MORPHOMETIC AND AUTORADIOGRAPHIC ANALYSIS OF PROTEIN BIOSYNTHESIS AND TRANSCRIPTION IN SYMPATHETIC NEURONS OF NORMAL AND PARTIALLY DESYMPATHIZED RATS

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The structural features and intensity of function of the apparatus responsible for plastic metabolism are largely determined by the functional state of the neuron [2, 8]. Partial chemical desympathization of animals, increasing the load on those neurons which remain, necessarily leads to intensive functioning of their protein-synthesizing apparatus [6]. The writers have undertaken a differential morphometric analysis of the ultrastructural components for the protein-synthesizing apparatus: the rough endoplasmic reticulum (RER) and the ribosomes, polysomes, and monoribosomes in the cytoplasm of sympathetic nerve cells, connected with it, in rats aged 1 and 6 months in which different degrees of chemical desympathization were induced by guanethidine. Since changes in the pattern of function may be linked with changes in transcription [10], the effect of desympathization on template activity of the sympathetic neurons also was studied.

EXPERIMENTAL METHOD

Desympathization was carried out by subcutaneous injection of guanethidine (isobarin, from Pliva, Yugoslavia) in the animals from the 1st through the 3rd, 10th, or 14th day after birth, in a dose of 15 mg/kg daily. The rats were killed on reaching the age of 1 month (immature) or 6 months (young, mature). In the month-old animals the total number of cells was counted in every 5th serial section through the superior cervical sympathetic ganglia, taken from control and experimental rats. The ganglia were fixed in a 4% solution of paraformaldehyde in Hanks' buffer, pH 7.4, for electron microscopy, rinsed with the same buffer, and treated with the writers' modification of the OTO method (1% OsO_4 solution for 1 h, 1% thiocarbohydrazide solution for 1 h, 1% OsO_4 solution again for 1 h), followed by dehydration in alcohols of increasing strength and acetone, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were prepared and studied in the Hitachi-300 electron microscope (Japan). The area of the cytoplasm and the surface area of membranes of the RER were determined on electron micrographs with a magnification of 9000 with the aid of a morphometric grid with 10-mm step, and the coefficient of fragmentation of cisterns of the RER was calculated. Under a magnificantion of 30,000, the degree of granulation of the RER membranes (the number of ribosomes per micron length of the membrane) and the number of polysomes and monoribosomes per square micron area of the cytoplasm were measured. Altogether 12 cells were analyzed from each of four animals of each group, and cells in which the section passed through a nucleus with a nucleolus were chosen. For a comprehensive assessment, the coefficient of protein-synthesizing activity (K_{ps}) was calculated [4]:

$$K_{ps} = \frac{S_v^e \cdot N_L^{RE}}{K_{fr} \cdot 10} + \frac{N^{PR}}{10} + \frac{N^R}{100},$$

where K_{ps} denotes the coefficient of functional activity of the protein-synthesizing apparatus, S_v^e the surface area of RER membranes, N_L^{RE} the degree of granulation of the RER membranes (the number of ribosomes per micron length of membrane), K_{fr} the coefficient of fragmentation of the cisterns of RER (the ratio of the number of closed cisterns to its surface area), N^{PR} the number of polysomes per square micron area of the cytoplasm, and N^R the number of mono-

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TABLE 1. Effect of Partial Fractional Desympathization by Guanethidine on Ultrastructural Components of Protein-Synthesizing System of Neurons in Cranial Cervical Sympathetic Ganglion of Rats Aged 1 and 6 Months

Parameter	Rats aged 1 month				Rats aged 6 months			
	Control	Desympathization			Control	Desympathization		
		3 Days	10 Days	14 Days		3 Days	10 Days	14 Days
S_v^e	136,8±5,9	169,8±13,7	196,4±7,9	244,2±18,9*	222,7±18,8*, **	221,7±14,2*, **	244,0±10,1**	231,1±9,6*, **
K_{fr}	1,5±0,3	1,1±0,1	0,9±0,1	0,8±0,03	1,0±0,1	0,8±0,04	0,6±0,3**	0,5±0,02**
N_L^{RE}	40,9±1,7*, **	41,3±1,0*, **	56,4±1,4**	54,2±1,0**	37,0±1,6*	41,3±0,5*	46,3±0,7**	48,3±1,2**
N^{PR}	17,6±0,9	22,2±0,9	36,7±1,1**	34,3±0,9**	28,6±0,9	33,6±0,8	60,9±2,2**	56,9±1,6**
N^R	29,7±2,7	12,4±0,7	15,8±0,7	10,9±1,2	20,5±1,5	15,7±0,4	10,1±0,5**	10,0±0,5**
K_{ps}	407,0±39,8	686,3±90,7	1420,4±142,2	1999,2±175,9	895,2±100,2**	1034,7±88,8**	1969,1±145,6**	2113,8±110,3**

Legend. *P > 0.01 for comparison of values between rats aged 1 and 6 months, **P > 0.01 for comparison of values in rats of the same age.

ribosomes per square micron area of the cytoplasm.

