gliocytes was very slightly increased on the 3rd day, but on the 7th-14th days there were 5.2-4.3 gliocytes surrounding one neuron.

On the 14th day of experimental myocardial necrosis abundant satellite infiltration took place with proliferation of connective tissue and death of some neurons. Starting with the 3rd hour of the experiment the area of contact between neurons and capillaries increased to reach a maximum on the 3rd day. On the 7th day the area was reduced, but by the 14th day it had again increased (Table 1). Similar changes were found in the stellate ganglia of persons dying from myocardial infarction. Small and medium-sized neurons, moreover, were predominantly affected. In the lower thoracic ganglia, used as the control, no changes were found.

This combined approach to the study of pathological manifestations of experimental myocardial necrosis in the cervico-thoracic ganglia could also be used to study and to identify the particular features not only of qualitative [1-4, 6, 8, 9], but also of quantitative changes in the histological characteristics of the capillary–glia–neuron complexes in the course of development of the pathological state. Definite correlation was found between the development of the electrocardiographic indices and the histopathological changes successively developing in the course of time in the cervico-thoracic ganglia of the sympathetic trunk. This metric asymmetry in the dimensions of the neurons in the right and left cervico-thoracic ganglia may point to the more important functional role of the latter in determining the pattern of extracardiac reflex regulation of cardiac activity. The absence of pathological manifestations in the lower thoracic ganglia indicates that the changes observed are specific for the cervico-thoracic ganglia. The results extend and deepen our existing ideas of the role of the morphological and functional integrity of the cervico-thoracic ganglia of the sympathetic trunk in the mechanism of development of myocardial infarction.

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ULTRASTRUCTURAL MANIFESTATION OF EARLY METABOLIC DISTURBANCES IN THE MYOCARDIUM OF DOGS WITH ALLOXAN DIABETES

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KEY WORDS: alloxan diabetes; glyconeogenesis; lysosomes; ultrastructure of the myocardium.

Metabolic changes in the myocardium play an important role in the development of cardiovascular lesions accompanying diabetes. The study of the state of the subcellular structures of the myocardium in the initial period of development of the disease is of great importance for clarification of the pathogenesis of these lesions and for the search for methods of rational treatment.

In the investigation described below the ultrastructure of the myocardium was studied in dogs after production of alloxan diabetes.

EXPERIMENTAL METHOD

Altogether 12 experimental dogs (with diabetes) and nine control dogs of both sexes weighing from 12.5 to 32.5 kg were used. Alloxan diabetes was induced by the method described in [1]. Observations began 1 month after the animals had developed diabetes. In blood taken from the aorta and coronary sinus of the control and experimental animals the insulin concentration was determined by a radioimmune method, glucose by the orthotoluidine method, nonesterified fatty acids (NEFA) by Duncombe's method [5], β-lipoproteins after Ledvina [4], and ketone bodies after Natelson [11]; in the myocardial tissue from the control and experimental animals activity of the following enzymes was determined: hexokinase [14] and phosphorylase [9]. Respiration coupled with oxidative phosphorylation was determined polarographically in the mitochondrial fraction isolated from the myocardium by differential centrifugation. Pieces of tissue were taken for electron microscopy from the subendocardial zones of the left ventricles. The material was processed in the usual way. Parallel studies were made of semithin sections from the same blocks, stained by McManus' method for the simultaneous detection of lipid and glycogen inclusions in the heart muscle cells.

EXPERIMENTAL RESULTS

With the model of alloxan diabetes used it was possible to produce diabetes of average and severe degrees in all the experimental dogs, with marked clinical manifestations. The

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