Effects of Hydra Peptide Morphogen and Its Analogue and Fragments on DNA Synthesis in Tracheal Epithelium and Smooth Muscle Cells in Newborn Albino Rats

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Hydra peptide morphogen, its Arg^7 analogue, and 6C, 3C, and 5N fragments were injected intraperitoneally in a dose 10^{-7} mol/kg to newborn rats from the 2nd to 6th day of life. Autoradiography with ^{3}H-thymidine showed that hydra peptide morphogen and its 6C fragment stimulated DNA synthesis in tracheal epitheliocytes, but inhibited this process in smooth muscle cells. 5N fragment inhibited DNA synthesis in both tissues, while 3C and Arg^7 were ineffective.

Key Words: *hydra peptide morphogen; DNA synthesis; epithelium; smooth muscle cells; trachea*

Hydra peptide head activator or hydra peptide morphogen (HPM, pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe) was isolated from neurosecretory cells of *Hydra attenuata* [13]. HPM regulating morphogenesis, regeneration, and reproduction is a universal factor of growth and differentiation of cells (including epitheliomuscular cells) in hydra [9,15]. HPM was found not only in coelenterates, but also in mammals. It should be emphasized that the concentration of HPM in mammalian tissues decreases during postnatal ontogeny [14]. HPM was also revealed in human placental trophoblasts. It was shown that blood content of HPM correlates with gestational age [12]. Our previous studies demonstrated that HPM is involved in the development and maintenance of structural homeostasis during the antenatal period [3,4]. It is known that age-related structural and functional peculiarities of the tracheobronchial system contribute to an increased risk of bronchoconstriction at the early postnatal ontogeny. Taking these facts into account, we analyzed the involvement of HPM in the development of epithelium-smooth muscle interrelations in the respiratory tract. Here we studied the effects of HPM, its analogue, and biologically active fragments on the intensity of lipid peroxidation (LPO) and DNA synthesis in tracheal epitheliocytes and smooth muscle cells in newborn albino rats.

MATERIALS AND METHODS

HPM, its analogue Arg^7 HPM, structural C-terminal fragments 6C (Glu-Pro-Pro-Gly-Gly-Ser) and 3C (Glu-Pro-Pro), and N-terminal fragment 5N (Lys-Val-Ile-Leu-Phe) were used. According to published data, Arg^7 HPM does not cross the blood-brain barrier because of the presence of arginine residue, and 5N fragment is a functional antagonist of native HPM [5]. Experiments were performed on 126 newborn albino rats. Control and experimental groups were composed by the method of litter separation to decrease genetically determined differences between litters. The animals received daily intraperitoneal injections of peptides in a dose of 10^{-7} mol/kg at 10.00-11.00 for 5 days (from day 2 to day 6 of life). Control animals received an equivalent volume (0.1 ml) of sterile isotonic NaCl. The rate of DNA synthesis in epitheliocytes and smooth muscle cells of the trachea was determined autoradiographically 24 h after the last injection. The rats were intraperitoneally injected with ^{3}H-thymidine in a dose

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of 1 μCi/g (molar activity 1570 TBq/M) 1 h before euthanasia. Autoradiographs were prepared routinely. The number of S-phase cells (index of labeled nuclei, ILN) and mean number of silver grains over the nucleus (labeling intensity, LI) were counted. Taking into account the important role of the LPO-antioxidant defense (LPO-AO) system in the maintenance of structural homeostasis, we measured the concentration of total lipids (using Lachema kits), α-tocopherol [10], lipid hydroperoxides [1], and malonic dialdehyde (MDA) [8] in the blood and lung homogenates. The results were analyzed by Student's *t* test.

RESULTS

HPM stimulated cell proliferation in the tracheal epithelium of newborn rats: ILN increased by 1.2 times compared to the control. 6C fragment produced a more potent mitogenic effect than native HPM and 1.2-fold increased ILN in tracheal epitheliocytes of rat pups compared to native HPM. At the same time, HPM and 6C fragment did not affect LI in epitheliocytes (Table 1). Experiments with 5N fragment (antagonist of native HPM) indirectly indicated the involvement of endogenous HPM in the development of structural homeostasis in the respiratory system. This peptide inhibited DNA synthesis in tracheal epitheliocytes: ILN and LI decreased by 1.2 times compared to the control (Table 1). Similar changes were revealed in other epithelial tissues of newborn rats [7].

Evaluation of proliferative activity of tracheal smooth muscle cells showed that HPM significantly decreased (by 1.7 times) ILN compared to the control. 6C fragment produced more pronounced effects: ILN decreased compared not only to the control (by 2.6 times), but also to that in HPM-treated rats (by 1.5 times). LI in smooth muscle cells remained practically unchanged under the effect of HPM, but decreased by 1.2 times after administration of 6C fragment (Table 1). Unlike HPM and 6C fragment, 5N fragment inhibited DNA synthesis in tracheal epitheliocytes and smooth muscle cells and significantly suppressed proliferative activity of smooth muscle cells: ILN decreased by 2.3 times compared to the control, while LI remained unchanged (Table 1). Arg^7 HPM and 3C fragment produced no effect on DNA synthesis (Table 1).

