DNA CONTENT IN NEURONS OF INTRAMURAL GANGLIA OF THE MOUSE HEART DURING STIMULATION OR INJURY TO THE SYMPATHETIC NERVOUS SYSTEM

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After massive injury to the sympathetic nervous system, hypertrophy and polyploidization take place in neurons of the intramural ganglia of the mouse heart. The effect observed is a unique response produced by massive blocking of the sympathetic innervation. In response to stimulation of the symptathetic nervous system no significant changes were found in the DNA content in the neurons studied.

Quantitative cytochemical determination of DNA has demonstrated the widespread occurrence of polyploid neurons in different parts of the nervous system. Tetraploid neurons have been found in the ganglionic layer of the retina, among the Purkinje cells, among neurons in the anterior horns of the spinal cord, in spinal ganglia, and in ganglia of the sympathetic chain [2, 3, 7, 8, 12]. Comparison of polyploid and diploid neurons shows that the former have nuclei and cell bodies of larger volume. The metabolism of the polyploid cell corresponds fully to its increased mass [4, 5]. The increase in number of polyploid cells in the nervous system has been shown to occur in various compensatory processes resulting from death of a considerable number of neurons [4, 5, 7, 8].

This paper describes the results of cytophotometric analysis of the DNA content in the nuclei of neurons in the intramural cardiac ganglia of mice during stimulation of or injury to the sympathetic nervous system.

The sympathetic nervous system was stimulated by injecting nerve tissue growth factor (NTGF) – a protein preparation isolated from the submandibular salivary glands of male mice – into newborn animals. This produces marked hypertrophy of the bodies of the sympathetic neurons and hypoplasia of their processes [13]. An increase in the intensity of ribonucleoprotein and protein synthesis is observed in the hypertrophied neurons. Polyploidization of the nuclei of some sympathetic neurons has been recorded [8, 9, 13].

After injection of antibodies against NTGF into newborn mice, massive selective injury to the cell population of ganglia of the sympathetic chain is observed [10-16].

The fact that experimental animals with different levels of function of the sympathetic nervous system can be produced in this way is of considerable interest on its own account, but it also enables the responses to these changes to be studied in another component of the system – the intramural part. A direct response of this part to NTGF has been denied [16].

Analysis of unit responses of the intramural part to such powerful injury to or hypertrophy of the sympathetic component can shed light on the functional relations between these parts of the system, and the investigation described below was carried out for this purpose.

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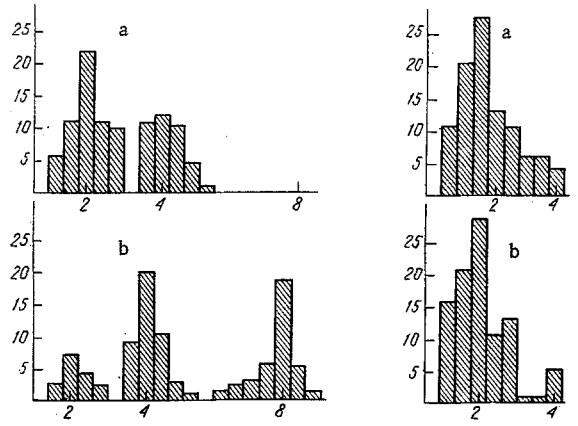

Fig. 1

Fig. 2

Fig. 1. Histogram of DNA content in intramural neurons of heart of month-old normal mice (a) and mice receiving antiserum against NTGF (b). Here and in Fig. 2: abscissa, DNA content (in ploidy units); ordinate, number of cells.

Fig. 2. Histogram of DNA content in intramural neurons of the heart of 14-day normal mice (a) and mice receiving NTGF (b).

