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EXPERIMENTAL BIOLOGY

Natural and Synthetic Cytomedines in Nerve Tissue Culture

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Effect of cortexin, epithalamine, and human cortical and pineal peptides on the growth of sensory neurons and the development of cortex and subcortical structures is studied in organotypic culture of chick embryo brain (embryonic days 10-11). In doses of 2-200 ng/ml these preparations exhibit stimulating effect on spinal ganglia, which is seen on day 3 in culture. Cortexin (100 ng/ml) and brain peptide (20 ng/ml) stimulate growth of cultured brain cortex explants. Epithalamine (200 ng/ml) and pineal peptide (100 ng/ml) stimulate the development of subcortical structures in culture. These peptides can be used as stimulators of neurite regeneration.

Key Words: *brain peptides; nerve tissue culture*

Cytomedines, peptides regulating structural and functional homeostasis of cell populations receive now much attention in current neurobiology and medicine [1]. Organotypic culture of spinal ganglia (SG) is a standard test system for the study of bioactive substances [4,7,8]: nanomolar concentration of bioactive proteins enhance neurite growth in sensory neurons of SG.

We studied complex polypeptide preparations cortexin and epithalamine isolated from cattle brain cortex and pineal gland, respectively, and little studied synthetic brain and pineal peptides, analogs of natural cortical and pineal peptides

The method of nerve tissue culture allows us to neglect reactions of the whole organism and to distinguish stimulating and inhibiting effects of test preparation on some fragments of the central and peripheral nervous system. Our goal was to study the development of explants from the peripheral and central nervous system in the presence of effective concentrations of test preparations under conditions of tissue culture.

MATERIALS AND METHODS

Experiments were carried out on 350 SG and 250 fragments from the cortex and subcortical structures of chick embryos (embryonic days 10-11) as described elsewhere [2]. The explants were cultured in the medium containing 35% Eagle medium, 25% fetal calf serum, 35% Hanks' saline, and 5% chick embryo extract, and supplemented with glucose (0.6%), insulin (0.5 U/ml), penicillin (100 U/ml), and glutamine (2 mM). SG were cultured on collagen substrate in rotating tubes at 36.7°C for 3 days. Fragments of the central nervous system were cultured at 36.7°C for 2 days on Petri dishes. Cortexin, epithalamine, and brain and pineal peptides were added to the cultures in concentrations of 0.5, 1, 2, 20, 50, 100, 200, 400, 800, and 1000 ng/ml. Biological activity was evaluated by the area index (AI) calculated as the ratio of the total area of the explant with the outgrowth zone to the initial explant area and expressed in percents. Control IP was taken as 100%. Significance of differences were assessed using the Student t test.

RESULTS

In the control, i.e., in the absence of cytomedines the SG explants flattened on the collagen substrate. Two zones were seen: the central zone consisted of nonmigrating differentiating neuroblasts, while the

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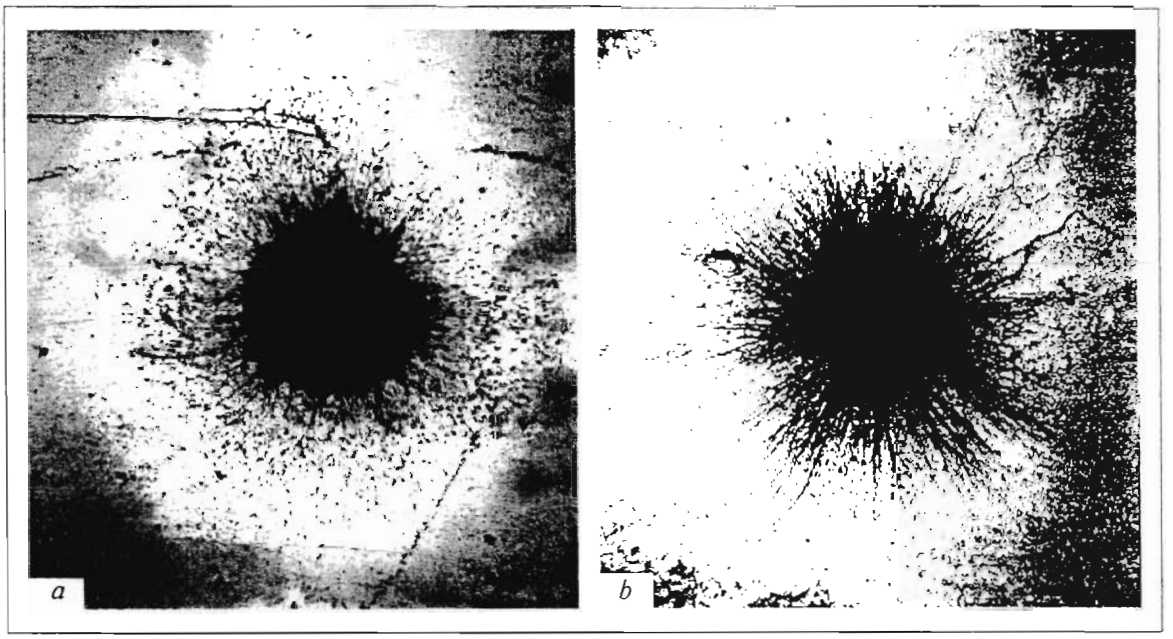

