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EFFECT OF ESTROGENS ON NEURONAL DEVELOPMENT IN NUCLEI OF THE TRACTUS SOLITARIUS TRANSPLANTED INTO THE ANTERIOR CHAMBER OF THE EYE IN RATS

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Evidence has been obtained of sex differences in the morphology and fraction of brain regions involved in the regulation of sexual behavior and cyclic gonadotropin secretion [4]. The manifestation of these differences depends on the action of sex hormones on the developing brain in the early stages of individual development. Androgens, which undergo aromatization in the brain into estrogens, stimulate growth of neurons and their processes during this period in sex-dependent brain structures, and they also modulate synaptogenesis [1, 2 5, 7, 8].

Evidence has been obtained to show that one of the nonhypothalamic structures involved in the mechanisms regulating cyclic secretion of gonadotropic hormones is the nucleus of the tractus solitarius (NTS) in the medulla [3]. It can accordingly be postulated that the neurons of these nuclei also serve as the target for estrogens, which will exert a neurotropic action on their development. This hypothesis has been tested on a model of a nerve tissue graft cultured in an immunoprivileged region of the recipient animal, namely the anterior chamber of the eye.

EXPERIMENTAL METHOD

Experiments were carried out on 40 mature, preliminarily ovariectomized female Wistar rats. Animals of the experimental group 1 week before transplantation were anesthetized with ether and a silastic capsule ("Dow Corning Corp.," USA) 3 cm long, filled with crystalline estradiol-17β (USSR) was implanted subcutaneously. The authors are grateful to Dr. Y. Arai (Japan) for providing the silastic capsules and to Dr. Biol. Sci. K. K. Pivnitskii for providing the estradiol, synthesized by himself. A capsule not containing the hormone was implanted in animals of the control group. Tissue samples containing NTS were removed under control of a stereoscopic microscope from the corresponding region of section of the lower brain stem in 20-day embryos. By means of a glass needle the extirpated tissue was injected through an

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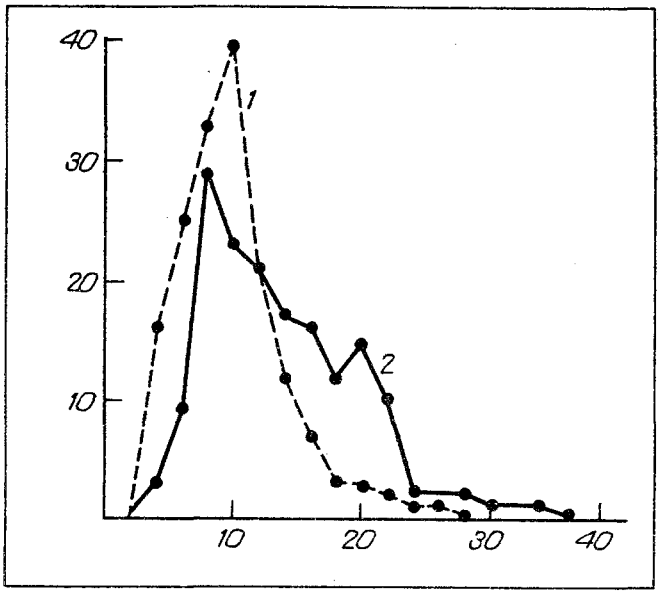

Fig. 1. Intensity of incorporation of ^{3}H-uridine triphosphate into nucleoplasm of neurons of intraocular grafts of NTS, not exposed (1) and exposed (2) to estrogens. Abscissa, number of grains of silver; ordinate, number of neurons.

incision in the cornea into the angle of the anterior chamber of the eye (ACE). After incubation of the nerve tissue in ACE for 4 weeks the animals were killed by decapitation. The level of synthetic activity of the neurons was estimated by an autoradiographic method of detection of activity of endogenous RNA-polymerases in fixed cells [6]. For electron microscopy the graphs were fixed in 2.5% glutaraldehyde solution in cacodylate buffer and in 1% osmium tetroxide solution and embedded in Epon-Araldite. Ultrathin sections were stained with lead citrate and examined in the "Hitachi-7A" electron microscope. Material for light-optical study was fixed in Susa fluid and embedded in paraffin-celloidin. Sections were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

The grafted tissue 4 weeks after transplantation was abundantly vascularized by vessels of the recipient's iris. At the ultrastructural level, bodies of neurons and glial cells and also the neuropil of the graft, consisting of dendrites, axons, and glial processes, did not differ significantly from those in tissue developing in situ.

Analysis of the autoradiographic data demonstrated the significant difference in activity of nuclear RNA-polymerases in the control and experimental groups. Analysis of variance curves of nucleoplasmic labeling of the nuclei of the neurons revealed the appearance of a group of neurons in the experiment with increased endogenous polymerase activity compared with the control (Fig. 1). Comparison of the results by Pearson's chi-square test showed a significantly larger number of neurons with a higher level of nucleoplasmic labeling by the radioactive isotope in grafts exposed to estrogens, irrespective of whether male or female tissue was transplanted into ACE.

Outlines of dendrites in neurons of the experimental group of grafts as a rule were greatly indented on account of spines and pseudopodial outgrowths. The number of axo-dendritic synapses, counted in an area of 10,000 μ^2 of graft tissue, exposed to estrogens, was 308 ± 20 for grafts obtained from females and 312 ± 18 for those from males. This is greater ($p < 0.05$) than in grafts from animals not so exposed (250 ± 18 for females and 268 ± 21 for males).

The results are thus evidence that signs of synthetic activity are significantly enhanced in some cells of intraocular grafts of NTS exposed to estrogens. At the electron-microscopic level, many lysosomes and mitochondria, a well-developed rough endoplasmic reticulum, and a well-marked Golgi complex with established granular vesicles, whose morphology is similar to that in catecholaminergic neurons, were present in these neurons.

It can be concluded from these results that induction of synaptogenesis in the stimulated neurons is based on intensification of synthetic processes, as we concluded from the structural and autoradiographic investigations of neurons in the grafts. It is also probable that the physiological manifestations of the action of sex steroids on the developing NTS are earlier maturation of catecholaminergic neurons and a larger number of synaptic junctions formed by these neurons. This last factor may lie at the basis of mechanisms of sexual differentiation of the brain, determining the sex specific type of innervation of the hypothalamo-hypophyseal system from catecholaminergic neurons in the brain stem.

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