Effects of Regulatory Peptides on DNA Synthesis in Duodenal Smooth Muscle Tissues of Albino Rats during the Early Postnatal Period

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Effects of angiotensin II, endothelin-1, dermorphin A10 analogue, dalargin, and hydra peptide morphogen on DNA synthesis in duodenal smooth muscle cells of newborn albino rats were studied by ^{3}H-thymidine autoradiography. Angiotensin II and endothelin-1 increased the number of DNA-synthesizing myocytes and did not affect the labeling intensity. Dermorphin A10 analogue, dalargin, and hydra peptide morphogen had no effect on these indexes.

Key Words: *regulatory peptides; DNA synthesis; smooth muscles; ontogeny*

Our previous experiments demonstrated that various regulatory peptides (RP) are involved in the establishment of tissue homeostasis during early ontogeny. RP modulate DNA synthesis in the epithelium [5] and myocardium [2,3] of newborn albino rats. These peptides affect proliferation of vascular smooth muscle cells [11]. However, the effects of RP on proliferation of visceral smooth muscle cells received little attention.

Here we studied the effects of various RP on proliferation of duodenal smooth muscle cells in newborn albino rats.

MATERIALS AND METHODS

Experiments were performed on 217 newborn albino rats. Control and experimental groups were formed by the method of offspring separation to reduce genetically determined differences.

Three groups of RP were studied: vasoactive peptides, opioid peptides, and hydra peptide morphogen (HPM). Angiotensin II and endothelin-1 (vasoactive peptides) were intraperitoneally injected in a daily dose of 5×10^{-8} mol/kg body weight from the 2nd to the 6th day of life at 10.00 a.m. Control animals were injected with an equivalent volume (0.01 ml) of the solvent (sterile isotonic NaCl).

Selective agonist of μ-opioid receptors tetrapeptide A10 (H-Tyr-DOrn-Phe-Gly-OH), nonselective δ,μ-agonist dalargin (D-Ala^2-Leu^5-Arg^6-enkephalin), and hydra morphogen undecapeptide (pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe) were intraperitoneally injected in single doses of 10^{-8} and 10^{-7} mol/kg to 4-day-old rats.

DNA synthesis in duodenal smooth muscle tissues was studied by autoradiography 24 h postinjection (24 h after the last injection for repeated administration). ^{3}H-Thymidine (1570 TBq/mol) was injected intraperitoneally in a dose of 1 μCi/g body weight 1 h before euthanasia. Duodenal segment (1 cm distally from the pyloric sphincter) was fixed in Carnoy fluid. Histological preparations and autoradiographs were prepared using routine technique. The labeling index of nuclei (LIN) was determined in duodenal circular muscles. The labeling intensity (LI) was analyzed using autoradiographs coated with M-emulsion.

The results were analyzed by Student's *t* test.

RESULTS

The repeated administration of angiotensin II to newborn albino rats stimulated DNA synthesis in duodenal

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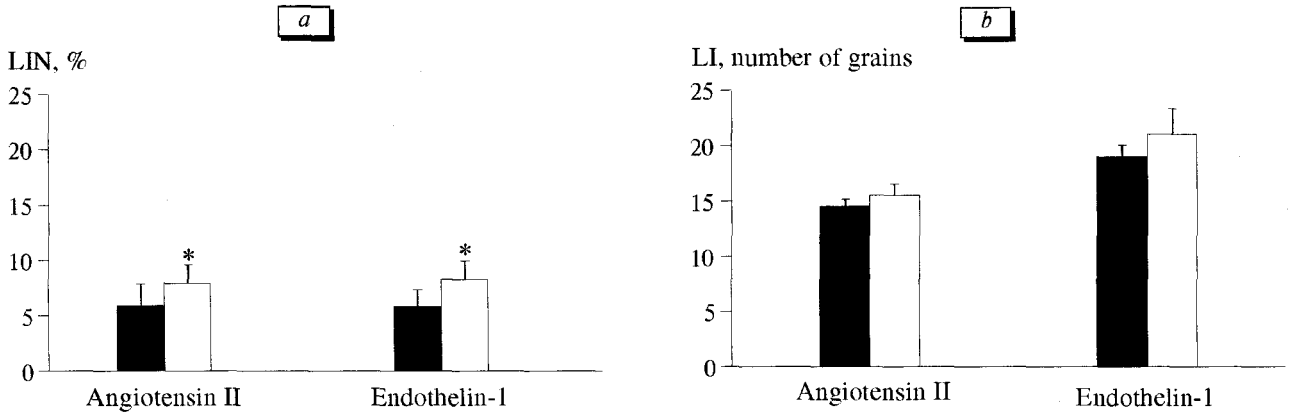