Fig. 1. Spinal ganglion explants on day 3 in culture. *a*) control; *b*) in the presence of 2 ng/ml brain peptide. Hematoxylin and eosin staining. ×35.

peripheral zone consisted of numerous axons radially growing from the ganglion in all directions. Axonal growth was accompanied by outspreading of proliferating fibroblast-like and satellite cells and resulted in the formation of specific halo around the ganglion (Fig. 1).

Growing zone of cortical and subcortical explants consisted of short neurites and glial fibroblast-like cells.

Significant neurite-stimulating effect (NSE) of cortexin (2-100 ng/ml) was noted on the 3rd day in culture: AI in SG explants increased by on average 70% in comparison with the control. The most pronounced NSE (by 123±7%, n=18, p<0.05) (control NSE, n = 15) was attained with the concentration of 20 ng/ml (Fig. 2). A decrease of NSE at a cortexin concentration of 50 ng/ml and its rise at a concentration 100 ng/ml are probably due to the presence of two types of polypeptides in the preparation.

Epithalamine in a concentration of 200 ng/ml produced a significant NSE: AI surpassed the control level by 28±8% (n=19, p<0.05), in comparison with the control (n=17) (Fig. 2).

The NSE of test preparations was dose-dependent: cortexin in concentration of 2, 10, and 50 ng/ml was less effective than in concentration 20 ng/ml and in concentrations of 50 and 200 ng/ml less effective than in concentration of 100 ng/ml (Fig. 2).

Epithalamine in concentrations of 2 and 50 ng/ml was less effective than in concentration 20 ng/ml and in concentrations of 100 and 400 ng/ml less effective than in concentration of 200 ng/ml (Fig. 2).

The effective concentration of brain peptide was very low; however, AI in the presence of this concentration was higher than in the control by 40±5% (n=25, p<0.05). Concentrations of 0.1 and 1 ng/ml were ineffective, while in concentrations above 10 ng/ml brain peptide inhibited neurite growth (Fig. 2).

Pineal peptide stimulated neurite growth only in a concentration of 20 ng/ml, while other concentrations were ineffective (Fig. 2).

In cortical explants, cortexin in a concentration of 100 ng/ml (effective concentration for SG),

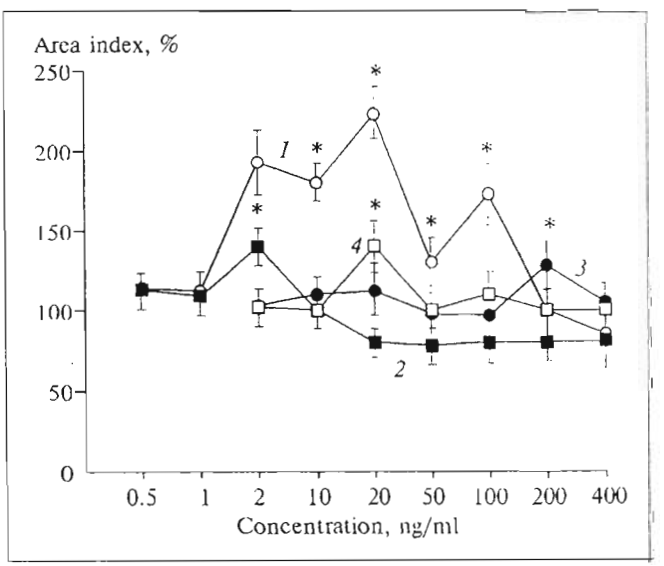

Fig. 2. Effect of cortexin (*1*), epithalamine (*3*), and brain (*2*) and pineal (*4*) peptides on spinal ganglions in a culture tissue. Here and in Fig. 3, *p<0.05 compared with the control.

