Ontogenetic Peculiarities of the Effect of Endothelin-1 on Tissue Homeostasis of Various Cell Populations in Albino Rats

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The effects of endothelin-1 on tissue homeostasis in newborn and adult albino rats were studied by ^{3}H-thymidine autoradiography, staining with $AgNO_3$, computer-assisted morphometry, and biochemiluminescence. In adult rats the ability of endothelin-1 to activate DNA synthesis in epithelial cells decreased compared to that in newborn animals; inversion of the stimulatory effects of endothelin-1 on the nucleolar apparatus of cardiomyocytes and pronounced intensification of free radical oxidation in the heart were also seen in adult animals. These differences can be related to ontogenetic peculiarities of endothelin-1 reception.

Key Words: *endothelin-1; ontogeny; proliferation; free radical oxidation*

Regulatory peptides are involved in the maintenance of tissue homeostasis. Vasoactive peptide endothelin-1 (ET-1) modulates proliferative activity of various cells *in vitro* [9,10,13] and *in vivo* [5,6]. Most studies of mitogenic activity in ET-1 were performed onh cultured cells. In our previous experiments [5,6] we assayed the effects of ET-1 on cell proliferation in various tissues of newborn mammals. The influence of ET-1 on proliferative processes in tissues of adult albino rats remains unclear. On the other hand, previous studies revealed marked ontogenetic differences in the effects of regulatory peptides on tissue homeostasis [1]. Studies of ET-1-induced changes in proliferative activity at different periods of ontogeny are of theoretical and practical importance: endothelin preparations are now proposed for introduction in clinical practice.

Here we compared the effects of ET-1 on tissue homeostasis at different stages of postnatal ontogeny.

MATERIALS AND METHODS

ET-1 was synthesized at the Laboratory of Peptide Synthesis (Russian Research-and-Production Center for Cardiology). Series I was performed on newborn male and female random-bred albino rats. Control and experimental groups were composed by the method of litter separation to decrease genetically determined differences between litters.

To estimate the effects of ET-1 on proliferative processes, this peptide was injected intraperitoneally in a single dose of 10 μg/kg on day 4 of life; some rats received ET-1 from the 2nd to 6th day of life. Control animals received an equivalent volume of 0.9% NaCl. DNA synthesis in the ectodermal epithelium of the skin and tongue and endodermal epithelium of the duodenum was determined by ^{3}H-thymidine autoradiography 24 h after treatment (or 24 h after the last injection) [2]. ^{3}H-Thymidine was injected intraperitoneally in a dose of 1 μCi/g (specific activity 1570 TBq/mol) 1 h before euthanasia. The index of labeled nuclei reflecting the ratio of DNA-synthesizing nuclei (ILN) and labeling intensity showing the mean number of silver grains over the nucleus (LI) were calculated on routinely prepared autoradiographs.

We studied the effects of repeated intraperitoneal treatment with ET-1 in a dose of 50 μg/kg (days 2-6 of life) on morphometric parameters of the myocardium in 7-day-old animals. Fragment of the left ventricle was subjected to alkaline dissociation and mor-

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phometry on a MEKOS-Ts computerized image scanner. Standard histological preparations of the myocardium were stained with 50% $AgNO_3$ to reveal regions of the nucleolar apparatus [3].

We assayed free radical processes in the myocardium from 7-day-old rats receiving ET-1 in a dose of 10 μg/kg from the 2nd to 6th day of life. The intensity of free radical oxidation was evaluated by H_2O_2-induced luminol-dependent chemiluminescence measured on an LS 50B luminescence spectrometer (Perkin Elmer) [4]. The intensity of H_2O_2-induced chemiluminescence and maximum flash amplitude (I_{max}) were measured at room temperature, calculated per 1 mg lipids, and expressed in arbitrary units. Signals were normalized using Finlab software.

Series II was performed on adult 3-month-old male rats weighing 140-180 g. The effects of ET-1 on DNA synthesis in the epithelium were studied by autoradiography. ET-1 in a dose of 10 μg/kg was injected intraperitoneally 2 times a day (24 and 4 h before euthanasia). Other animals received daily injections of the peptide for 5 days. Morphometric parameters of the myocardium and nucleolar organizer and the intensity of free radical processes in the heart of adult rats were studied after 5 injections of ET-1 in a dose of 10 μg/kg as described above.

The experiments were performed on 172 rats. The results were analyzed by Student's *t* test.

RESULTS

ET-1 in a single dose of 10 μg/kg markedly stimulated DNA synthesis in various epithelial tissues from newborn albino rats (Table 1). In the epithelium of the skin, tongue, and duodenum ILN increased by 33, 59, and 75%, respectively. In the intestinal epithelium a significant increase in the count of proliferating epithelial cells was accompanied by an increase in LI (by 47%). Fivefold treatment with the peptide in a dose of 10 μg/kg also stimulated DNA synthesis. LI in the skin epithelium increased by 41%. In the tongue epithelium LI tended to increase by 20.5%.

Our previous studies showed that 5-fold treatment with ET-1 in a dose of 100 μg/kg (4×10^{-8} mol/kg) stimulates proliferative processes in the epithelium of the skin, tongue, and duodenum [6]. ET-1 also intensified DNA synthesis in the myocardium of newborn albino rats [5]. ET-1 stimulates proliferative processes in various cells of albino rats during the early postnatal ontogeny, which is consistent with published data on mitogenic effect of this peptide in cell and tissue cultures [9,10,13].

