

MICROSPECTROPHOTOMETRIC ANALYSIS OF CYTOPLASMIC RIBONUCLEOPROTEINS OF AUTONOMIC NEURONS DURING DISTURBANCE OF HORMONAL REGULATION

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It is shown by the method of comparative microspectrophotometry that gonadectomy in rats reduces the RNA content in the cytoplasm of autonomic neurons in the intramural plexus of the prostate gland. This change in the RNA content is the result of exclusion of specific hormonal influences.

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Regular changes have been found in the content of protein and RNA in neurons at rest, during excitation [3, 10-12, 16], and during regeneration of their processes [8], and the level of ribonucleoprotein and protein metabolism has been shown to provide an indicator of the functional activity of the neuron [4, 17]. The region of the perikaryon of the nerve cell is a place of intensive protein synthesis maintaining the structural integrity of the neuron and ensuring its adequate function. Hormonal factors can substantially modify the functional state of the central and peripheral nervous system and can lead to the appearance of structural changes in these divisions [5, 14].

One such factor is the level of androgenic hormones in the body. Changes in the content of ribonucleoproteins in the cytoplasm of peripheral autonomic neurons have not previously been studied during a disturbance of the balance of androgenic steroids.

The object of the present investigation was to make a microspectrophotometric analysis of the content of ribonucleoproteins in nerve cells of the autonomic nervous plexus of the prostate gland in rats during blocking of the testosterone effect.

Experimental disturbance of various components of the prostatic nervous apparatus has been shown to cause a development of degenerative changes in the parenchyma and stroma of this gland [6, 13]; this leads to marked disturbances of its function. At the same time, experiments [15] have shown that gonadectomy, by disturbing the hormonal regulation of prostatic function, causes definite changes also in the nervous apparatus of the gland. The character of these changes and their reversibility have not been investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 40 noninbred sexually mature male rats. In the animals (18) of group 1 the hormonal regulation of prostatic function was disturbed by gonadectomy. The animals (18) of group 2 received testosterone propionate in a daily dose of 10 mg regularly after gonadectomy. Group 3, consisting of four rats, was the control.

Removal of the material and its subsequent histological analysis were strictly standardized [1]. The ganglia of the prostate from animals of the groups to be compared were embedded in one block. Sections

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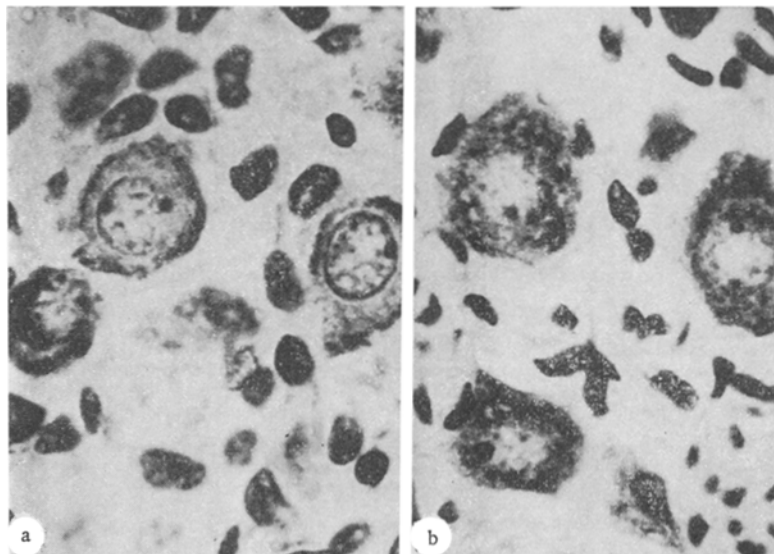


Fig. 1. Cytoplasmic ribonucleoproteins of neurons of intramural prostatic plexus 21 days after gonadectomy. a) Animal of group 1: decrease in RNA content; b) animal of group 2: no difference from control. Stained by Einarson's method, 1080 \times (immersion).

were cut to a thickness of 5-7 μ . Ribonucleoproteins in the cytoplasm of the neurons were demonstrated by staining with galloxyanin - chrome alum by Einarson's method (pH 1.64). This staining method enables the ribonucleoprotein content to be determined quantitatively by microspectrophotometry [9].

To measure the optical density of the stained structures in the cytoplasm of the neurons, an original model of integrating microspectrophotometer [2] was used. Sections of cells containing a nucleus and nucleolus were selected for measurement. In each section 20 cells were measured, the mean optical density being determined in relative units, allowing for the mean area of the cytoplasm of the neurons. Photometry was carried out in monochromatic light at $\lambda = 557 \text{ m}\mu$ (magnification 200 times, diameter of beam 5 μ).*

EXPERIMENTAL RESULTS

The mean optical density of the cytoplasm of autonomic nerve cells of the prostatic plexus, stained by Einarson's method, in animals not receiving testosterone propionate, 3 and 6 h after gonadectomy, was sharply reduced, indicating a decrease in the ribonucleoprotein content. The ratio between the mean optical density of the cytoplasm of neurons in the animals of groups 1 and 2 (D_2/D_1) was 2.39 after 3 days, 3.42 after 5 days, 1.77 after 14 days, 1.91 after 30 days, and 1.13 after 60 days.

The greatest difference between the mean optical density values was obtained during the first week after the beginning of the experiment. Later it gradually diminished. No differences in the ribonucleoprotein content in the cytoplasm of the prostatic neurons were found between the control rats and the rats of group 2.

The results of microspectrophotometric analysis indicate that the decrease in RNA content in the cytoplasm of the prostatic neurons in the animals of group 1 was due to disturbance of the testosterone balance, and not to the operative interference. The results are inadequate to allow conclusions to be drawn regarding the character of disturbances of RNA metabolism in the investigated neurons. It is nevertheless possible that the disturbance of hormonal regulation may cause a decrease in RNA synthesis. This is shown by the results of previous experiments [7], showing that gonadectomy leads to the development of a specific atrophy of nerve cells of the intramural prostatic plexus. This is manifested as a progressive decrease in size of the neurons and of their nuclei, and also as structural changes and redistribution of the tigroid granules in their cytoplasm (Fig. 1).

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These changes were associated with blocking of the androgenic, and not of the anabolic action of testosterone. This conclusion is confirmed by the fact that a parallel study of autonomic ganglia unrelated to the reproductive system in these same animals (the superior cervical sympathetic ganglion) revealed no structural changes in the neurons forming them.

It can thus be concluded that the combination of changes produced by a disturbance of the androgenic hormone balance in rats includes a disturbance of the structure of the nervous apparatus of the reproductive organs and, in particular, of neurons of the prostatic plexus. This is expressed as a decrease in the RNA content in their cytoplasm.

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