Studies of the effects of HPM on LPO-AO system showed that this agent increased blood content of total lipids by 1.2 times and decreased the amount of lipid hydroperoxides in the lungs by 1.7 times compared to the control. Arg^7 HPM considerably decreased (by 2 times) blood MDA content compared to the control. 6C fragment elevated the contents of MDA (by 1.9 and 1.8 times), α-tocopherol (by 1.6 and 1.5 times), and total lipids (by 1.4 and 1.5 times) in rat lungs compared to the control and HPM-treated animals, respectively. 5N fragment decreased blood α-tocopherol concentration and the content of total lipids in the lungs by 1.6 and 1.3 times, respectively, and increased MDA content in the lungs by 1.3 times compared to the control. 3C had no effect on these parameters of the LPO—AO system (Table 2).

Thus, HPM and 6C fragment stimulated DNA synthesis in tracheal epitheliocytes and inhibited this process in smooth muscle cells against the background of activation of the blood AO system. In addition, HPM inhibited LPO, while 6C fragment intensified LPO and activated AO system in the lungs. This effect of exogenous HPM is probably mediated by changes in endogenous regulatory peptides functionally related with HPM (*e.g.*, via the angiotensin-converting enzyme system) [11]. It should be emphasized that under similar experimental conditions, exogenous angiotensin II displays mitogenic properties in relation to populations of epitheliocytes and smooth muscle cells of various origins and localizations [2,6]. It was shown that 5N fragment inhibits cell division in various cell populations, activates LPO, and suppresses AO system in the blood and lungs. These data suggest that shifts in the LPO—AO system towards LPO activation contri-

TABLE 1. Effects of HPM and Its Analogue and Fragments on DNA Synthesis in Tracheal Epitheliocytes and Smooth Muscle Cells in Newborn Albino Rats (*M±m*)

Parameter	Control	HPM	Arg^7 HPM	Fragments		
				5N	6C	3C
ILN, %						
epitheliocytes	1.88±0.10	2.29±0.11*	1.78±0.10	1.50±0.09*	2.76±0.09+	1.94±0.11
myocytes	0.633±0.047	0.363±0.029*	0.664±0.049	0.271±0.019*	0.246±0.018+	0.672±0.050
LI						
epitheliocytes	24.12±0.65	23.21±0.97	24.70±0.97	19.40±0.94*	25.37±0.91	23.24±0.79
myocytes	19.82±0.95	21.13±1.03	20.74±0.96	17.40±0.92	15.76±0.89+	21.68±1.07

Note. $p<0.05$: *compared to the control, +compared to HPM-treated rats.

TABLE 2. Effects of HPM and Its Analogue and Fragments on LPO—AO System in the Blood and Lungs of Newborn Albino Rats ($M\pm m$)

LPO-AO parameter	Control	HPM	Arg^7 HPM	Fragments		
				5N	6C	3C
Total lipids						
blood, g/liter	7.73±0.63	9.42±0.48*	7.09±0.48	7.82±0.34	8.21±0.38	8.48±0.69
lungs, mg/g	1.65±0.12	1.57±0.16	2.07±0.29	1.23±0.12*	2.38±0.21+	1.93±0.11
Lipid hydroperoxides, mmol/g lipids						
blood	0.053±0.003	0.044±0.007	0.050±0.006	0.052±0.007	0.050±0.007	0.043±0.007
lungs	1.11±0.21	0.66±0.08*	0.95±0.14	0.83±0.14	0.80±0.09	0.85±0.11
MDA, fluorescence units/g lipids						
blood	152.9±30.4	109.5±15.4	77.2±6.1*	170.3±24.4	123.3±20.2	101.5±18.6
lungs	1571±173	1698±257	1610±277	2209±176*	3004±272*+	1599±150
α-Tocopherol						
blood, μmol/liter	19.43±2.41	18.81±1.80	18.53±2.11	12.41±1.11*	20.85±1.94	21.62±2.97
lungs, μg/g lipids	20.70±3.44	22.77±3.11	26.87±5.62	15.87±2.05	34.05±3.70*+	22.04±2.8

Note. $p<0.02$: *compared to the control, +compared to HPM-treated rats.

bute to inhibition of DNA synthesis in the epithelium and smooth muscle cells induced by 5N fragment.

Modulation of DNA synthesis in neonatal rats by HPM and its fragments suggests the population selectivity and heterogeneity of HPM receptors, whose development and expression change after contact with active domains of HPM and its functionally important fragments.

Our findings indicate that HPM is involved in the development of tracheal epitheliocytes and smooth muscle cells in newborn rats. The ability of exogenous HPM to modulate proliferative activities of epitheliocytes and smooth muscle cells against the background of high AO activity in the organ and in the whole body offers strong possibilities of using this substance for correcting epithelial disorders and hyperplasia of the muscle layer in airways of patients with bronchopulmonary diseases.

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