EXPERIMENTAL BIOLOGY

Effect of Neonatal Testosterone on the Chromatin Matrix Activity and Perikaryon Size of Rat Sympathetic Neurons

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 12, pp. 683-685, December, 1997
Original article submitted October 25, 1996

Testosterone administered to neonatal rats increased the number of neurons, their growth rate, and chromatin matrix activity in the cranial cervical ganglion. Transcription activity in the test animals decreased to the 3-month age and was below control value to the end of the first year of life. Testosterone is thought to accelerate neuron differentiation in the cranial cervical ganglion, and the increased number of neurons makes it possible for individual cells to maintain matrix activity at a lower level.

Key Words: *cranial cervical ganglion; testosterone*

Similar to the entire sympathetic nervous system, maturation of neurons in sympathetic ganglia is not completed at the moment of birth. Formation of definitive phenotype and stabilization of cell population in the cranial cervical ganglion (CCG) in rodents is finished mainly at the end of the first month of life [2]. During the early postnatal ontogenesis, some neurons die as a result of apoptosis, and in the females this process is more intense [4,9]. Exogenous testosterone inhibits the natural process of cell death when administered to the neonatal rat females [6,9].

Our aim was to ascertain whether the effect of testosterone depends on its total dose and to study the age dynamics of some cytological indexes of CCG neurons under neonatal androgenization.

MATERIALS AND METHODS

Experiments were carried out on 42 female Wistar rats of four age-grades: juvenile (1 month), young (3 month), young reproductive (6 month), and mature reproductive (12 month) in accordance with classification [1].

Oil solution of testosterone propionate (TP) was injected subcutaneously in a dose of 80 mg/kg starting from day 3 after birth and then every 48 h according to the scheme [6]. Some animals were given different numbers of injections (from 8 to 14) in order to study dose-dependence of TP effect. The animals were killed on day 30 under ether narcosis. Other animals received TP during the entire first month of life (14 injections) and were used for investigation at different ages. Control animals were injected with sterile olive oil according to the same scheme.

Cells in CCG were counted on serial paraffin slices with the width of 7 μ. The neurons with nucleoli were counted for every 5th slice; the resulting data were averaged. This mode of counting takes into account only those neurons that were cut through geometric center of the soma. The maximum and minimal cell diameters were measured with the help of an MOV-1 screw ocular micrometer at ×1500. The product of these values corresponds to the neuron profile field in relative units.

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0007-4888/97/0012-1229$18.00

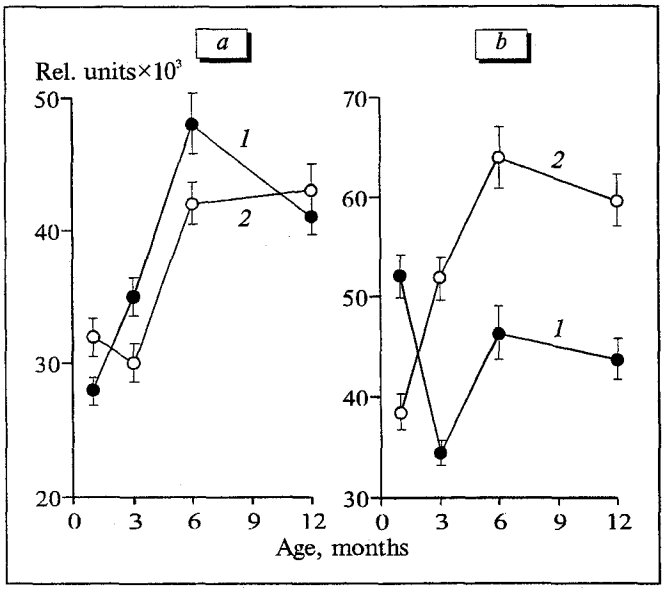

Fig. 1. Age dynamics of perikaryon size (*a*) and overall nuclear chromatin matrix activity (*b*) of neurons in the cranial cervical ganglion: *1*) test, *2*) control. Ordinate: *b*) number of silver reduced grains over nucleus.

