7. A. M. Sagirov, A. Yu. Romakov, A. B. Sumarokov, and N. A. Mazur, Kardiologiya, **24**, No. 11, 91 (1984).
8. B. I. Tkachenko, D. P. Dvoretskii, V. I. Ovsyannikov, et al., (No title given), Leningrad (1971), p. 295.
9. P. D. Henry, Am. J. Cardiol., **46**, No. 6, 1047 (1980).
10. M. Holck, S. Thorens, and G. Hausler, Eur. J. Pharmacol., **85**, No. 3/4, 305 (1982).
11. F. Malpartida, Rev. Esp. Cardiol., **38**, No. 3, 176 (1985).
12. W. G. Naylor, Calcium Antagonists, Academic Press, London (1988).
13. D. C. Pand and N. Sperelakis, Eur. J. Pharmacol., **81**, No. 3, 403 (1982).
14. H. Yasul, S. Omote, A. Takizawa, et al., Am. J. Cardiol., **43**, No. 3, 647 (1979).

HYDRA PEPTIDE MORPHOGEN ACTIVATES Na/H EXCHANGE IN HUMAN ERYTHROCYTES

A. Yu. Khomichuk, S. S. Timoshin, S. N. Orlov, N. I. Pokudin, and A. A. Kubatiev

UDC 612.841.014:612.6.014.43

KEY WORDS: Hydra peptide morphogen; Na/H exchange

Hydra peptide morphogen (HPM), which consists of 11 amino-acid residues, was first; isolated from Hydra and sea anemones (Coelenterata) [7]. It was later found in the plasma, intestine, and brain (hypothalamus and pons) of mammals and man [1, 6, 12]. HPM has now been placed in the neuropeptide class. Its functions are being studied from all aspects.

HPM in a dose of 20 μg/kg has been shown to activate ornithine decarboxylase in the liver of intact and partially hepatectomized rats [5], evidence of its involvement in the regulation of growth and regeneration. It has been suggested that the peptide is produced and utilized by tumor cells of nervous and endocrine tissue as a growth factor [13]. In previous investigations we found that HPM activates cell division of different kinds of albino rat epithelium over a wide range of doses. The modulating action of HPM on concentrations of cyclic nucleotides in regenerating liver and muscle has been noted [4]. However, the pattern of intracellular transmission as a whole under the influence of HPM has not been adequately studied.

Much information indicating involvement of the Na/H exchange system in the regulation of proliferative activity of many tissues had been obtained [2]. Accordingly, it was decided to study the effect of HPM on the velocity of Na/H antitransport.

EXPERIMENTAL METHOD

Fresh donor's blood containing heparin (50 U/ml) was used. Erythrocytes were sedimented (1000 g, 10 min, 2-4°C), the plasma and white blood cells were removed, and the erythrocyte suspension was washed with physiological saline and kept on ice. The velocity of Na/H exchange was determined as the amiloride-inhibited component of the rate of proton efflux under conditions of creation of an electrochemical proton gradient, with values of the intra- and extracellular pH of 6.45 and 8.00 respectively (H-induced Na/H exchange), and estimated in microequivalents H/min. The techniques used and the order of the calculation are given elsewhere [2, 9]. The amiloride was obtained from "Sigma" (USA) and the anion transport inhibitor (SITS) from "Serva" (West Germany). Heparin was obtained from "Gedeon Richer" (Hungary). HPM

Central Research Laboratory, Khabarovsk Medical Institute. Department of General Pathology and Pathological Physiology, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, pp. 586-587, June, 1991. Original article submitted November 29, 1990.

