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Inductive Activity of Retinal Peptides

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We studied the effect of retinal polypeptide Retinalamin on multipotent ectodermal cells of the early gastrula of *Xenopus laevis*. Neuronal differentiation of the early gastrula ectoderm including the brain, retina, and pigment epithelium depended on Retinalamin concentration.

Key Words: *Retinalamin; induction; early gastrula ectoderm; differentiation*

The formation of various types of cells during organism's development from fertilized oocyte *i.e.*, the problems of tissue differentiation and molecular factors regulating this process are a difficult and very interesting problems of biology. In 1956 H. Tiedemann showed that induction is realized via the action of low-molecular-weight proteins. Further studies devoted to identification these substances showed that the role of inductor is played by activin (tgf-β) produced in vessels of adult mammals [10,12,14]. This discovery raised many questions. Apart from activin (tgf-β), mesodermal structures are induced by other growth factors. Some of them induce tissues the upper and other the lower parts of the body. Different doses of the same substance also produced different effects [11]. Apart from biochemical isolation of the growth factors we used a method based on induction of tissues with proteins secreted by cells of living tissues [4, 7,13] or direct treatment of the multipotent ectodermal tissue (MET) at the early gastrula stage. This procedure most adequately reflects the effect of tissue-specific factors [6,7]. The question arises whether tissue-specific differentiation is associated with activin-like substances (tgf-β) and growth factors or it involves other proteins?

A new class of preparations, peptide bioregulators isolated from various organs and tissues and containing polypeptides with a molecular weight of 1-10 kDa, recently appeared [8,9]. Experiments with tissue cultures showed that peptide bioregulators produce tissue-specific effects [1,3-5]. Probably, peptide preparations from various tissues or organs include substances mediating autoinduction (maintenance of tissue specificity in the adult organism).

Here we studied inductive activity of the peptide bioregulator Retinalamin isolated from the retina and administered in various concentrations in relation to MET in clawed frogs *Xenopus laevis*.

MATERIALS AND METHODS

Experiments were performed on *Xenopus laevis* embryos. The eggs were obtained by artificial stimulation of males and females with gonadotropin. We selected pairs of frogs that produced not less than 70% living eggs over the last 6 months. Gonadotropin in doses of 200 and 400 U was administered into spinal lymph sacs in males and females, respectively.

Embryos at the certain stage of development were sterilized in 70% ethanol for 30 sec, washed 2 times with sterile water, and placed in sterile Petri dishes (diameter 3.5 cm) with sterile amphibian Niu-Twitty solution containing antibiotics. After sterilization we removed embryonic membranes and isolated tissue samples.

The ectoderm of the early gastrula of *X. laevis* was used as the test system. This multipotent tissue is

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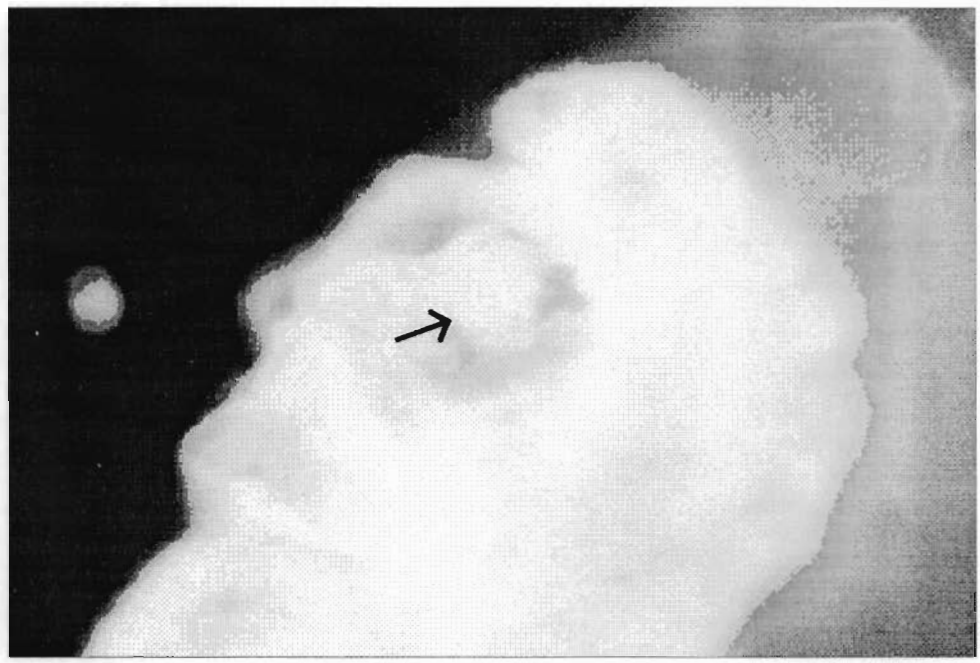

