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Effects of Peptides on Proliferative Activity of Retinal and Pigmented Epithelial Cells

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We studied the effects of Retinalamin (polypeptide preparation isolated from the retina) and a synthetic peptide Epithalon (Ala-Glu-Asp-Gly) on proliferative activity of retinal and pigmented epithelial cells. Experiments showed that Retinalamin and Epithalon (in certain concentrations) tissue-specifically stimulated proliferation of retinal and pigmented epithelial cell in culture.

Key Words: *peptides; retinalamine; epithalone; retinal and pigmented epithelium cell culture*

Two classes of bioactive substances are known: growth factors (stimulating cell growth in cell cultures) and induction factors (regulating differentiation pathways during embryogenesis) [9,10,13]. All these substances are low-molecular-weight proteins. The appearance of a new class of preparations — peptide regulators with a molecular weight of 1-10 kDa isolated from organs and tissues and short peptides synthesized on their basis - extended the range of regulatory factors involved in intricate processes of cell interactions [6,7, 11]. The induction effect of Retinalamin (polypeptide preparation isolated from the retina) on polypotent ectodermal cells of early gastrula of *Xenopus Laevis* was studied [8]. Retinalamine promoted neural differentiation in the early gastrula ectoderm, including the brain, retina, and pigmented epithelium. We studied the effect of Retinalamin and Epithalon (synthetic peptide Ala-Glu-Asp-Gly) on retinal and pigmented epithelial cell in culture.

MATERIALS AND METHODS

Experiments were carried out on primary retinal and pigmented epithelial cell cultures derived from rats [4,5]. Type I collagen (Sigma) served as the substrate for cell culturing. The cells were cultured in sterile medium 199 with 10% serum albumin and standard antibiotics. Retinal or pigmented epithelium cells (3×10^5) were put into each culturing well. Peptide preparations Retinalamin (Samson-Med Firm) and Epithalon (St. Petersburg Institute of Bioregulation and Gerontology) were used in concentrations of 2, 10, 20, 50, 100, and 200 ng/ml. The peptides were added into cell culture twice a week during 1 month. The cells were incubated in a CO_2 incubator at 100% humidity and 37°C [2].

Mitogenic tissue-specific activity of the studied peptides in retinal and pigmented epithelial cell culture was evaluated by spectrophotometric quantitation of viable cells in suspension. The cells were stained with 0.75% methylene blue in 75% ethanol. 0.5 M HCl in 50% ethanol (0.5 ml) was added to the precipitate of stained cells washed in distilled water, and optical density of the solution was measured.

The number of cells in suspension was determined from optical density of the sample by the calibration curve.

Optical density was measured in flat-bottom 96-well plates (Costar) on a Multiscan ex Primary EIA V 2.1-0 device with Labsystems Genesis V3.03 software at λ=650 nm. The results were statistically processed using Student's parametrical *t* test.

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RESULTS

Analysis of the results of the studied peptide effects on retinal and pigmented epithelial cell cultures revealed a significant relationship between mitogenic activity of cells and concentrations of Retinalamin and Epithalon. Pigmented epithelial cells proliferated more actively than retinal cells. The results recorded after week 1 of culturing showed that the maximum mitogenic activity was observed in cultures exposed to 200 ng/ml Retinalamin. After one week of culturing with Epithalon the maximum mitogenic activity was observed in retinal cells exposed to 10 and 100 ng/ml and in pigmented epithelial cells exposed to 10 and 20 ng/ml Epithalon. During the next 3 weeks the maximum mitogenic activity of pigmented epithelial and retinal cells was observed in cultures grown in the presence of 50 and 100 ng/ml Retinalamin and 2 and 10 ng/ml Epithalon (Tables 1, 2).

These data attest to high tissue-specific capacity of Retinalamin and Epithalon in certain concentrations to induce active proliferation of cultured retinal and pigmented epithelial cells. These peptide preparations can regulate proliferative activity of cells of a certain tissue and trigger the process of differentiation in polypotent tissue. Moreover, they can trigger not only differentiation, but also a cascade of interrelated differentiations, for example, in ocular tissues [3]. Presumably, the peptide signal modulates functional activity of the genome by inducing the expression of genes participating in the process of ocular tissue differentiation. It seems that the effects of peptide preparations depend on cell functions and degree of differentiation and, hence, on their membrane-receptor complex. Treatment of differentiated adult cells in a short-term culture stimulates proliferative activity of cells without modulating their tissue specificity. We should like to emphasize that the proliferative process does not suppress the maintenance of tissue-specific differentiation, that is, two mechanisms work synchronously and the cells in the culture do not loose the signs of differentiated tissue.

