Effect of Endothelin-1 on DNA Synthesis in Tracheal Epithelium and Smooth Muscle Cells in Newborn Albino Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 3, pp. 294-296, March, 2000
Original article submitted January 12, 2000

Repeated (5-fold) intraperitoneal injections of 5×10^{-9} mol/kg endothelin-1 inhibited DNA synthesis in tracheal epitheliocytes and activated lipid peroxidation in the lungs of newborn rats. Endothelin-1 in a dose of 5×10^{-8} mol/kg stimulated proliferative activity of tracheal smooth muscle cells and intensified lipid peroxidation in the blood, which aggravated observed changes.

Key Words: *endothelin; DNA synthesis; epithelium; smooth muscle cells; trachea*

The interaction between epithelial and smooth muscle elements in the airways is of particular importance. The epithelium modulates the contractile activity of smooth muscles in the respiratory tract and maintains adequate lung inflation at various periods of postnatal ontogeny [2,3]. The respiratory epithelium controls these processes via production of various biologically active substances, in particular endothelins (ET, bicyclic regulatory peptides consisting of 21 amino acids). ET-1 plays a special morphofunctional role in the development of respiratory organs. Overproduction of ET-1 contributes to the pathogenesis of bronchopulmonary dysplasia, pulmonary hypertension, and respiratory distress syndrome in newborns [9,10]. Furthermore, ET-1 is involved in the regulation of tracheobronchoconstriction, which in turn depends to a certain extent on the state of smooth muscles and epithelial layer in the airways. Here we studied the influence of various doses of ET-1 on DNA synthesis in epitheliocytes and smooth muscle cells (SMC) of the trachea in newborn rats, and analyzed some biochemical mechanisms of these effects.

MATERIALS AND METHODS

Experiments were performed on 64 newborn albino rats of various strains. Control and experimental groups were composed by the method of litter separation to decrease the genetically determined differences between various litters. According to recommendations for *in vivo* studies of the effects of regulatory peptides, ET-1 was applied in doses of 5×10^{-10}, 5×10^{-9}, and 5×10^{-8} mol/kg. Since continuous infusion of the peptide requires anesthesia and immobilization of animals and, therefore, is not convenient for analyzing cell division, the animals received daily intraperitoneal injections of the peptide at 10-11 a.m. for 5 days (starting from the 2nd to 6th day of life). Control animals received an equivalent volume of sterile isotonic NaCl. Proliferative activities of epitheliocytes and SMC were determined autoradiographically 24 h after the last injection. The rats were intraperitoneally injected with ^{3}H-thymidine in a dose of 1 μCi/g (molar activity 1570 TBq/M) 1 h before euthanasia. Autoradiographs were prepared routinely [2]. The number of S-phase cells (index of labeled nuclei, ILN) and the mean number of silver grains over the nucleus (labeling intensity, LI) were counted. Serum total lipids were measured using Lachema kits and contents of α-tocopherol [8], lipid hydroperoxides [1], and malonic dialdehyde (MDA) [5] were measured in the blood and lung homogenates to analyze the state of lipid peroxidation-antioxidant defense (LPO-AO) system. The results were analyzed by Student's *t* test.

RESULTS

Repeated injection of 5×10^{-10} mol/kg ET-1 produced no significant changes in studied parameters (Table 1).

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0007-4888/00/0003-250$25.00

ET-1 in a dose of 5×10^{-9} mol/kg inhibited cell proliferation in the tracheal epithelium: ILN decreased 1.3-fold compared with the control, while LI remained unchanged. Increasing the dose of ET-1 to 5×10^{-8} mol/kg potentiated its antiproliferative effects: ILN and LI decreased by 1.5- and 1.3-fold, respectively (Table 1). Thus, ET-1 in a dose of 5×10^{-9} mol/kg restricted accumulation of DNA-synthesizing epitheliocytes by reducing the number of cells entering S-phase of the cell cycle. ET-1 in a dose of 5×10^{-8} mol/kg produced similar effect not only due to these changes, but also due to deceleration of epitheliocyte progression through S-phase of the cell cycle. Taking into account previous data on the epithelium-dependent modulation of smooth muscle contraction in the airways caused by exogenous ET-1 [15], we concluded that the proliferative reaction of tracheal SMC depends on epitheliocyte division. The most profound changes in spatial organization of the epithelial layer produced by 5×10^{-8} mol/kg ET-1 were accompanied by marked proliferative response of SMC: ILN and LI increased 1.4- and 1.2-fold, respectively (Table 1).

