Effects of Angiotensin II on Proliferative Activity of Epitheliocytes and Smooth Muscle Cells of the Tracheobronchial System in Newborn Albino Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 7, pp. 28-30, July, 2000
Original article submitted April 13, 2000

Angiotensin II was injected intraperitoneally in a dose of 5×10^{-8} mol/kg to newborn rats from the 2nd to 6th day of life. Autoradiography with ^{3}H-thymidine showed that angiotensin II stimulated DNA synthesis in epitheliocytes and smooth muscle cells of the trachea and large (cartilaginous) and small (noncartilaginous) bronchi, intensified lipid peroxidation, and activated the antioxidant defense system in the lungs.

Key Words: *angiotensin; DNA synthesis; epithelium; smooth muscle cells; respiratory tract*

Angiotensin II (AT-II) and other neuropeptides are involved in morphogenetic processes, including regulation of cell division. Previous studies demonstrated that AT-II produces different effects on DNA synthesis in various cell populations. Some authors reported that this peptide possesses mitogenic properties [6, 11,14], while others observed its antiproliferative activity [10,15]. The involvement of AT-II in the regulation of growth, development, and regeneration explains dysmorphogenetic abnormalities under conditions of impaired AT-II expression and production. Changes in activity of angiotensin-converting enzyme, which crosses the placental barrier and modulates the balance of maternal, fetal, and uteroplacental renin-angiotensin systems, produce damaging effects on the respiratory system in the fetus and newborn [12]. Abnormal development of structural homeostasis in population of epithelial smooth muscle cells in airways during the early ontogeny contributes to an increased risk of bronchoconstriction at the late postnatal ontogeny [3]. Here we studied the effects of repeated administration of AT-II on DNA synthesis in epitheliocytes and smooth muscle cells of the trachea and large (cartilaginous) and small (noncartilaginous) bronchi and the intensity of lipid peroxidation (LPO) in the lungs and blood of newborn rats.

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MATERIALS AND METHODS

Experiments were performed on 37 random-bred newborn albino rats. Control and experimental groups were composed by the method of litter separation to reduce genetically determined differences between various litters. The animals received daily intraperitoneal injections of AT-II in a dose of 5×10^{-8} mol/kg at 10-11 a.m. for 5 days (from the 2nd to 6th day of life). Control animals received an equivalent volume (0.1 ml) of sterile isotonic NaCl. ^{3}H-Thymidine (1 μCi/g, molar activity 1570 TBq/M) was injected intraperitoneally 1 h before euthanasia. Autoradiographs were prepared routinely. DNA synthesis in epitheliocytes and smooth muscle cells of the trachea and large (cartilaginous) and small (noncartilaginous) bronchi was evaluated 24 h after the last injection. 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. To study the state of LPO-antioxidant defense (LPO-AO) system in the blood and lung homogenates, we measured the concentration

0007-4888/00/0007-636$25.00

TABLE 1. Effects of Repeated Administration of AT-II on DNA Synthesis in Cells of the Tracheobronchial System in Newborn Albino Rats (*M±m*)

Structure			Epitheliocytes		Myocytes	
			control	AT-II	control	AT-II
Trachea		ILN, %	1.94±0.12	2.61±0.13*	0.647±0.037	0.976±0.076*
		LI	23.81±0.75	23.01±1.01	21.19±0.89	24.57±1.23*
Bronchi	large	ILN, %	1.02±0.07	1.52±0.11*	0.588±0.064	0.854±0.083*
		LI	21.78±1.35	23.94±1.65	19.14±0.91	21.35±0.92
	small	ILN, %	0.77±0.07	1.12±0.09*	0.510±0.060	0.746±0.062*
		LI	16.71±0.88	21.48±1.25*	19.31±1.07	22.11±1.18

Note. *$p<0.05$ compared to the control.

of total lipids (Lachema kits) and contents of α-tocopherol [7], lipid hydroperoxides [1], and malonic dialdehyde (MDA) [5]. The results were analyzed by Student's *t* test.

RESULTS

Repeated injection of AT-II to newborn rats increased ILN in the epithelium of the trachea and large and small bronchi by 1.4, 1.5, and 1.5 times, respectively, compared to the control. LI significantly increased only in epitheliocytes of small bronchi (by 1.3 times, Table 1). Our previous studies revealed stimulatory effects of AT-II on proliferation of epitheliocytes in the duodenum and skin of newborn rats [2].

AT-II induced proliferation of smooth muscle cells of the tracheobronchial system: ILN and LI in the trachea increased by 1.6 and 1.2 times, respectively, compared to the control, while in the large and small bronchi these parameters increased by 1.5 times (Table 1). Published data indicate that 8-h incubation with 100 nM AT-II induces the synthesis of DNA and protein in cultured smooth muscle cells from human bronchi [9]. Our previous experiments showed that AT-II activates DNA synthesis in duodenal smooth muscle tissue [4].

Repeated administration of AT-II elevated the contents of MDA and α-tocopherol by 1.5 and 1.4 times, respectively (Table 2). These data suggest that AT-II stimulates mobilization of endogenous α-tocopherol from the liver and its further redistribution in other tissues of newborn rats (e.g., accumulation in the respiratory system), which maintains the content of lipid hydroperoxides in lung parenchyma at the control level. Phenol antioxidants affect only certain stages of LPO and, therefore, cannot provide a multilevel defense from reactive oxygen species. One of the side effects of AT-II on LPO-AO system is the increase in MDA level, an integral parameter of LPO intensity. These data attest to complex interrelations between LPO and proliferative processes. In our experiments, AT-II produced no statistically significant changes in blood parameters.

AT-II is functionally related to endothelin-I activity. A common gene structure of AT-II and endothelin-I receptors provides the molecular basis for this relationship [13]. In our previous experiments, endothe-

TABLE 2. Effects of Repeated Administration of AT-II on LPO-AO System in the Blood and Lungs of Newborn Albino Rats (*M±m*)

Parameter		Control	AT-II
Total lipids	g/liter blood	5.69±0.33	5.00±0.32
	mg/g lung tissue	1.52±0.11	1.41±0.08
Hydroperoxides, mmol/g lipids	blood	0.082±0.010	0.098±0.012
	lung	1.30±0.12	1.40±0.09
MDA, fluorescence units/g lipids	blood	49.60±3.05	50.70±5.67
	lungs	1425±191	2116±257*
α-Tocopherol	μmol/liter blood	24.25±2.74	26.86±2.27
	μg/g lung lipids	22.26±3.32	32.91±3.18*

Note. *$p<0.02$ compared to the control.

lin-I under the same experimental conditions inhibited proliferative activity of tracheal epitheliocytes and stimulated proliferation of smooth muscle cells against the background of marked activation of LPO in the lungs and blood of newborn rats. It is known that AT-II elevates the basal level of endothelin-I [8]. Probably, the effects of AT-II observed by us are mediated through the endogenous endothelin-I system.

Hence, AT-II produces adverse effects on the development of structural and spatial interrelations between epitheliocytes and smooth muscle cells in the respiratory tract during the neonatal period. Hyperplasia of smooth muscle cells caused by AT-II can lead to hyperreactivity of airways.

Our findings indicate that AT-II produces similar effects on epitheliocytes and smooth muscle cells in the trachea and large and small bronchi of newborn rats and confirm mitogenic effect of AT-II on epitheliocytes and smooth muscle cells of various origins and localizations.

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