Effects of Opiate Receptor Ligands on DNA Synthesis in Tracheal Epitheliocytes and Smooth Muscle Cells of Newborn Albino Rats

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The dermorphin analogue A10 injected to newborn rats from the 2nd to 6th day of life increased the index of labeled nuclei in epitheliocytes and smooth muscle cells and labeling intensity in smooth muscle cells. Dynorphin A_{1-13} increased the index of labeled nuclei and labeling intensity in epitheliocytes and labeling intensity in smooth muscle cells. Dalargin increased the labeling intensity in epitheliocytes, but had effect on DNA synthesis in muscle cells.

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

Under normal and pathological conditions, functional state of respiratory organs, including the respiratory tract, is regulated by not only the central, but also peripheral opioidergic system [9,15]. Morphogenetically active endogenous opiates modulate the growth, development, and regeneration of organs and tissues [4,13]. We found no published data on proliferotropic activity of opiate peptides (OP) in relation to the structural homeostasis in respiratory organs. It was reported that OP modulate tumor growth during neoplastic processes [11]. Since OP-secreting neuroendocrine cells are localized in the epithelium [9] and various subpopulations of opiate receptors are expressed by smooth muscle cells (SMC) of airways [15], OP are probably involved in the formation of epithelial and smooth muscle structures in the respiratory mucosa. The interest in morphogenetic properties of opiate receptor agonists during the neonatal period is due to the fact that the μ-, κ-, and δ-receptor systems are differently developed in the early ontogeny [7]. Here we studied the effects of opiate receptor ligands (μ-, κ-, and δ-receptor agonists) on DNA synthesis in tracheal epitheliocytes and SMC and the state of LPO-antioxidant defense (LPO—AO) system in newborn rats.

MATERIALS AND METHODS

The dermorphin A10 analogue (H-Tyr-D-Orn-Phe-Gly-OH, highly selective μ-receptor agonist), dynorphin A_{1-13} (H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys, biologically active dynorphin A fragment, selective κ-receptor agonist), and dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg, leu-enkephalin analogue, δ-receptor agonist) were obtained from the Laboratory of Peptide Synthesis (Research Center for Cardiology, Russian Academy of Medical Sciences).

Experiments were performed on 78 newborn albino rats. Control and experimental groups were composed by the method of litter separation to reduce 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 the 2nd to 6th day of life). Control animals received an equivalent volume (0.1 ml) of sterile isotonic NaCl. The rate of DNA synthesis in tracheal epitheliocytes and SMC was determined autoradiographically 24 h after the last injection. The rats

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TABLE 1. Effects of Repeated Administration of A10, Dynorphin A_{1-13}, and Dalargin (10^{-7} mol/kg) on DNA Synthesis in Tracheal Epitheliocytes and Smooth Muscle Cells of Newborn Albino Rats (*M*±*m*)

Cells		Control	A10	Dynorphin A_{1-13}	Dalargin
Epitheliocytes	ILN, %	1.70±0.10	2.52±0.13*	2.71±0.14*	1.81±0.09
	LI	18.14±0.85	20.21±0.98	25.25±1.04*	25.34±1.10*
SMC	ILN, %	0.763±0.062	0.990±0.074*	0.864±0.063	0.695±0.078
	LI	16.30±0.92	21.14±1.04*	22.82±0.96*	17.40±0.92

Note. *p<0.05 compared to the control.

were intraperitoneally injected with ^{3}H-thymidine in a dose of 1 mCi/g (specific activity 1570 TBq/mol) 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. To study the state of LPO—AO system in the blood and lung homogenates, the concentrations of total lipids (Lachema kits), α-tocopherol [8], lipid hydroperoxides [1], and malonic dialdehyde (MDA) were measured [5]. The results were analyzed by Student's *t* test.