TABLE 1. Effects of Single (Numerator) and Repeated (Denominator) Treatment with ET-1 on DNA Synthesis in Different Epithelial Cells of Newborn Albino Rats (*M*±*m*)

Tissue	Control		ET-1	
	ILN, %	LI	ILN, %	LI
Skin epithelium	18.05±1.1 / 17.87±1.23	15.75±0.9 / 10.54±0.54	24.00±1.03* / 20.29±1.77	16.6±0.64 / 14.88±0.78*
Tongue epithelium	13.78±0.99 / 16.2±0.94	15.58±1.14 / 9.8±0.44	21.9±1.24* / 15.83±1.32	17.03±1.00 / 11.81±0.92
Duodenal epithelium	17.51±1.65 / 27.07±1.15	13.65±1.62 / 11.75±0.72	30.69±1.78* / 27.59±0.95	20.13±1.53* / 11.82±0.99

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Effects of ET-1 (Numerator, Twofold Treatment; Denominator, Repeated Treatment) on DNA Synthesis in Different Epithelial Cells of Adult Albino Rats (*M*±*m*)

Tissue	Control		ET-1	
	ILN, %	LI	ILN, %	LI
Skin epithelium	4.06±0.71 / 3.87±0.66	9.86±0.52 / 9.45±0.52	3.96±0.29 / 4.98±0.53	8.95±0.54 / 9.86±0.54
Tongue epithelium	15.58±1.21 / 12.12±1.56	12.42±0.76 / 16.21±1.08	9.32±0.82* / 13.74±1.62	15.27±0.80* / 21.26±0.89*
Duodenal epithelium	28.72±1.29 / 35.68±6.88	17.77±1.81 / 19.46±2.17	31.2±1.43 / 44.11±3.87	14.81±1.03 / 17.95±0.79

TABLE 3. Effects of ET-1 on Morphometric Parameters of Cardiomyocytes in Newborn and Adult Albino Rats

Parameter	Newborn		Adult	
	control	experiment	control	experiment
Cardiomyocyte perimeter, μ	145.81±5.25	154.01±6.48	252.03±8.63	254.87±22.55
Cardiomyocyte area, μ^2	690.67±45.34	775.40±78.70	2005.1±348.9	2430.7±465.3
Nucleus perimeter, μ	22.56±0.63	23.61±0.45	32.44±1.33	31.30±0.60
Nucleus area, μ^2	33.63±1.75	35.38±1.60	47.95±2.83	43.92±2.12
Nucleolus area, μ^2	2.28±0.07	2.58±0.10*	3.44±0.21	2.79±0.09*
Number of nucleoli	2.61±0.08	2.75±0.11	2.49±0.10	2.05±0.06*

ET-1 produced different effects on DNA synthesis in adult albino rats (Table 2). ET-1 in a dose of 10 μg/kg did not change DNA synthesis in the epithelium of the skin and duodenum. ET-1 injected 2 times a day produced different changes in ILN and LI in the tongue epithelium. The count of proliferating epithelial cells decreased by 67%, while LI increased by 23%. Administration of the peptide for 5 days increased LI in the tongue epithelium by 31%.

These results indicate that ET-1 produces different changes in the proliferative processes in epithelial tissues from newborn and adult animals. Probably, these differences are tissue specific and typical of epithelial cells in the skin and intestine.

We studied the influence of ET-1 on the myocardium from newborn and adult rats to evaluate whether ontogenetic peculiarities of peptide-induced changes in tissue homeostasis are systemic. Proliferative processes in the myocardium of adult rats are minimum and cannot be assayed by autoradiography. Ontogenetic peculiarities of ET-1-induced changes in the heart were studied by morphometrical, histochemical, and biochemical methods.

Administration of ET-1 in a dose of 50 μg/kg from the 2nd to 6th day of life markedly increased the density of cardiomyocyte nucleoli, which indicates that this peptide activates protein synthesis in myocardial cells (Table 3). These results are consistent with published data that ET-1 stimulates protein synthesis in cultured cardiomyocytes from newborn albino rats [11,13].

By contrast, treatment of adult albino rats with ET-1 for 5 days suppressed anabolic processes in the myocardium (Table 3). The number and area of cardiomyocyte nucleoli markedly decreased in ET-1-receiving rats.

Biochemiluminescence of heart tissues from newborn and adult rats revealed activation of free radical oxidation. It should be emphasized that prooxidant changes in adult rats were more pronounced than in newborn animals. In the myocardium of 7-day-old rats H_2O_2-induced chemiluminescence and I_{max} surpassed the control by 21 and 30%, respectively. In adult animals these parameters surpassed the control by 49 and 82%, respectively.

Probably, the contribution of the direct cellular effect of ET-1 into changes observed after its administration is diminished in adult rats, which unmasked changes associated with its vasoconstrictor activity of the peptide.

Ontogenetic peculiarities of endothelin receptors were studied in the renal cortex of albino rats. The count of specific receptors markedly decreases with age. It should be emphasized that the ratio between various subtypes of receptors also undergoes considerable changes. The number of ETa receptors mediating the mitogenic effect of this peptide decreases, while the content of ETb receptors increases. ET loses its ability to stimulate hydrolysis of phosphoinositols in kidney cells with age [7]. Changes in other cell populations are similar. ET-1 increases intracellular cAMP content and stimulates proliferation of smooth muscle cells of the pulmonary artery in newborn, but not in adult animals [8].

Our results demonstrate considerable ontogenetic peculiarities of ET-1-induced changes. In newborn rats this peptide stimulates DNA synthesis in various cells and protein synthesis in the myocardium. In adult animals ET-1 produces no stimulatory effect on proliferative processes, suppresses nucleolus-organizing activity in cardiomyocyte nuclei, and significantly intensifies free radical oxidation in the heart.

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