14. N. Payet and J.-G. Lehoux, J. Physiol. (Paris), 78, No. 3, 317 (1982).
15. A. Tahri-Joutei and G. Pointis, J. Reprod. Fertil., 82, 247 (1988).
16. G. Zerek-Melen, A. Lewinski, M. Pawlinkowski, et al., Res. Exp. Med., 187, 415 (1987).

EFFECT OF HYDRA UNDECAPEPTIDE MORPHOGEN ON CELL DIVISION IN THE ALBINO RAT CORNEAL AND LINGUAL EPITHELIUM

A. Yu. Khomichuk and S. S. Timoshin

UDC 617.713+616.313]-018.7-018.15-02[615.324:593.711:547.96]-092.9

KEY WORDS: cell proliferation, hydra peptide morphogen, DNA synthesis.

Hydra peptide morphogen (HPM) is an undecapeptide Glp-Pro-Pro-Gly-Gly-Ser-Lys-Val-Lle-Leu-Phe. HPM was first isolated from Hydra and the sea anemone, in which it has a marked stimulating action on reparative regeneration [5].

HPM has been found in the mammalian brain [6] and also in human blood serum and in neurons of the cranial part of the human brain [1]. The role of the peptide in regulation of physiological functions in mammals remains largely unclear. There is information in the literature on the role of HPM in the activation of anabolic processes. HPM significantly increases activity of ornithine decarboxylase, an enzyme limiting polyamine synthesis in the rat liver [4]. No information could be found on the effect of HPM on cell renewal processes.

The aim of this investigation was to study the character of the effect of HPM on cell division in the albino rat corneal and lingual epithelium.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-190 g. HPM was injected intraperitoneally in a dose of 10 μg/kg. Control animals received an injection of the same volume of isotonic sodium chloride solution. An intraperitoneal injection of ^{3}H-thymidine (specific radioactivity 0.6 μCi/g) was given to the rats 1 h before sacrifice, and in addition, 5 μCi of ^{3}H-thymidine was applied to the cornea. To estimate the rate of mitosis, some animals were given an intraperitoneal injection of colchicine (0.2 μg/100 g) 2 h before sacrifice. Cell division was studied 4 and 24 h after injection of colchicine. In the experiments of group 2, HPM was injected in the same dose in the course of 5 days and the animals were decapitated 24 h after the last injection of the peptide. Preparation of autoradiographs, determination of the index of labeled nuclei (ILN, %), obtaining total preparations, and determining the mitotic index (MI, %) were undertaken by methods described previously [3]. The mitotic index of blocked metaphases (MI_{col}) and the duration of mitosis t_m were determined by the usual method [2] in the lingual epithelium only, for complete blocking of mitosis did not take place in the cornea with the dose of colchicine used. Altogether 110 animals took part in the experiments. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The results are evidence that 4 h after injection of HPM an increase took place in the number of dividing cells in the lingual epithelium: MI_{col} was increased by 1.8 times. MI_{col} in the tongue 24 h after injection of colchicine was 1.9 times higher than in the control (Table 1). Together with an increase in the number of dividing cells in the lingual epithelium, injection of HPM led to acceleration of mitosis itself: t_m in the control was 116 min, whereas 4 and 24 h after injection of HPM it was reduced to 75 and 85 min respectively. Evi-

Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 425-427, October, 1990. Original article submitted May 12, 1989.

 0007-4888/90/0010-1424$12.50

TABLE 1. Effect of a Single Injection of HPM on Cell Division in the Albino Rat Lingual and Corneal Epithelium

Group of animals	MI, %	ILN, %	LI	MI, %	t_m, min
		Lingual epithelium			
Control	10,9±1,0	7,5±0,624	14,0±0,73	10,9±1,1	116
Experimental					
4 h	13,05±1,4	5,9±0,33	11,3±0,81	20,9±1,7*	74
24 h	14,0±1,2	10,4±0,21*	11,4±0,54	19,8±1,6*	85
		Corneal epithelium			
Control	20,8±1,5	3,6±0,17	15,2±1,7	—	—
Experimental					
4 h	19,2±1,9	4,5±0,4*	14,4±1,4	—	—
24 h	14,2±1,2*	7,3±0,5*	20,4±2,2	—	—

Legend. Asterisk indicates significant differences compared with control.

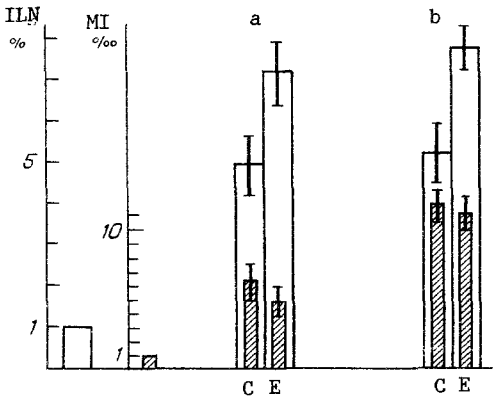

Fig. 1. Effect of five injections of hydra peptide morphogen on cell division in corneal (a) and lingual (b) epithelium of albino rats. C) Control, E) experiment.

dently the acceleration of mitosis was responsible for the significant decrease in MI in the cornea 24 h after injection of colchicine.