Fig. 1. Culture of the early gastrula ectoderm developed 5 days after 1-h exposure to Retinalamin in a concentration of 2 ng/ml. Arrow points to the brain.

can differente under the influence of inducing factors. We excised regions of the blastocoel roof (early gastrula, stage 10.5) without marginal ectoderm, since it could be partially induced due to tangential influence of surrounding components. The samples were placed in test solutions and incubated for 1 h until they formed closed vesicles. These vesicles were placed in sterile amphibian Niu-Twitty solution containing antibiotics and cultured in an incubator at 20°C for 4-5 days. Blastocoel roof from early gastrula *X. laevis* at stage 10.5 cultured in sterile amphibian Niu-Twitty solution for 4-5 days served as the control. Explants were fixed with Bouin fluid and treated with alcohols. Sections (5 μ) were stained with azocarmine and Mallory's agent.

The experiments were performed with the standard solution of Retinalamin in concentrations of 200, 100, 50, 20, 10, and 2 ng/ml. Each dose was tested on 30 cultures. In control cultures (n=20) only atypical epidermis developed.

In series I we evaluated the dependence of reception of inducing signals by multipotent cells on geometric characteristics of the tissue; various experimental schemes were tested.

In variant I the early gastrula ectoderm at stage 10.5 was placed in the test solution active side up; the ectoderm folded over 1 h. In two variants, the ectoderm was placed on a filter the active side up or down, and pressed with a weight preventing folding for 1 h. Then the folded or non-folded ectoderm was placed in

TABLE 1. Tissues Induced by Retinalamin in Early Gastrula Ectoderm (%)

Parameter	Retinalamin concentration, ng/ml						
	0 (control)	200	100	50	20	10	2
Number of examined/ accounted cultures	20/20	90/64	90/85	90/60	90/40	90/80	90/70
Tissue type							
atypical epidermis	100	100	90	90	100	83	92
epidermis	—	39	—	15	40	42	38
melanophores	—	—	40	12	20	10	8
nervous	—	—	12	—	—	33	15
brain	—	—	—	12	—	8	8
retina	—	—	—	—	—	3	—
pigment epithelium	—	—	—	—	—	1	—

