Effect of Endothelin-1 on DNA Synthesis in Various Cell Populations of Newborn Albino Rats

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Effect of intraperitoneal injection of endothelin-1 on DNA synthesis in various cell populations of newborn albino rats was studied by ^{3}H-thymidine autoradiography. The number of DNA-synthesizing cells increased in the epitheliums of the tongue and skin, while labeling intensity remained unchanged. Both parameters were elevated in the duodenal epithelium; the index of labeled nuclei changed in the duodenal smooth muscle layer. We revealed no significant changes in these parameters in the myocardium.

Key Words: *endothelin-1; proliferative processes; epithelium; smooth muscle tissue*

Our previous studies showed *in vivo* effects of atrial peptides and angiotensin II on cell proliferation [1,3]. These peptides are closely related to the endothelin (ET) system [10,12]. It was shown that ET produces a variety of proliferative responses in cultured cells [5, 9,14]. However, in available literature we found no data on *in vivo* effects of ET on proliferative processes in mammals.

Here we studied the effects of ET-1 on DNA synthesis in some cell populations of albino rats during the early postnatal ontogeny.

MATERIALS AND METHODS

ET-1 was injected intraperitoneally in a dose of 4×10^{-8} mol/kg body weight at 10 a.m. starting from the 2nd to 6th day of life. There is a great body of data on biological activity of ET-1 in various concentrations. Maximum and minimum doses of ET-1 used *in vitro* were 10^{-6} [11] and 10^{-12} mol/liter [8], respectively. In *in vivo* experiments, ET-1 was applied in concentrations from 5 ng/kg/min [7] to 10 pmol/min (prolonged infusion) [6]. Since prolonged infusion is inconvenient for studying proliferative processes, in our experiments ET-1 was injected repeatedly in a dose of 4×10^{-8} mol/kg. This dose was shown to be optimal for analyzing the effects of regulatory peptides on proliferation [1-3]. Control animals received an equivalent volume (0.01 ml) of sterile isotonic NaCl. Control and experimental groups were composed by the method of litter separation to decrease the genetically determined differences between various litters.

The rats were intraperitoneally injected with ^{3}H-thymidine in a dose of 1 μCi/g body weight (molar activity 1570 TBq/M) 1 h before euthanasia. Samples were taken from the myocardium, liver, duodenum (1 cm distally to the pylorus), tongue, and ear skin. The animals were weighted before injection and euthanasia. Organs were weighed after dissection.

Tissue samples were fixed in Carnoy's fluid. Histological preparations and autoradiographs were prepared by routine techniques. The index of labeled nuclei reflecting the ratio of DNA-synthesizing cells (ILN) and labeling intensity showing the number of silver grains over the nucleus (LI) were calculated. Experiments were performed on 64 rats.

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

RESULTS

Repeated injection of ET-1 in a dose of 4×10^{-8} mol/kg significantly inhibited weight gain. Body weight on day 2 was 6.42±0.13 and 6.29±0.13 g in the control

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TABLE 1. Effect of ET-1 on DNA Synthesis in Epithelium and Myocardium of Albino Rats during Early Postnatal Ontogeny (*M*±*m*)

Tissue		ILN, %		LI, %	
		control	experiment	control	experiment
Epithelium	duodenum	25.23±2.12	34.03±2.53*	10.83±0.77	14.46±1.01*
	tongue	6.59±0.62	10.42±1.19*	9.59±0.36	9.55±0.31
	skin	13.28±0.77	17.48±1.10*	15.36±0.63	16.27±0.95
Myocardium	left	4.078±0.448	5.456±1.207	14.532±0.772	15.433±0.872
	right	4.348±0.506	5.349±1.127	15.0650±0.0831	16.649±0.802
Ventricle	left	7.172±0.712	6.116±1.172	19.673±1.33	21.077±1.190
	right	5.090±0.404	5.649±1.148	15.334±0.640	16.668±0.751
Interventricular septum		8.320±1.104	6.643±1.086	19.525±1.154	21.731±1.226
Duodenal muscular layer		5.810±0.617	8.280±0.662*	18.66±1.10	21.510±0.951
Liver		3.500±0.287	3.000±0.315	21.000±0.721	22.000±0.495

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

and experimental groups, respectively; on day 7, this parameter increased to 10.6±0.3 and 8.54±0.27 g, respectively.

ET-1 stimulated DNA synthesis in the epithelium. In the endodermal epithelium of the duodenum, ILN and LI increased by 34.9 and 33.59%, respectively (Table 1). The rise of ILN indirectly indicates accelerated cell passage through S-phase of the cell cycle. The reaction of the duodenal epithelium indicated increased number of cells entering S-phase of the cell cycle and their accelerated passage through this phase.

In the ectodermal epithelium (ear skin and tongue), ILN increased by 31.56 and 58.06%, respectively (Table 1), while LI remained unchanged.

ET-1 produced no significant changes in DNA synthesis in the liver parenchyma. In treated rats, ILN only slightly increased (2.313±0.315 *vs.* 2.765±0.287% in the control) and LI remained unchanged (Table 1). This is inconsistent with previous data of M. Pinzani *et al.* [13] showing that ET-1 stimulates DNA synthesis in cultured stellate cells. This contradiction requires further investigations.

The reaction of duodenal myocytes to ET-1 was similar to that of the epithelium: ILN increased by 42.47% compared to the control, while LI remained unchanged (Table 1).

It was shown that DNA synthesis in the myocardium of newborn rats changes in response to systemic administration of atrial peptides [1], angiotensin II [3], opioid receptor ligands [2], and hydra peptide morphogen [4]. In our experiments, ET-1 produced no significant changes in ILN and LI in comparable myocardial zones (Table 1).

Stimulatory effects of ET-1 on proliferation of cultured glioma [5] and mesangial cells [9] were described. Similar reactions revealed in our studies and observed in *in vitro* experiments indicate direct stimulation of *in vivo* DNA synthesis with ET-1. However, the role of a decline in cell population (as a manifestation of injuries induced by high doses of ET-1) followed by compensatory stimulation of tissue proliferation can not be excluded. On the other hand, it was shown that ET-1 inhibits apoptosis [15]. Furthermore, direct effects of this peptide are also confirmed by stimulation of DNA synthesis in the duodenal epithelium induced by ET-1 in a dose of 4×10^{-10} mol/kg. This dose of ET-1 is 100-fold lower than that used in our experiments and has no effect on the dynamics of body weight of rat pups. The mechanisms of *in vivo* effects of ET-1 on cell proliferation require further detailed studies.

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