To assess template activity of the chromatin, which was done on the animals aged 6 months, Moore's autoradiographic method [9] was used. To sections through a ganglion 8 μ thick, cut on a freezing microtome at -20°C and fixed in alcohol and acetone (1:1) for 5 min, 0.02 ml of a mixture containing (in 1 ml) 100 μmoles Tris-HCl buffer, pH 7.9, 150 μmoles sucrose, 80 μmoles ammonium sulfate, 12 μmoles 2-mercaptoethanol, 0.02 μmole ^{3}H-UTP, 0.6 μmole of each of the unlabeled triphosphates, 8 μmoles $MgCl_2$, and 2 μmoles $MnCl_2$ was applied. The sections were incubated at 37°C for 30 min. The reaction was stopped, the sections were thoroughly washed in distilled water, and they were then postfixed for 30 min in ethanol and acetic acid (3:1). Unincorporated triphosphates were removed with 5% TCA (15 min at 4°C), after which the sections were washed for 60 min in running water. The sections were dried and coated with 5% photographic emulsion. The level of template activity of the chromatin was determined as the number of grains of reduced silver counted separately above the nucleolus and nucleoplasm. The significance of differences between means was estimated by the Fisher–Student method.

EXPERIMENTAL RESULTS

Injection of guanethidine in a dose of 15 mg/kg from the 1st through the 3rd, 10th, or 14th days after birth caused death of 26.7, 60.2, and 75.2% of cells respectively in the cranial cervical sympathetic ganglion of the experimental rats, using a comparable weight of innervated tissue, reflected in the time course of the animals' body weight, which was 327.1 ± 2.5, 326.2 ± 3.3, 310.6 ± 5.4, and 330.5 ± 4.2 g in the control and at the above-mentioned times of injection of guanethidine respectively. At the age of 1 month, an increase in area of the RER membranes and in the number of ribosomes bound with them and of free polysomes in the cytoplasm, was observed in all the experimental animals (Table 1). The degree of this increase correlated with the level of desympathization. The number of membrane-bound and free polysomes did not differ significantly in the groups receiving injections of guanethidine for 10 and 14 days. The coefficient of fragmentation of the RER cisterns and the number of monoribosomes in the cytoplasm were less in the desympathized animals than in the control. The coefficient of functional activity of the protein-synthesizing apparatus of the cell, calculated on the basis of these parameters, was increased by 1.7, 3.5, and 4.9 times after desympathization for 3, 10, and 14 days respectively.

At the young reproductive age (6 months) the area of the RER membranes did not differ significantly in the control and all the experimental rats. The number of membrane-bound ribosomes and of polysomes in the cytoplasm remained higher in the desympathized animals. The ultrastructural correlates of the neuronal protein-synthesizing apparatus in the experimental rats receiving guanethidine for 10 and 14 days did not differ significantly (P > 0.01). The coefficient of functional activity of the protein-synthesizing system in these rats was more than twice as high as the control value. Activity of the protein-synthesizing system in the experimental rats receving guanethidine for 3 days was comparable with that in the control.

Thus the area of the RER membranes in neurons of the control animals and of rats receiving guanethidine for 3 days increased significantly between the 1st and 6th months of life, it was increased only a little in rats receiving guanethidine for 10 days, and unchanged in rats receiving the compound for 14 days. The area of the RER membranes increased with age in neurons of the control and experimental animals, as shown by a fall in the coefficient of fragmentation of the RER cisterns toward 6 months, but this process was more marked in the sympathized rats. The number of ribosomes associated with the RER membranes did not change between the 1st and 6th months in the control or in the experimental rats receiving the compound for 10 and 14 days. By 6 months the number of polysomes in the cytoplasm of the nerve cells also was increased in both the experiment and the control; this effect was more marked in the animals receiving guanethidine for 14 days. An increase in the coefficient of activity of the protein synthesizing system between the 1st and 6th months was observed in animals of all groups, but it was more marked (by 2.3 times) in the control, and less so in the experimental rats (by 1.5, 1.4, and 1.1 times, determined 3, 10, and 14 days respectively after desympathization).