RESULTS

Repeated administration of A10 significantly stimulated division of tracheal epitheliocytes in newborn rats, which was confirmed by a 1.5-fold increase in ILN (Table 1). Dynorphin A_{1-13} produced a mitogenic effect on epitheliocytes increasing ILN and LI by 1.6 and 1.4 times, respectively. Dalargin increased LI by 1.4 times, but had no effect on ILN. Thus, A10 and dynorphin A_{1-13} increased the count of DNA-synthesizing epitheliocytes. Dalargin produced no effect on the number of S-phase epitheliocytes, but accelerated DNA synthesis (similarly to dynorphin A_{1-13}). Stimulation of physiological regeneration in the epithelium of airway mucosa by μ- and κ-receptor agonists probably underlies the opiate-mediated regulation of mucociliary clearance [10] and stress-protective effects of these regulatory peptides.

Studies of proliferative activity of tracheal SMC showed that A10 increased ILN and LI by 1.3 times (Table 1). Dynorphin A_{1-13} 1.4-fold increased LI, but had no effect on ILN. Dalargin did not modulate cell division. Thus, only A10 increased the count of ^{3}H-thymidine-containing SMC. Dynorphin A_{1-13} accelerated DNA synthesis, while dalargin had no effect on DNA synthesis. It should be emphasized that μ-receptor agonists can modulate induced tracheobronchoconstriction [14]. Our previous experiments showed that endothelin-I enhancing contractile activity of the respiratory tract stimulates DNA synthesis in tracheal SMC [3].

Thus, highly selective μ-receptor agonist A10 exhibits the highest morphogenetic activity, while the δ-receptor agonist dalargin was least potent. Despite pronounced affinity for δ-receptors, dalargin also weakly binds to μ-receptors, which probably determines its effects on DNA synthesis in tracheal epitheliocytes. As differentiated from μ- and κ-receptors, δ-receptors are least developed [7] and not involved in the regulation of respiratory functions in the neonatal period [12].

TABLE 2. Effects of Repeated Administration of A10, Dynorphin A_{1-13}, and Dalargin (10^{-7} mol/kg) on LPO—AO System in Newborn Albino Rats (*M*±*m*)

Parameter		Control	A10	Dynorphin A_{1-13}	Dalargin
Total lipids	g/liter blood	6.53±0.48	7.44±0.51	7.22±0.58	6.81±0.45
	mg/g lung tissue	1.43±0.11	1.51±0.13	1.87±0.19*	1.41±0.18
Lipid hydroperoxides, mmol/g lipids					
	blood	0.045±0.004	0.051±0.007	0.027±0.005*	0.044±0.007
	lungs	1.17±0.15	0.98±0.07	0.77±0.10*	0.98±0.19
MDA, fluorescence units/g lipids					
	blood	146±28	155±30	160±33	59±15*
	lungs	1690±190	3200±280*	1180±160*	1899±200
α-Tocopherol	μmol/liter blood	16.53±1.52	17.40±2.13	16.79±2.11	15.5±1.00
	μg/g lung lipids	17.33±1.63	25.71±3.05*	20.12±2.50	19.90±2.41

Note. *p<0.02 compared to the control.

Our results and published data [4] show that these OP receptor agonists produce codirected effects and stimulate cell division in the cardiorespiratory system of newborn animals. The κ-receptor agonist dynorphin A_{1-13} produced the most pronounced effect on the myocardium, while the μ-receptor agonist A10 was most active in the tracheal epithelium-SMC population. This was probably related to peculiarities of development of these receptors in various organs.

Studies of biochemical parameters showed that OP produced marked changes in the LPO—AO system (Table 2). A10 activated LPO and stimulated the AO system in rat lungs: the contents of MDA and α-tocopherol 1.9- and 1.5-fold surpassed the control, respectively. Dynorphin A_{1-13} inhibited LPO and activated the AO system, which was manifested in a 1.4-fold decrease in MDA level in lungs, 1.6- and 1.5-fold decrease in lipid hydroperoxide contents in the blood and lungs (respectively), and 1.3-fold increase in total lipid concentration in lungs. The inhibitory effect of this peptide on LPO is probably related to the presence of 3 arginine residues displaying antioxidant activity [6]. Dalargin had no effect on the LPO—AO system in rat lungs, but 2.5-fold decreased MDA level in the blood, which is consistent with its antioxidant properties [2].

Thus, dermorphin A10 and dynorphin A_{1-13} to a various extent activated DNA synthesis in tracheal epitheliocytes and SMC of newborn rats and modulated the state of LPO—AO system.

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