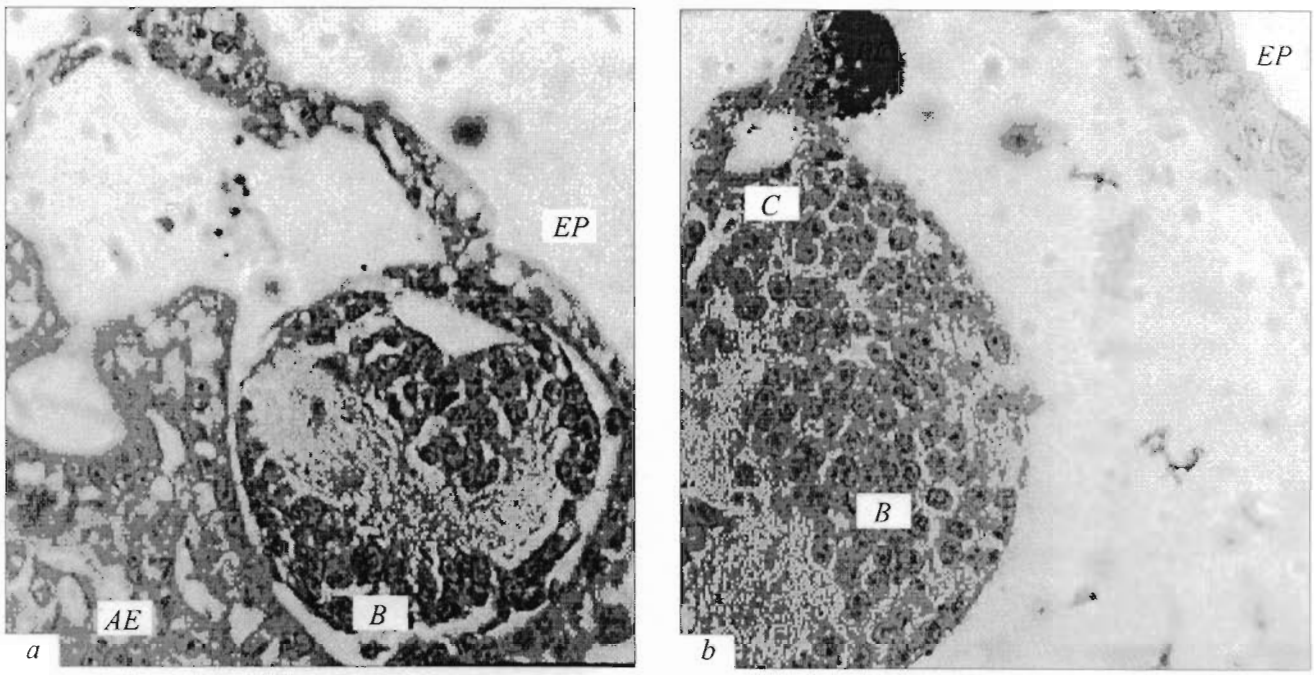

Fig. 2. Histological sections (5 μ) of cultured early gastrula ectoderm developed 5 days after 1-h exposure to Retinalamin in concentrations of 2 (*a*) and 10 ng/ml (*b*), ×250. Atypical epidermis (*AE*), epidermis (*EP*), brain (*B*), retina (*R*), and pigment epithelium (*PE*).

amphibian Niu-Twitty solution with antibiotics and cultured as described above.

Series I showed that tissue differentiation did not depend on its geometric characteristics during reception of the inducing signals. Therefore, the next 2 series were performed on folded ectoderm.

RESULTS

Retinalamin triggered neuronal induction including the brain, retina, and pigment epithelium (Table 1, Fig. 1, Fig. 2, *a*). The percentage of induced ectoderms was low (12-15), but specific for the peptides.

Treatment with Retinalamin in a concentration of 50 ng/ml induced the development of not only the brain, but also the pituitary. However, this assumption should be confirmed by immunohistochemical tests. After incubation with Retinalamin in a concentration of 10 ng/ml the retina developed only in 2 of 80 test cultures and the pigment epithelium developed in 1 culture (Fig. 2, *b*).

When comparing the results of our experiments with previous studies of peptide bioregulators we found that inducing proteins in concentrations of below than 10 ng/ml were ineffective, while Retinalamin was effective in concentrations of 5, 10, and 2 ng/ml. Therefore, the induced response indirectly depended on the concentration of this inducing factor. Tissue-specific differentiation was observed after exposure to Retinalamin in minimum doses.

Our results indicate that treatment of multipotent and stem cells with organ peptides is a promising approach to directed tissue-specific differentiation.

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