Studies of Cytokines in Nerve Tissue Cultures

N. I. Chalisova and V. Kh. Khavinson

The effects of cortexin, epithalamin, and synthetic peptides on the growth of processes in sensory neurons and on the development of fragments of cortical and subcortical brain structures were studied in organotypic cultures from 10-11-day chick embryos. Cortexin (20 and 100 ng/ml), epithalamin (20 and 200 ng/ml), polypeptides M and P (2 and 20 ng/ml) had neurite-stimulating actions, evident on day 3 of dorsal root ganglion culture. Addition of cortexin (100 ng/ml) or polypeptide M (20 ng/ml) to the culture medium of cerebral cortex explants stimulated explant development. Addition of cortexin at the same concentration to explants of subcortical formations suppressed their development. Epithalamin (200 ng/ml) or polypeptide M (100 ng/ml) stimulated the development of explants from subcortical formations, the existence of the neurite-stimulating effect effects of these cytokines provided the basis for identifying the mechanism of action of brain peptides.

KEY WORDS: Brain peptides, nerve tissue culture.

The cytokines, a class of biologically active substances, often termed growth factors, are currently of great interest in neurobiology and medicine. These include fibroblast growth factor, transforming growth factor, a large group of interleukins, as well as neurotrophins and cytokedins. These latter two groups have great potential for clinical applications.

Cytomedins are peptide bioregulators which maintain structural and functional homeostasis of the cell populations which contain and produce these factors. Cytomedins provide the basis of a class of preparations containing complex peptide fractions, i.e., cortexin and epithalamin, which are extracted respectively from the bovine cerebral cortex and epithalamus and are included in the State Pharmacopoeia of the Russian Federation [6, 7].

Experimental and clinical studies have demonstrated that complex use of cerebral cortex and epiphyseal peptides can "slow the body's biological clock," decreasing the frequencies of tumors and age-related diseases. Cytomedins have been used with success in geriatric medicine [6]. However, the neurotrophic properties of these agents have not yet been investigated.

The best method for studying biologically active substances in experimental conditions is provided by tissue culture, where the effects of defined concentrations of substances on nerve tissue can be identified. The actions of humoral, nervous, hormonal, and other influences acting at the whole-body level, are excluded. The intensity of neurite growth in tissue cultures allows the stimulating or inhibiting effects of one or another growth factor to be measured precisely. Organotypic cultures of nerve tissue (i.e. cultures retaining the structural organization of tissue fragments) can yield data on the neurotrophic properties of cytokines.

The classical test system for the bioassay of neurotrophic activity consists of organotypic cultures of dorsal root ganglia [8, 13, 16]. Sensory neurons in these ganglia respond to the addition of nanomolar concentrations of nerve growth factors with increases in neurite growth rate.

The aim of the present work was to study the development of explants from the peripheral and...
central parts of the nervous system in organotypic cultures in the presence of effective concentrations of cortexin, epithalamin, and their synthetic analogs polypeptide M and polypeptide P.

METHODS

Experiments were performed using 300 explants of dorsal root ganglia and 250 fragments of cerebral cortex and subcortical formations from 10-11-day chick embryos as described previously [8].

Nutritive medium for explant cultivation consisted of 35% Eagle’s solution, 25% fetal calf serum, 35% Hank’s solution, and 5% chick embryo extract, and was supplemented with glucose (0.6%), insulin (0.5 U/ml), penicillin (100 U/ml), and glutamine (2 mM). Chick embryo dorsal root ganglia, cerebral cortex fragments, and fragments of subcortical formations were placed in this medium. Ganglia were cultured on collagen supports in rotating tubes in an incubator at 36.6°C for 3 days. Central nervous system fragments were cultured in Petri dishes in an incubator at 36.7°C for 2 days. The experimental medium was supplemented with cortexin, epithalamin, polypeptide M and polypeptide P (from the Institute of Bioregulation and Gerontology, St. Petersburg) at concentrations of 2, 20, 50, 100, 200, 400, 800, and 1000 ng/ml. Biological activity was assessed in terms of the area index—the ratio of the area of the whole explant including the growth zone to the initial area of the ganglion or brain fragment. The significance of differences between mean values of the area index were assessed using Student’s t test. The area index was expressed as a percentage and control values were taken as 100%.

RESULTS

The development of control explants of dorsal root ganglia growth in the absence of cortexin or epithalamin involved spreading over the collagen support and the appearance of two surrounding zones. The central zone consisted of non-migrating differentiating neuroblasts, and the peripheral zone consisted of a multitude of axons growing around the ganglia in all directions. Axon growth was accompanied by displacement of proliferating satellite cells and fibroblast-like cells, leading to the formation of a characteristic areola around the ganglia. The growth zones of explants of cortex and subcortical formations contained shorter neurites than those of dorsal root ganglion explants; glial cells and fibroblast-like cells were again displaced.

On cultivation day 3, two concentrations of cortexin had clear growth-stimulating effects: at 20 ng/ml, when the area index of dorsal root ganglion explants was 123 ± 7% (n = 8, p < 0.05) higher than in controls (n = 10), while at 100 ng/ml, the area index was increased by 73 ± 2% (n = 12, p < 0.05) compared with controls (Fig. 1). The lower neurite-stimulating activity of cortexin at 50 ng/ml compared with 20 ng/ml, and the increase in activity at 100 ng/ml, might be associated with the presence of two different polypeptides in the preparation, as discussed below. The area index was statistically significantly smaller, by 85 ± 5%, at 50 ng/ml (n = 11, p < 0.05) than at 20 ng/ml (n = 8) and statistically significantly greater, by 35 ± 3% at 100 ng/ml (n = 12, p < 0.05) than at 50 ng/ml (n = 11).

Investigation of the development of spinal root ganglia in the presence of epithalamin again revealed significant neurite-stimulating effects at two concentrations: at 20 ng/ml, when the area index of dorsal root ganglion explants was 123 ± 7% (n = 8, p < 0.05) higher than in controls (n = 10), while at 100 ng/ml, the area index was increased by 73 ± 2% (n = 12, p < 0.05) compared with controls (Fig. 1). The lower neurite-stimulating activity of cortexin at 50 ng/ml compared with 20 ng/ml, and the increase in activity at 100 ng/ml, might be associated with the presence of two different polypeptides in the preparation, as discussed below. The area index was statistically significantly smaller, by 85 ± 5%, at 50 ng/ml (n = 11, p < 0.05) than at 20 ng/ml (n = 8) and statistically significantly greater, by 35 ± 3% at 100 ng/ml (n = 12, p < 0.05) than at 50 ng/ml (n = 11).

Investigation of the development of spinal root ganglia in the presence of epithalamin again revealed significant neurite-stimulating effects at two concentrations: at 20 ng/ml, when the area index of dorsal root ganglion explants was 123 ± 7% (n = 8, p < 0.05) higher than in controls (n = 10), while at 100 ng/ml, the area index was increased by 73 ± 2% (n = 12, p < 0.05) compared with controls (Fig. 1).

The presence of two peaks of neurite-stimulating activity in both preparations might be associated with the presence in each of them of two types of neurite-stimulating polypeptides with different effective concentrations.

Each of the effective concentrations showed clear dose-dependent effects. Cortexin concentra-