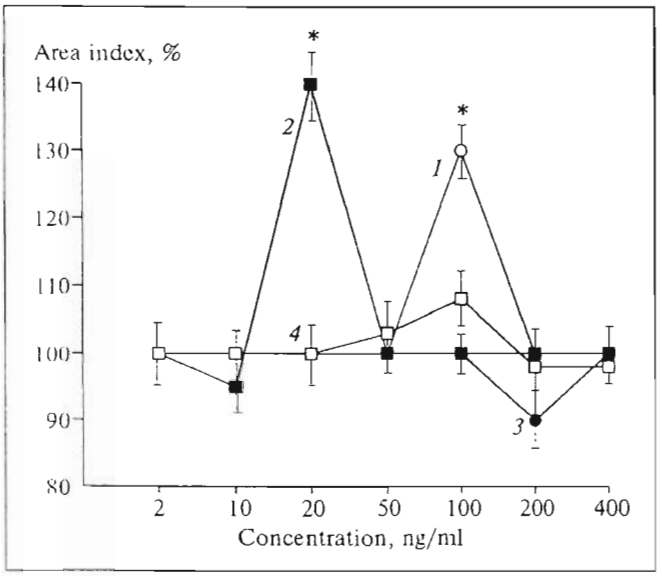

Fig. 3. Effect of cortexin (*1*), epithalamine (*3*), and brain (*2*) and pineal (*4*) peptides on cultured cortical explants.

induced neurite growth: AI increased by 30±2% (n=21, p<0.05) in comparison with the control (n=18). Other concentration were ineffective (Fig. 3). Brain peptide in a concentration of 20 ng/ml markedly (by 40±7%, p<0.05) stimulated neurite growth. Epithalamine and pineal peptide in the entire concentration range had no stimulating effect on cortical explants, while epithalamine in a concentration of 200 ng/ml even inhibited their growth (Fig. 3).

In subcortical explants, epithalamine in a concentration of 200 ng/ml increased AI by 27±7% (n=22, p<0.05) in comparison with the control (n=19), while NSE of 20 mg/ml was insignificant. Pineal peptide in concentration of 100 ng/ml increased AI of subcortical explants by 25±5(n=18, p<0.05) in comparison with the control (n=14).

Cortexin in a concentration of 100 ng/ml inhibited growth of subcortical explants by 67±3% (n=12, p<0.05) in comparison with the control (n=14). In this concentration, cortexin stimulated growth of SG and cortical explants and inhibited growth of subcortical explants. Brain peptide was ineffective in the entire concentration range.

The same NSE in the same concentration ranges were noted when the explants were cultured for 7 days. A slight decrease in AI (insignificant) can be attributed to retraction of nerve fibers in long-term culturing.

Possible potentiation of NSE in the presence of various combinations of test peptides was carefully studied. The effect of a combination of cortexin (10 ng/ml) and brain peptide (2 ng/ml) on SG was equal to that of individual preparations. No potentiation was observed, when SG were cultured in the presence of 200 ng/ml epithalamine and 20 ng/ml pineal peptide. Combined application of synthetic peptides (2 ng/ml brain peptide and 20 ng/ml pineal peptide) yielded no potentiation in comparison with their isolated application.

No potentioation was found in cortical explant exposed to a combination of cortexin (100 ng/ml) and brain peptide (20 ng/ml).

These data suggest that extracts from the brain (cortexin) and pineal glands (epithalamine) contain peptide fractions stimulating the development of the corresponding brain structures. The existence of some peaks of NSE, especially for cortexin, indicates the presence of several active peptide fractions. It can be hypothesized that neurotrophic factors, regulators of neurone functions [3,5], also contribute to the stimulating effect of cortexin and epithalamine. The neurotrophic factors, which are present in epithalamine and cortexin, probably stimulated neurite growth in organotypic cultures of chick SH. This agrees with the finding that these factors are not species-specific [6], therefore preparations from human brain stimulate the growth of SG and brain explants from other animal species.

On the other hand, the synthetic peptides were characterized by a narrower range of effective concentrations: 2 and 200 ng/ml for brain and pineal peptides, respectively. These concentrations produced strong and similar NSE on cultured SG (40% for each peptide).

Effective concentrations of synthetic peptides were lower than those of extracts. In particular, stimulation of cortical explants was produced by 100 ng/ml cortexin and 20 ng/ml brain peptides. Analogously, epithalamine stimulated growth of subcortical explants in a concetration of 200 ng/ml, while pineal peptide exhibited NSE in a concentration of 100 ng/ml. The absence of potentiation in combined application of extracted and synthetic preparations implies that these preparations influence the same cell populations, i.e., cortexin and brain peptides affect the same corticocytes, while epithalamine and pineal peptide influence the same cells in subcortical structures.

These data open prospects for using not only extracted (containing several peptide fractions), but also synthetic preparations in some pathologies of the central nervous system in geriatric practice. Recombinant neurotrophic factors have been recently applied for the treatment of Alzheimer and Parkinson diseases, post-stroke states, etc. [4,7]. Synthetic peptides have some advantages, since they are not immunogenic and do not cause allergic complications.

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