Autoradiographic analysis indicated differences in the level of template activity of the chromatin in the control and desympathized animals aged 6 months. For instance, the total intensity of labeling of the nucleus and nucleoplasm was significantly higher than in the control, by 1.9, 2.6, and 2.7 times after administration of guanethidine for 3, 10, and 14 days respectively. This result was achieved mainly on account of an increase in the intensity of nucleolar labeling, which was increased by 2.3, 3.4, and 3.6 times, whereas the nucleoplasmic labeling was increased by 1.5, 1.7, and 1.8 times (the difference was not significant between groups of animals receiving guanethidine for 10 and 14 days in both cases). The intensity of total and nucleolar labeling differed significantly in neurons of animals receiving guanethidine for 3 days, on the one hand, and for 10 and 14 days, on the other hand.

According to data in the literature, development of chemically partially desympathized animals is not impaired [1, 3, 7], and this is confirmed by our results of determination of the time course of the body weight of the experimental rats. In this case the decrease in the number of neurocytes in animals receiving guanethidine ought to be accompanied by an increase in the functional load on the individual cell, leading to reorganization of its protein-synthesizing apparatus. In fact, hypertrophy of all the subcellular organelles of the protein-synthesizing apparatus, as well as the coefficient of functional activity calculated on their basis, demonstrated intensification of its function after desympathization. Marked correlation was observed between the level of desympathization and the morphometric parameters of intensity of functioning of the protein-synthesizing system at the age of 1 month, but at the age of 6 months differences between the ultrastructural parameters of intensity of protein synthesis in the control and in rats receiving guanethidine for 3 days were not statistically significant. This can evidently be explained on the grounds that after this period of desympathization a reduction in the cell population by one-third is insufficient to produce lasting compensatory changes in the remaining neurons. In the sympathetic ganglia of animals receiving guanethidine for 10 and 14 days death of more than two-thirds of the neurons leads to long-lasting ultrastructural changes; differences between the values of the parameters studied in animals of these two groups, moreover, were not statistically significant.

Intensification of the function of the apparatus for plastic metabolism in nerve cells subjected to different loads (including desympathization) has been observed by many investigators [2, 5, 7, 8]. However, the state of template activity under these circumstances has been studied only sporadically [10]. Yet it was natural to expect that the increase in the number of ribosomes which we found should have been brought about by corresponding changes in transcription. Autoradiographic study of sympathetic neurons in animals treated with guanethidine showed that the changes affect not only the structures which are responsible for translation, but also DNA-dependent RNA synthesis. The increase in the intensity of transcription was most marked for nucleolar chromatin. However, in the situation under examination the increase in template activity also affected extranucleolar sites, evidence of possible changes in the spectrum of synthesized proteins. The increase in template activity of neuronal chromatin of desympathized animals correlated to some extent with the degree of desympathization.

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MORPHOLOGICAL ASSESSMENT OF STRUCTURAL CHANGES IN ARTERIES AND ARTERIOLES OF THE KIDNEY IN EXPERIMENTAL RENAL ISCHEMIA

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The effect of experimental ischemia of varied duration on the structural organization of the renal parenchyma has been studied by several investigators [2, 4, 7, 9, 11]. Previous publications [5, 6] have contained descriptions of ultrastructural changes in the glomerular capillaries of the kidney and certain parts of the nephron in ischemia with or without restoration of the circulation it it.

In the present investigation structural changes in the walls of the intramural arteries and glomerular arterioles of the kidney, which characteristically give a marked spastic response [1], were studied during ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 84 adult Wistar albino rats with 7 animals used at each time. Animals of group 1 were killed 30 min and 1, 2, and 3 h after ligation of the left renal artery and vein, animals of group 2 were killed 3 and 30 days after restoration of the circulation through the artery. At the same time the right kidney was removed in order to bring the experimental conditions close to those of clinical transplantation of the kidney. The control group consisted of 10 animals. Pieces of kidney were fixed, dehydrated, and embedded in paraffin wax blocks. Sections 5-6 μ thick were stained with hematoxylin and eosin, with Weigert's fuchselin, and counterstained by Van Gieson's method. Material for electron-microscopic study was fixed in 1% OsO_4 solution and, after dehydration, embedded in a mixture of Epon and Araldite. Sections from the blocks were studied with the IEMB-100B and JEM-100B electron microscope with an accelerating voltage of 75 kV. For an objective analysis of changes in the parameters of the vessel wall, the vessels were subjected to morphometry, followed by statistical analysis of variance of the numerical data.

EXPERIMENTAL RESULTS

In the first 30 min after occlusion of the renal artery and vein of the left kidney appreciable changes were observed in the endothelial cells in the wall of the interlobar arteries (they swelled and projected into the lumen of the vessel). The inner elastic membrane (IEM) had clearly distinguishable coils. The smooth-muscle cells (SMC) in the media followed a circular course and their nuclei remained elongated in shape. The outer elastic membrane contained shallow indentations (Fig. 1a). In the smaller arteries (arcuate, interlobular)

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