The results of autoradiographic analysis helped to explain the mechanism of stimulation of cell division in response to injection of HPM. In the cornea the number of DNA-synthesizing nuclei (ILN) was increased by 1.3 times as early as 4 h after injection of the peptide. ILN was increased by an even greater degree (by 2.1 times) after 24 h. In the tongue the number of DNA-synthesizing nuclei was increased (by 1.4 times) only after 24 h. No significant changes were found 4 h after injection of HPM.

Stimulation of proliferation in the corneal and lingual epithelium also took place 24 h after five injections of HPM (Fig. 1). This was shown by an increase in ILN in the cornea by 1.9 and in the tongue by 1.5 times. No significant changes in the mitotic index were observed under these circumstances. This can evidently be attributed to the fact that, just as after a single injection of HPM, mitosis itself was accelerated. Support for this view is given by the increase in the relative number of prophases in the cornea of the experimental animals to 44%, whereas in the control it was only 28%. The number of prophases in the lingual epithelium rose to 20% compared with 15% in the control.

The results of this investigation demonstrate that HPM has a stimulating action on cell division in the corneal and lingual epithelium. Injection of the peptide leads to acceleration of mitosis itself. HPM also stimulates the entry of cells into the S period. This leads to an increase in the proliferative pool.

The high efficacy of the peptide in minimal doses suggest that HPM is involved in the maintenance of structural homeostasis.

LITERATURE CITED

1. A. A. Varaksin and V. A. Vinogradov, Arkh. Anat., No. 9, 34 (1987).
2. O. I. Epifanova, Hormones and Cell Multiplication [in Russian], Moscow (1965), pp. 163-169.
3. G. M. Kalivetskaya, S. S. Timoshin, A. L. Lykov, et al., Byull. Éksp. Biol. Med., No. 4, 48 (1982).
4. K. N. Yarygin, A. N. Kazimirskii, et al., Byull. Éksp. Biol. Med., No. 6, 680 (1986).
5. H. Shaller and H. Bodenmöller, Proc. Natl. Acad. Sci. USA, 78, 7000 (1981).
6. H. Shaller, Trends. Neurosci., 2, 120 (1979).

INCREASE IN THE NEGATIVE CHARGE OF ERYTHROCYTE MEMBRANE PROTEINS IN HEREDITARY NEUROMUSCULAR DISEASES

V. Yu. Khrennikov, A. I. Deev, V. F. Sitnikov, and A. F. Tagiev

UDC 616.74-009-756.7-07/616.155.1-018.1: 576.314/-008.939.6

KEY WORDS: dystrophy; cell membranes.

The muscular dystrophies are monogenic inherited human diseases which, according to most authorities [7-9], are based on a generalized defect of cell membranes. On account of this view, many attempts have been made to discover changes in those membranes most accessible for clinical analysis of the cells, namely erythrocytes [3]. Interest in erythrocytes is also due to the fact that these cells, like target cells, contain a contractile apparatus, consisting of the contractile proteins spectrin and actin [4]. A whole range of disturbances in the erythrocyte membrane have not been discovered in hereditary muscular dystrophies: changes in shape and deformability of the cells, in cation transport, and in activity of membrane-bound protein kinases [3]. Meanwhile data on phosphorylation of membrane proteins are contradictory. For instance, intensification of phosphorylation of spectrin and of band 3 protein has been found in Duchenne progressive muscular dystrophy (PMD) [10, 11], although in another publication [5] no such effect could be found.

Since an increase in phosphorylation of proteins must lead to an increase in the negative charge on their surface, this effect can be used as the basis for estimating the level of protein phosphorylation. In the investigation described below, the protein surface charge was studied by measuring the efficiency of quenching of protein fluorophores by nitrate anions.

EXPERIMENTAL METHOD

The diagnosis of various forms of hereditary muscular dystrophies was based on clinical, clinico-genealogic, and electromyographic analysis. Erythrocytes were washed twice in physiological saline and hemolyzed by the addition of 4 volumes of 0.01 M Tris-HCl (pH 7.4), incubated for 20 min at 4°C, after which the hemolysate was passed through a column (0.5 × 8.0 cm) filled with sepharose 4B, and the erythrocyte membrane fraction was collected. The protein content in the membrane was estimated spectrophotometrically by measuring absorption of the cell ghost suspension in 1% SDS at 280 and 310 nm [6]. Fluorescence was measured on a "Hitachi MPF-2" spectrofluorometer (Japan) in cylindrical microcuvettes with a capacity of 0.4 ml. Protein fluorescence was excited at 262 nm in the region of minimal absorption of nitrate, and recorded at 335 nm, in the region of emission of tryptophanyls. Allowing for the geometry of the cuvettes used, a correction for optical screening under the conditions of excitation and recording used was introduced [1], in accordance with the equation:

$$F = F_{meas} / 1 - 1.68 \cdot M^{-1} \cdot C, \quad (1)$$

N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. O. Badalyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 427-428, October, 1990. Original article submitted February 6, 1990.

 0007-4888/90/0010-1426$12.50