PARTICIPATION OF HYDRA PEPTIDE MORPHOGEN IN REGULATION OF PROLIFERATION OF VARIOUS ALBINO RAT TISSUES

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UDC 611-0.18.1-003.93:615.36

KEY WORDS: hydra peptide morphogen; proliferation; DNA synthesis

Hydra peptide morphogen (HPM) is an undecapeptide with the following amino acid sequence: *p*Glu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe. It was first isolated from a freshwater hydra and from polychetes [5], but subsequently was found in virtually all mammals and in man [7, 8]. In a few publications a wide range of effects of HPM has been described. It alters the parameters of the estrous cycle in rats [1], stimulates pancreatic secretion of amylase in vitro [6], and considerably increases synthesis of polyamines, by activating ornithine decarboxylase (ODC) in the liver of intact and partially hepatectomized rats [2]. In previous investigations the writers showed that HPM activates proliferative processes in the corneal and lingual epithelium of albino rats [4].

The aim of this investigation was to determine the effect of HPM on cell proliferation depending on its dose, and to study the effect of HPM on proliferative processes in other organs.

EXPERIMENTAL METHOD

To elucidate the mechanism of action of HPM on cell division, it was decided to study the possibility of its direct action by application to the cornea. Experiments were carried out on 130 male albino rats weighing 160-180 g. To determine dependence of the effect on dose the peptide was dissolved in isotonic solution and injected intraperitoneally in doses of 1, 10, and 100 μg/kg body weight. Control animals received the equivalent volume of sodium chloride solution. In the study of the effect of HPM on cell division in epithelium of the stomach and thymus, just as in our previous experiments we used a dose of 10 μg/kg. To assess the possibility of a direct action of the peptide on the cornea, 20 μl of a 100 nM solution of HPM was applied to the cornea 3 times (in the course of 1 h), for 5 days. DNA synthesis was judged by autoradiography with ^{3}H-thymidine. For this purpose, ^{3}H-thymidine was injected intraperitoneally 1 h before sacrifice in a dose of 0.6 μCi/g (specific activity 84 Ci/mmole), and 5 μCi ^{3}H-thymidine was additionally applied to the cornea. The index of labeled nuclei (ILN) was expressed as a percentage, and the labeling intensity (LI) as the average number of grains of silver above the nucleus. The mitotic index (MI, in promille) and the mitotic index of colchicine mitoses (MI_{col}, in promille) and the duration of mitosis (in minutes) also were determined. For this purpose, a group of animals 2 h before sacrifice was given colchicine by intraperitoneal injection in a dose of 0.2 mg/100 g. Histological sections and autoradiographs were prepared by methods described previously. Cell division was studied 4 and 24 h after a single application and 24 h after the final application. Dependence of effect on dose was determined 24 h after exposure. Colchicine was obtained from "Fluka" (Switzerland), and HPM was synthesized in the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR by Professor M. I. Titov. The significance of differences was determined by Student's t test.

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Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman*.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 307-309, September, 1991. Original article submitted February 5, 1991.

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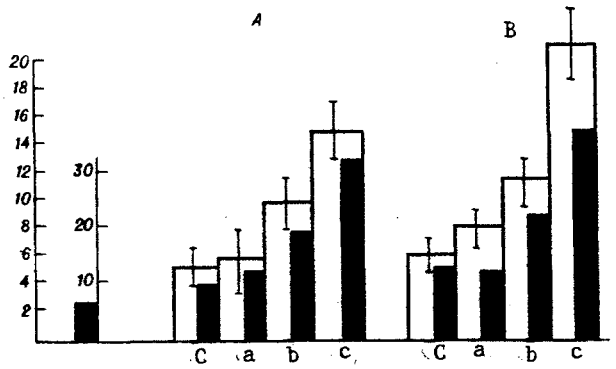

Fig. 1. Effect of different doses of HPM on DNA synthesis in corneal (A) and lingual (B) epithelium. Ordinate, on left, ILN (in %), on right — LI. C) Control; a) dose of HPM 1 μg/kg, b) dose of HPM 10 μg/kg, c) dose of HPM 100 μg/kg.