It should be noted that in the culture of polypotent cells peptide preparations interact with embryonal cells chanracterized by maximum and specific number of receptors on membranes [8]. This determines the characteristics of polypotent tissue, whose cells at this moment are "waiting" for a signal triggering a specific cascade of cell transformations eventuating in the appearance of differentiated cells, but not for a signal for active proliferation.

Hence, Retinalamin and Epithalon are multifunctional preparations. Peptide regulators can influence differentiated cells of adult organism by triggering cell proliferation and maintaining tissue specificity and the polypotent embryonal cells by inducing tissue-specific differentiation [8]. The effect of peptide drugs is tissue-specific, but the form of its manifestation (cell proliferation or differentiation) depends on functions of the target biosystem.

An important result of this experiment is that it to a certain measure disclosed the mechanism of high therapeutic efficiency of Retinalamin and Epithalon in humans and animals with retinal diseases [12].

TABLE 1. Effect of Peptides on Growth Dynamics of Retinal Cell in Tissue Culture of Rat Eye ($M\pm m$, $\times 10^6$/ml)

Peptide concentration, ng/ml		Day			
		7	14	21	28
Control (normal saline)		0.30±0.01	2.80±0.11	4.60±0.23	9.40±0.51
Retinalamin	2	0.50±0.03	3.70±0.30	5.70±0.41	10.40±0.46
	10	2.20±0.19*	3.69±0.24	11.20±1.57*	7.90±0.84
	20	0.60±0.04*	5.90±0.27*	10.90±0.76*	10.10±0.29
	50	4.20±0.27*	8.70±0.26*	16.30±0.91*	34.20±0.93*
	100	5.90±0.36*	8.40±0.23*	17.80±1.21*	40.80±1.02*
	200	8.70±1.63*	1.70±0.11	10.40±1.46*	5.70±0.83*
Epithalon	2	0.20±0.05	4.50±0.36*	33.40±0.91*	40.20±0.88*
	10	4.50±0.24*	5.40±0.13*	33.40±0.27*	79.20±1.43*
	20	2.70±0.14*	4.20±0.20*	9.50±0.53*	19.80±1.28*
	50	0.70±0.09*	1.50±0.22	4.90±0.32	11.30±0.68*
	100	4.50±0.20*	6.70±0.31*	8.70±0.25*	7.20±0.61
	200	1.50±0.16*	4.90±0.21*	6.70±0.21*	9.20±0.11

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Effect of Peptides on Growth Dynamics of Pigmented Epithelium Cell in Tissue Culture of Rat Eye ($M\pm m$, $\times 10^6$/ml)

Peptide concentration, ng/ml		Day			
		7	14	21	28
Control (normal saline)		0.30±0.01	3.30±1.01	4.90±1.52	14.20±1.64
Retinalamin	2	6.90±1.70*	8.90±0.53*	10.40±0.38*	13.60±0.61
	10	8.50±0.49*	9.50±1.02*	11.60±0.90*	13.10±0.34
	20	6.70±1.53*	5.60±0.53*	7.20±0.98*	13.10±0.56
	50	7.70±0.96*	13.90±0.28*	23.20±3.30*	33.60±1.20*
	100	8.50±0.41*	16.80±1.02*	30.10±2.91*	48.60±3.55*
	200	8.70±0.22*	10.30±0.63*	12.60±1.25*	16.40±1.58
Epithalon	2	3.50±0.19*	9.20±0.36*	25.60±1.60*	42.10±1.24*
	10	4.50±0.22*	9.40±0.24*	28.30±1.06*	81.20±3.29*
	20	4.5±0.1*	7.20±0.25*	17.80±0.34*	33.40±3.46*
	50	1.00±0.12*	8.40±1.47*	16.30±0.32*	16.60±0.42
	100	1.20±0.19*	4.50±0.70	7.70±0.45*	12.10±0.85
	200	3.50±0.15*	6.30±0.39*	8.70±0.43*	19.10±1.63*

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