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Effects of Analogues of Hydra Peptide Morphogen on DNA Synthesis in the Myocardium of Newborn Albino Rats

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DNA-synthetic activity of myocardial cells was studied by ^{3}H-thymidine autoradiography in newborn albino rats after intraperitoneal injection of hydra peptide morphogen and its analogues. Administration of hydra peptide morphogen stimulated proliferative activity in the myocardium. Short analogues of hydra peptide morphogen, 6C and 3C peptides, produced a similar effect. Administration of arginine-containing analogue of hydra peptide morphogen significantly reduced the number of DNA-synthesizing nuclei in the ventricular myocardium of newborn albino rats. The role of the structure of the peptide molecule in the realization of the morphogenetic effects of hydra peptide morphogen is discussed.

Key Words: *DNA synthesis; proliferation; L-arginine; regulatory peptides*

Hydra peptide morphogen (HPM), a bioactive peptide, is a factor of growth and differentiation in invertebrates [14]. HPM-binding receptors are presented in mammalian tissues and are involved in the regulation of gene expression [11,12], in particular during embryogenesis [13]. Our earlier experiments demonstrated the influence of HPM on cell proliferation in some organs of newborn albino rats [8].

Here we compared the effects of structural analogues of HPM on DNA-synthetic activity in the myocardium of newborn albino rats.

MATERIALS AND METHODS

Experiments were performed on random-bred newborn albino rats, offspring of 3-month-old intact females (n=48).

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In two experimental series we used the following peptides: 1) HPM, pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-ILeu-Leu-Phe; 2) HPM arginine analogue (Arg-HPM) (pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-ILeu-Leu-Phe-Arg). Binding of L-arginine residue to HPM molecule modifies some its properties [7]. The role of terminal L-arginine in the realization of the effects of opioid peptides on tissue homeostasis, in particular during the early postnatal ontogeny [3,4], has been previously shown [5,9]. The involvement of NO synthesis system was assumed as the main mechanism underlying this relationship; 3) C-terminal fragments of HPM: 6-amino acid (6C peptide) Ser-Lys-Val-Ile-Leu-Phe; 3-amino acid (3C peptide) Ile-Leu-Phe.

In experimental series II with short HPM analogues, only ventricular myocardium was analyzed, because the changes observed in the atrial and ventricular myocardium were similar.

The peptides were injected intraperitoneally (5 injections on postnatal days 2-6) in an equimolar dose

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9×10^{-8} mol/kg of body weight. Controls received an equal volume of sterile isotonic NaCl. Each series of experiments had its own group of controls.

Twenty-four hours after the last injection, parameters of DNA synthesis in the myocardium were evaluated autoradiographically. To this end, ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 84 Ci/mol) was injected 1 h before the sacrifiction. Radioautographs were prepared according to the methods accepted in the laboratory. Index of labeled nuclei (ILN) was calculated (in %); 1000 cells were analyzed. Labeling intensity (LI) was calculated as the mean number of tracks per 50 labeled nuclei. ILN was counted separately for the left and right atria and in the subendocardial layers of ventricles

Differences between the groups were significant at $p<0.05$ (Student's t test).

RESULTS

Fivefold administration of HPM to newborn rats stimulated proliferative processes in the myocardium. LI significantly increased, which attested to intensification of DNA synthesis (Table 1). We have previously demonstrated stimulation of proliferation under the influence of HPM in the myocardium, epithelium of the tongue, trachea, and stomach of newborn animals [8].

The effect of 6C peptide was similar to that of HPM: LI in the right ventricular myocardium significantly increased by 15%. After administration of 3C peptide, stimulation of DNA synthesis in the myocardium was significantly more pronounced. In the left ventricle, ILN significantly increased by 40.8%, and there was a distinct statistical trend toward an increase in ILN in the right ventricle (by 45.2%). LI increased by 14.8% in the left ventricular myocardium and by 12.8% in the right ventricular myocardium. Thus, short 3-amino-acid analogue of HPM not only accelerated DNA synthesis, but also increased the proliferative pool of cardiomyocytes.

The study showed that the stimulating effect of short HPM analogues on DNA synthesis in neonatal myocardium increased with decreasing the length of the amino acid chain. C-terminal HPM tripeptide produced a stronger effect on planarian regeneration than the full-length peptide. The results of this study suggest that the active morphogenetic center of the HPM molecule is localized in the 3C fragment of the amino acid chain. It is known that, irrespective of the size of peptide regulatory molecule, its active site is formed by 6-8 amino acid residues. Their structure is more or less rigidly fixed in the form of quasi-cycle, β-bend or loop and corresponds to a "pocket" in the receptor molecule. A peculiarity of morphogenetic activity of regulatory peptides is mismatches in the molecular structure necessary for the realization of their "specific" effects (participation in nociception for opioid peptides, vasotonic activity for angiotensin II, *etc.*) and their effect on proliferation. The latter, is characterized by lower structural "requirements". For modulation of the proliferation processes, shorter chain of amino acid peptide is usually sufficient.

The effects of HPM arginine-containing analogue were different. Administration of Arg-HPM significantly reduced ILN, while indicators of LI ventricular myocardium in newborn albino rats were stable (Table 1). This indicates a decrease in the number of S-phase nuclei without changing the rate of DNA synthesis. Thus, L-arginine residue attached to the molecule leads to inversion of effect of regulatory peptide.

Sedatin and dalargin, other regulatory peptides containing terminal arginine, also inhibited DNA syn-

TABLE 1. Impact of HPM and Its Structural Analogues on DNA Synthesis in the Myocardium of Newborn White Rats

Experimental condition	Left atrium		Right atrium		Left ventricle		Right ventricle	
	ILN, %	LI	ILN, %	LI	ILN, %	LI	ILN, %	LI
Series I								
Control	5.9±0.5	21.4±1.0	5.0±0.5	20.0±1.0	6.9±0.6	19.4±0.7	6.5±0.6	21.1±1.1
HPM	6.7±0.6	26.4±1.5*↑	6.7±0.6	25.8±0.4*↑	7.8±0.5	25.1±0.7*↑	6.8±0.6	24.4±1.0*↑
Arg-HPM	5.8±0.6	22.6±1.6	5.2±0.5	22.0±1.0	5.4±0.3*↓	21.0±1.2	4.6±0.4*↓	21.1±0.9
Series I								
Control	—	—	—	—	4.8±0.5	16.2±0.4	4.2±0.5	15.6±0.6
6C peptide	—	—	—	—	5.5±0.6	17.3±0.6	4.9±0.4	18.8±1.1*↑
3C peptide	—	—	—	—	6.7±0.7*↑	18.6±1.1*↑	6.1±0.9+↑	17.6±0.8*↑

Note. *$p<0.05$, +$p<0.1$ compared with controls.

thesis in the myocardium of newborn animals [1,4]. At the same time, sedatin analogue lacking arginine had no effect [4]. Blockade of NO-synthase with L-NAME abolished the morphogenetic effects of dalargin and sedatin in airway and gastric epithelium [5,9]. These findings suggest that arginine-containing peptides can affect tissue homeostasis through regulation of NO synthesis activity. In the course of proteolysis, arginine residue can be cleaved from the peptide and serve as a substrate for NO-synthase [2]. Participation of NO in the regulation of cell division was previously reported [6]. In addition, we can not exclude that the inversed effect of arginine-containing peptide is related to its inability to cross the blood-brain barrier. The results confirm that the presence of L-arginine residue at terminal position of the regulatory peptide molecule is associated with its influence on the parameters of tissue homeostasis in mammals.

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