TABLE 1. Effect of HPM on Cell Division and DNA Synthesis during Five Applications to Albino Rat Cornea

Group	MI, %	MI_{col}, ‰	Duration of mitosis, min	ILN, %	LI
Control	10,61±0,70	14,84±0,73	85,5	4,79±0,30	13,2±1,6
Experiment	11,53±1,02	21,93±1,29*	63,1	7,92±0,33*	12,1±1,3

Legend. Here and in Table 2 asterisk indicates significant differences compared with control at $p < 0.01$ level.

TABLE 2. Effect of HPM on Cell Division in Gastric Epithelium and in Cortical Layer of the Thymus

Group	Stomach			Thymus		
	MI_{col}, ‰	ILN, %	LI	MI_{col}, ‰	ILN, %	LI
Control	22,8±1,15	6,28±0,51	22,83±2,1	21,38±0,6	3,63±0,4	17,6±1,1
Experiment (4 h)	18,8±1,75	5,33±0,34	20,48±1,4	9,65±1,01*	1,73±0,09*	15,9±1,02
Experiment (24 h)	30,4±1,7*	11,24±0,8*	27,16±2,5	19,75±0,4	5,37±0,32	19,4±0,9

EXPERIMENTAL RESULTS

The study of the effect of different doses of HPM on proliferative processes in the lingual and corneal epithelium revealed no significant changes in the parameters of DNA synthesis when the peptide was given in a dose of 1 μg/kg (Fig. 1). If HPM was given in a dose of 10 μg/kg ILN in the lingual epithelium was increased by 1.7 times, and in the cornea it was doubled. No significant changes were found in LI, which indirectly characterizes the velocity of DNA synthesis, after injection of HPM in the same dose. An increase in the dose of HPM to 100 μg/kg not only led to a significant increase in ILN in the tissues studied compared with the same parameter with a dose of 10 μg/kg, but also to a significant increase in LI, indicating an increase in the rate of DNA synthesis. The presence of a dose versus effect relationship is indirect evidence that HPM acts directly on proliferation. This conclusion was confirmed also in experiments with application of HPM to the cornea (Table 1). Application of HPM to the cornea led to a significant increase of 1.6 times in ILN compared with the control. Meanwhile MI_{col} in the experimental group was increased by 1.5 times. The fact that MI under these conditions did not change significantly can be attributed to a faster rate of passage of the cells through mitosis. The average duration of mitosis in the control was 85.5 min, in agreement with data in the literature [3], whereas in the experimental group it was shortened to 63 min, i.e., by 1.4 times.

Application of the peptide to the cornea did not rule out its possible action on different systems of the body, but comparison of the parameters of cell division in the cornea, to which HPM was applied, and the cornea to which isotonic sodium chloride solution was applied leads to the conclusion that HPM has a direct influence on cell division in this tissue.

In previous investigations the stimulating effect of HPM was discovered on cell division in epithelium of ectodermal origin, i.e., in epithelium of the cornea and tongue. The results of the present investigation show that HPM affects proliferative processes also in epithelium of endodermal nature. This is confirmed by the results of a study of cell division in the epithelium of the pyloric part of the stomach and of the cortex of the thymus. No significant changes in cell division processes were observed in the gastric epithelium 4 h after administration of HPM. Administration of HPM led after 24 h to a twofold increase in ILN, whereas MI_{col} rose by 1.5 times (Table 2). In this connection there are some interesting data [5] to the effect that the HPM concentration in the mammalian gastrointestinal tract is high. A somewhat different picture was found in a study of proliferative processes in the cortex of the thymus. MI_{col} 4 h after administration of the peptide was reduced by half, whereas ILN in the experimental group also was reduced by 2.1 times. Normalization of the values of MI_{col} was observed after 24 h, and ILN was significantly higher (by 1.5 times) than in the control. No significant changes in LI occurred in this series of experiments.

The experimental results described above confirm that HPM is involved in the regulation of proliferative processes. The high efficacy of HPM in low concentrations, and its systemic and direct effect suggest that it is involved in the regulation of structural homeostasis.

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