EXPERIMENTAL METHOD

NTGF and antiserum against it were injected subcutaneously into newborn BALB mice. The mice received NTGF for the first 14 days after birth in a dose of 500 biological units/g body weight. The antibodies were injected on the first 5 days after birth in doses of 0.07, 0.09, 0.1, 0.12, and 0.15 ml/g. Mice of the same litter and kept under the same conditions were used as control. The mice receiving NTGF were killed on the 14th day (immediately after the end of the injection) and those receiving antiserum against NTGF were killed 1 month after birth. The hearts of the control and experimental animals were fixed in a formalin–ethanol–acetic acid mixture (3 : 1 : 0.3) and embedded in paraffin wax. Serial sections were cut and the localization of the intramural ganglia determined. In each individual case the thickness of the section was measured. To determine the DNA content in the sections from the intramural ganglia the Feulgen reaction was carried out under strictly standard conditions. The DNA content in conventional and comparable units was calculated as the product of the optical density and the volume of the nucleus. To determine the volume the outlines of nuclei were traced with a drawing apparatus. The optical density of the nuclei was measured with a probe cytophotometer by the one-wave method [1]. Nuclei with the largest diameter in one of a series of sections were examined photometrically. The DNA content in the nuclei was compared with the diploid standard – the DNA content in the nuclei of small splenic lymphocytes – and expressed in ploidy units. Survey sections were stained with toluidine blue.

EXPERIMENTAL RESULTS

Investigation of the intracardiac ganglia after blocking of the sympathetic system by injection of antiserum against NTGF revealed several characteristic pictures. Nearly all the neurons contain more than the normal number of nucleoli (from 2 to 4). Hypertrophy of the bodies of the neurons and glial cells was observed. The glia formed dense capsules around the nerve cells. Whereas normally the cell population of the cardiac intramural ganglia can be divided into several subgroups by the character of distribution of the Nissl's substance, in the present experiments nearly all the neurons had the same type of distribution of the tigroid. This type of structural uniformity suggests increased functional activity of the neurons of the intramural ganglia. Similar conclusions have been drawn by other workers under different experimental conditions [6]. The results of measurement of the DNA content in nuclei of the intramural neuron control month-old mice are shown in Fig. 1a. Analysis of the histogram shows that the overwhelming majority of neurons have diploid nuclei. Few polyploid nuclei are found in animals of this age; about 15% of the total number of neurons have tetraploid nuclei.

The results of measurement of the DNA content in the nuclei of the intramural neurons after immunosympathectomy revealed a different pattern (Fig. 1b). Analysis of the histogram reveals a marked difference in the distribution of DNA compared with the control: the histogram is shifted to the right and most neurons of the cardiac intramural ganglia of the experimental mice have polyploid nuclei (tetraploid and octaploid). Only a very small proportion of the total number of nuclei measured were diploid. The group of tetraploid nuclei was much greater than in the control animals. There was a large group of octaploid nuclei, although in the control group not a single octaploid nucleus could be found.

Quantitative cytochemical determination of the DNA content thus revealed certain differences in the cytological organization of the intramural neurons after injury to the sympathetic nervous system.

Measurements of the areas of the neurons showed that in the immunosympathectomized mice the nerve cell bodies were much larger (420 ± 11.3) than in the control (318 ± 9.7).

Visual assessment of the sections of the intramural ganglia from animals of the second experimental group receiving NTGF and the control group showed no significant morphological differences between them. The results of measurements of the DNA content in nuclei of neurons of the intramural ganglia of the control 14-day mice are shown in Fig. 2a. In the animals of this age most neurons of the intracardiac ganglia were diploid.

The histogram (Fig. 2b) of the DNA content in the nuclei of the intracardiac neurons of mice receiving NTGF show that it was almost indistinguishable from the histogram in Fig. 2 (normal 14-day mice), i.e., after stimulation of the sympathetic nervous system no significant changes could be found in the absolute content of DNA in the nuclei of those neurons.

Massive blocking of the sympathetic innervation thus induces hypertrophy and polyploidization in the neurons of the intracardiac ganglia. These processes and the other morphological characteristics are analogous to those observed in the few neurons of the sympathetic chain remaining intact after administration of the antiserum; a definite morphological analogy can accordingly be drawn between the neurons of the intramural ganglia and the comparatively few neurons of the sympathetic chain.

It can be concluded from these experiments that NTGF has no specific action on neurons of the intramural system. Consequently, the effect observed after administration of antibodies against NTGF is a reaction to massive blocking of the sympathetic innervation. The results thus demonstrate reciprocity between the functional activity of the intramural system of the heart and its volume in the corresponding ganglia of the sympathetic chain.

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