In available literature we found no data on *in vivo* effects of exogenous ET-1 on DNA synthesis in the epithelium and SMC of the airways. *In vitro* experiments on cultured epitheliocytes and SMC produced ambiguous results. Some authors demonstrated direct mitogenic effect of this peptide (activation of DNA synthesis) [6,11], while others reported that exogenous ET-1 added into the culture medium did not affect DNA synthesis, but markedly enhanced the proliferative response during combined use with epidermal growth factor [12,13]. It is interesting that enhanced expression and production of ET-1 in bronchial asthma caused by antiinflammatory factors lead to destructive changes in the epithelial layer and SMC hyperplasia in the respiratory tract [14]. Our studies showed that exogenous ET-1 causes similar morphofunctional disorders *in vivo*.

Repeated injection of ET-1 in doses of 5×10^{-9} and 5×10^{-8} mol/kg not only modulated cell division, but also induced the reaction of LPO-AO system (Table 1). A 1.6-fold decrease in the content of total lipids in the lungs caused by 5×10^{-9} mol/kg ET-1 reflected activation of LPO at the organ level. A 10-fold increase in the dose of ET-1 promoted LPO activation and inhibited AO system not only in the organ, but also in the whole body: blood content of lipid peroxides increased by 1.5-2.3 times, and the content of total lipids in the lungs decreased by 1.3-1.6 times. It should be noted that NO-ergic mechanisms closely related to LPO play a role in the interaction between epithelial and smooth muscle elements. During bronchial asthma, destructive changes in the respiratory system are accompanied by multilevel activation of LPO [4]. Extremely potent oxidants, including peroxynitrite, formed in response to long-term expression of inducible NO synthase during inflammation are probably involved in above mentioned processes [7].

TABLE 1. Effects of Repeated Injection of ET-1 on DNA Synthesis in Epithelium and SMC and Activity of LPO-AO System in the Blood and Lungs of Newborn Albino Rats ($M\pm m$)

Parameter		Control	ET-1, mol/kg		
			5×10^{-10}	5×10^{-9}	5×10^{-8}
ILN, %	epitheliocytes	1.94±0.12	1.90±0.09	1.46±0.08**	1.30±0.08**
	SMC	0.647±0.037	0.629±0.056	0.755±0.086	0.936±0.076**
LI	epitheliocytes	23.81±0.75	24.02±0.85	22.76±0.78	18.96±0.72**
	SMC	21.19±0.89	20.79±1.09	23.69±1.04	25.24±1.02**
Total lipids	g/liter blood	5.80±0.21	6.11±0.38	6.18±0.40	4.62±0.31*
	mg/g lung tissue	1.61±0.07	1.50±0.06	1.06±0.08*	0.98±0.08*
Lipid hydroperoxides, mmol/g lipids					
	blood	0.068±0.003	0.062±0.004	0.064±0.004	0.099±0.005*
	lungs	1.26±0.08	1.45±0.10	1.56±0.16	2.87±0.29*
MDA, fluorescence units/g lipids					
	blood	50.8±2.59	51.94±3.26	54.70±3.18	53.3±3.13
	lungs	1372±112	1219±109	1697±178	1699±162
α-Tocopherol	μmol/liter blood	24.99±1.57	23.07±1.96	20.26±2.60	21.80±2.19
	μg/g lung lipids	21.36±2.31	24.56±3.31	25.22±3.08	24.74±3.13

Note. *$p<0.02$ and *$p<0.05$ compared with the control.

Thus, in neonatal rats ET-1 displays selective morphogenetic activity in relation to proliferation of tracheal SMC and epitheliocytes against the background of LPO intensification in the blood and lungs.

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