0007-4888/91/0006-0763$12.50 ©1991 Plenum Publishing Corporation

TABLE 1. Effect of HPM on Rate of Efflux of Protons from Human Erythrocytes (in μeq H/liter·min)

No. of donor	Incubation medium without amiloride		Incubation medium containing 0.5 mM		Amiloride-inhibited component (Na/H exch.)	
	control	HPM	control	HPM	control	HPM
1	118,0	190,0	82,0	100,0	36,0	90,0
2	106,2	165,2	94,4	88,5	11,8	76,7
3	112,1	171,1	94,4	88,5	17,7	82,6
4	135,7	165,2	88,5	88,5	47,2	76,7
5	94,4	147,5	88,5	76,7	5,9	70,8
6	100,3	141,6	88,5	76,7	11,8	70,8
7	82,6	112,1	47,2	59,0	35,4	53,1
$M \pm m$	106,9±6,5	156,1±8,7*	83,4±6,2	82,6±4,9	23,7±5,9	74,4±4,4*

Legend. Asterisk indicates values for which $p < 0.001$ compared with control.

was synthesized in the Laboratory of Peptide Chemistry, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Professor M. I. Titov.

EXPERIMENTAL RESULTS

Previous experiments showed that HPM in a concentration of 1-100 nM increases the rate of proton efflux from human erythrocytes. Further experiments were carried out with HPM in a concentration of 100 nM. The results of the investigation are given in Table 1. They show that the action of HPM is due to an increase in the amiloride-inhibited component of Na/H exchange, whereas the amiloride-noninhibited component is virtually unchanged. HPM in the concentrations studied increased the rate of Na/H exchange threefold. It must be pointed out, however, that activity of the carrier was increased seven-tenfold in donors (Nos. 2, 5, and 6) with a low basal rate of Na/H exchange.

The problem of the role of Na/H exchange in intracellular transmission processes leading to proliferation of cells of vertebrates has been discussed in the literature [8]. For invertebrate cells it has been shown that activation of Na/H exchange is an essential condition for the initiation of proliferation [8]. This suggests that the proliferative action of HPM is partly based on activation of Na/H exchange.

To shed light on the mechanism of the stimulating action of HPM on the velocity of Na/H exchange further experiments must be carried out on proliferating cell cultures. It has recently been shown that this carrier may be activated in erythrocytes by protein kinases C and A [3, 10, 11].

LITERATURE CITED

1. A. A. Varaksin, V. A. Vinogradov, P. A. Motavkin, et al., Arkh. Anat., No. 9, 34 (1987).
2. S. N. Orlov, I. Yu. Postnov, N. I. Pokudin, et al., Byull. Éksp. Biol. Med., **106**, 266 (1988).
3. S. N. Orlov, G. A. Skryabin, V. S. Kotelevtsev, et al., Biol. Membr., No. 6, 1261 (1989).
4. V. D. Slepushkin, I. A. Prum, V. A. Vinogradov, et al., Byull. Éksp. Biol. Med., No. 10, 488 (1989).
5. K. N. Yarygin, A. N. Kazimirskii, G. I. Kositskii, et al., Byull. Éksp. Biol. Med., No. 6, 680 (1985).
6. C. Birr, B. Zachmann, H. Bodenmuller, and H. C. Schaller, FEBS Lett., **131**, No. 2, 317 (1981).
7. C. J. P. Grimmelikhuizen and H. C. Schaller, Trends Biochem. Sci., **4**, 265 (1979).
8. S. Grinstein, D. Rotin, and M. J. Mason, Biochim. Biophys. Acta, **988**, 73 (1989).
9. S. N. Orlov, I. Yu. Postnov, N. I. Pokudin, et al., J. Hypertension, **7**, 781 (1989).
10. S. N. Orlov, N. I. Pokudin, P. V. Gulak, et al., Physiol. Bohemoslov., **39**, 15 (1990).
11. S. N. Orlov, N. I. Pokudin, S. V. Kotelevtsev, et al., J. Membr. Biol., **107**, 105 (1989).
12. H. C. Schaller and H. Bodenmuller, Naturwissenschaften, **68**, 252 (1981).
13. H. C. Schaller and H. Bodenmuller, Hoppe-Seylers Z. Biol. Chem., **366**, 1003 (1985).