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CYTOLOGICAL ANALYSIS OF SYMPATHETIC NUERONS OF NORMALLY DEVELOPING AND PARTIALLY DESYMPATHIZED RATS AGED 1 AND 5 MONTHS

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When administered to animals for several days after birth guanethidine induces a marked decrease in the number of sympathetic nerve cells [5]. Depending on the program of its administration, desympathatization in the rats may be partial or virtually complete [2]. Since the physical development of partially desympathized animals is affected only a little, they are convenient objects with which to study the development of sympathetic neurons when the volume of innervated tissues is increased.

In the investigation described below neurons of the cranial cervical sympathetic ganglion of sexually immature and young mature rats were studied during normal development and after partial chemical desympathization by guanethidine at an early age.

EXPERIMENTAL METHOD

Desympathization was induced by subcutaneous injection of guanethidine (Izobarin, from Pliva, Zagreb, Yugoslavia) subcutaneously from the 1st through the 14th day after birth in a dose of 15 mg/kg daily. When the rats of the experimental and control groups had reached the age of 1 month (immature) or 5 months (young mature; Zapadnyuk's classification [3]), their cranial cervical sympathetic ganglia were incubated *in situ* for 30 min in a solution of ^{3}H-leucine (specific activity 40 Ci/mmole). The animals were killed 1 h after the end of incubation and the cranial cervical sympathetic ganglia were fixed in Carnoy's fluid and embedded in paraffin wax. Sections 7 μ thick were coated with type M nuclear emulsion, exposed for 1 month at 4°C, and stained with methylene blue. Autoradiographs were analyzed by counting grains of silver per unit area of cytoplasm of the nerve cells. The number of neurons with a well outlined nucleus was counted in every 5th section in serial sections through the cranial cervical sympathetic ganglion of the experimental and control animals. Summation of the values for all the 5th sections served as an index of the number of cells in the ganglion. The volume of the perikarya was determined by regarding them as approximately an ellipsoid of rotation, by the formula $V = (\pi/6)AB^2$ where A is the major and B the minor diameter of sections of the perikaryon containing the nucleus with nucleolus, measured by means of a screw ocular micrometer.

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TABLE 1. Effect of Partial Chemical Desympathization by Guanethidine on Number and Size of Nerve Cells and Surface Area of Membranes of Cytoplasmic Reticulum in Neurons of Cranial Cervical Sympathetic Ganglion in Rats Aged 1 and 5 Months (M ± m)

Age of animals, months	Group of animals	Number of nerve cells, $\times 10^3$	Mean volume of body of nerve cells, μ^3	Surface density of membranes, μ^{-1}	Surface area of membranes, μ^2
1	Control	5,64±0,68	1510±53	3,06±0,12	4620±181
	Expt.	1,02±0,28* (18,1%)	2030±79*	3,20±0,10	6496±203*
5	Control	5,88±1,04	2658±69**	2,70±0,08**	7176±196**
	Expt.	1,15±0,48* (19,6%)	5090±167**	2,72±0,12**	13844±610**

*P < 0.01 compared with control group of rats of same age.
**P < 0.01 compared with rats aged 1 month.

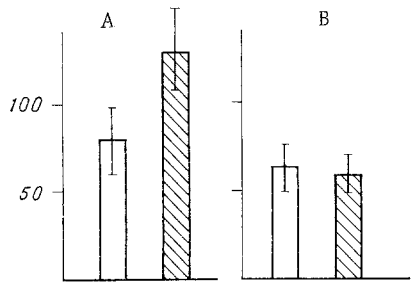

Fig. 1. Concentration of label above cytoplasm of neurons 1 h after injection of ^{3}H-leucine. Rats aged: A) 1 month, B) 5 months. Unshaded columns — control, shaded columns — experiment. Ordinate, concentration of label (in conventional units.)

The cranial cervical sympathetic ganglia of some of the experimental and control animals were fixed with potassium permanganate and embedded in Epon and Arladite. Ultrathin sections were stained with lead and photographed in an electron microscope. On electron micrographs of cross-sections through perikarya containing the nucleus with nucleolus, the surface density of membranes of the cytoplasmic reticulum was determined with the aid of a test grid. Knowing the mean volume of the perikarya, the total surface area of the membranes of the cytoplasmic reticulum could be determined [1].

EXPERIMENTAL RESULTS

The rate of physical development of the experimental and control rats was perfectly comparable. For instance, the weight of the desympathized and normally developing animals was 54.9 ± 2.0 and 58.2 ± 2.0 g respectively at the age of 1 month and 209 ± 7.7 and 212 ± 8.0 g at the age of 5 months. As Table 1 shows administration of guanethidine by the chosen program caused death of more than 80% of nerve cells in the cranial cervical sympathetic ganglion. The volumes of the perikarya of the viable neurons in the experimental animals were higher in both age groups. The intensity of labeling of the cytoplasm of cells of the desympathized rats after injection of ^{3}H-leucine exceeded ($P < 0.05$; Fig. 1A), and at the age of 5 months was equal to the corresponding intensity in the control animals (Fig. 1B).

The surface density of membranes of the cytoplasmic reticulum was the same in the experimental and control rats. However, because of an increase in the volumes of the nerve-cell bodies, the surface area of membranes of the cytoplasmic reticulum was greater in the desympathized rats (Table 1). The surface density of membranes of the cytoplasmic reticulum was lower in the control animals at the age of 5 months than at the age of 1 month, but since the volumes of the neurons increase with age, the total surface area of the membranes of the cytoplasmic reticulum was greater.

Age changes in the dimensions of the perikarya of nerve cells of the normal and desympathized animals revealed definite shifts in the parameters of the state of the translation apparatus (Table 1). The total surface area of membranes of the cytoplasmic reticulum and incorporation of the labeled precursor for protein biosynthesis by nerve cell bodies were

greater in partially desympathized rats at the age of 1 month than in control animals of the same age. When the control and experimental rats reached a young reproductive age, parameters such as the surface density of membranes of the cytoplasmic reticulum and the intensity of incorporation of ^{3}H-leucine were observed to fall and to equalize. Meanwhile, because of the increase in volume of the nerve-cell bodies, the total surface area of membranes of the cytoplasmic reticulum increased, more especially in the experimental rats.

The fact that the age dynamics of the neurons, as reflected in the parameters studied, coincided in normally developing and partially desympathized animals suggests that desympathization introduces no radical changes into the process of postnatal development of the sympathetic nerve cells and, in particular, of their cytoplasmic structures responsible for protein biosynthesis. In that case the quantitative differences, especially as regards the specific parameters in rats aged 1 month, could be evidence of a higher intensity of structural metabolism in animals receiving guanethidine, because of the disparity between the number of viable neurons and the quantity of tissue to be innervated. The metabolic potential of the nerve-cell perikaryon is known to be intended largely to provide for structural and functional parameters of the processes and terminals. Increased activity of structural metabolism observed in partially desympathized rats at the age of 1 month could be aimed at compensating for the deficit of sympathetic innervation of the organs through additional growth of axons. It is interesting to compare this idea with the results of an investigation carried out by Borisov et al. [2], who showed that the initial sharp depression of pressor reflexes in desympathized rats is followed by their partial recovery by the age of 4 months.

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