Autoradiographic determination of endogenous RNA polymerase activity [8] was used to study the chromatin matrix activity. The CCG slices with the width of 8 μ were cooled in a cryostat and used in the study. The preparations were exposed simultaneously, which made it possible to compare the results obtained in different age groups. Transcription activity of nuclear chromatin was evaluated by the number of reduced silver grains over a nucleolus and nucleoplasm at magnification 1000.

The results were statistically analyzed using Student's *t* test.

RESULTS

In our experiments, TP did not change the number of neurocytes in female rat CCG during the first 3 weeks of life. A significant increase in the number of nerve cells (from 18,092±1595 to 22,478±849, $p<0.05$) was observed only after 10 injections of the hormone, i.e., on postnatal day 21. After 14 injections (postnatal day 30), the number of neurocytes in CCG increased to 26,625±1069, which is consistent with the observations of others that TP increases the survival rate of CCG neurons when administered to neonatal males [9] and females [6].

This phenomenon was not accompanied by any increase in the perikaryon size. By contrast, the average index was somewhat greater in the control group. The size distribution histograms in one-month-old animals demonstrate an increased proportion of small and middle-size cells in comparison with the control group.

During the subsequent period the hypersympathetic animals demonstrated a larger growth rate of CCG neurons in comparison with the control group: at the age of 3 and 6 months, the average size of cells in the test group was larger than that in the control group ($p<0.05$), although this difference was leveled by the end of the 1st year of life (Fig. 1, *a*). It should be noted that in young and mature age, the CCG neurocytes are characterized by a large variety of size. For example, the profile field of the largest cells in 6-month-old animals is about 10 times as large as that of small neurons, the proportion of large neurons being higher in the test group.

The data on the chromatin matrix activity showed that the mean value of transcription rate in one-month-old test rats was larger both in nucleolar (9.8±0.3 silver grain) and extranucleolar (41.0±1.1) sites in comparison with the control values (8.9±0.3 and 28.7±1.1, respectively). During normal ontogenesis, enhanced transcription activity was observed in the nuclei of 6-month-old rats (Fig. 1, *b*). At the same time, prenatally androgenized rats demonstrated a drastic decrease in matrix activity to the 3-month age down to 7.6±0.3 in nucleoli and to 26.6±0.8 in nucleoplasm, while the respective control values were 13.5±0.5 and 37.6±1.2. During subsequent development, the dynamics of transcription activity was similar in test and control animals, though this index was low under hypersympathization.

Administration of TP to neonatal rodent females is known to increase the mass of submaxillary salivary gland (SMSG), which is one of the target organs for CCG neurons. Moreover, exogenous TP enhances the synthesis of nerve growth factor in SMSG [5,7]. Our data show that in 3-month-old rats treated with TP during the neonatal period, the ratio of SMSG mass to body mass was $5.53\pm0.33\times10^{-4}$ in experimental, and $3.80\pm0.40\times10^{-4}$ in control rats ($p<0.05$). The same was true at the end of the first year of life: $4.27\pm0.03\times10^{-4}$ for test and $2.87\pm0.23\times10^{-4}$ for control rats ($p<0.01$).

Previously it was shown that the increase in the matrix chromatin activity occurs in CCG neurons in the young and mature age [3]. This effect was attributed to cell adaptation to incremented functional load. In our experiments the enhanced number of CCG neurons may decrease the functional load per individual cell, and such a decrease took place under augmented trophic support by retrograde transport of the nerve growth factor from SMSG. This made it possible for neurons to maintain a lower intensity rate of transcription over the entire mature period of life in comparison with that in control animals. The high activity of matrix chromatin in one-month-old hypersympathetic animals can be explained by ac-

celerated neuronal differentiation and increased metabolic activity of neurons related probably to an earlier onset of the growth processes.

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