Fig. 1. Effect of repeated administration of vasoactive peptides on DNA synthesis in duodenal smooth muscles of newborn albino rats: the labeling index of nuclei (LIN, *a*) and labeling intensity (LI, *b*) in control (dark bars) and treated rats (light bars). *$p<0.05$ compared with the control.

smooth muscles (Fig. 1). LIN in treated animals increased by 34.2% in comparison with the control. Enlargement of the proliferative myocyte pool was not accompanied by acceleration of cell passage through the S-phase of the cell cycle (LI did not change). Recent studies confirm the involvement of the renin-angiotensin system in the maintenance of structural homeostasis in mammals. Angiotensin II exhibits a mitogenic effect in many cell populations [8,11].

Endothelin-1 is a functional synergist of the renin-angiotensin system [13]. This substance stimulates proliferation of cultured astrocytes and mesangial cells [8,10,13]. Administration of endothelin-1 to newborn albino rats increased the number of DNA-synthesizing smooth muscle cells (Fig. 1). LIN in duodenal muscles of treated rats 1.5-fold surpassed the control value. However, LI did not differ from the control (as in the case of angiotensin administration). Endothelin-1 is known to activate phosphoinositide hydrolysis in cultured tracheal smooth muscle cells [11]. These data provide insights into intracellular mechanisms of the observed effect.

The RP spectrum in the digestive tract is comparable with that revealed in the nervous system [4,6]. Mammalian intestine contains opioids and various subtypes of opioid receptors [9]. Opioids affect proliferation of enterocytes [5], absorption [9], and motor functions of the intestinal wall [7]. However, in our experiments opioid peptides had no effects on DNA synthesis in duodenal muscles (Table 1). Single injection of selective μ-opioid receptor agonist (tetrapeptide A10) and nonselective δ,μ-agonist (dalargin) did not change LIN and LI in smooth muscle cells.

HPM is a paracrine regulator of structural homeostasis in the intestine. In mammalian intestine, HPM is a potent stimulator of proliferative processes in the epithelial layer of the digestive tract [1]. The number of smooth muscle cells in the S-phase did not change after single administration of HPM in a dose of 10^{-8} mol/kg. LIN was similar in experimental and control groups (Table 1).

The absence of changes of the proliferative activity in intestinal smooth muscle tissues after administration of opioids and HPM was probably due to ontogenetic particularities of the reaction. Previously, we showed considerable age-related differences in the effects of opioid peptides on proliferative processes in the epithelium of albino rats [5]. The effects of HPM can be associated with similar ontogenetic specific features.

TABLE 1. Effect of RP on Proliferative Activity of Duodenal Smooth Muscle Cells of Albino Rats during the Early Postnatal Ontogeny (*M±m*)

Peptide, dose (mol/kg)		LIN, %		LI, mean number of grains above nucleus	
		control	experiment	control	experiment
Dermorphin A10	10^{-8}	10.14±0.8	7.68±0.8	19.49±1.45	15.45±1.63
	10^{-7}	7.72±1.04	6.92±1.11	16.78±1.08	17.28±1.03
Dalargin	10^{-8}	7.38±0.68	8.10±0.75	14.50±1.41	17.36±1.36
	10^{-7}	8.55±0.75	7.26±1.04	15.87±0.9	14.73±1.43
HPM	10^{-8}	7.23±2.34	7.22±0.68	—	—

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