BIOPHYSICS AND BIOCHEMISTRY

Effect of Endothelin-1 on DNA Synthesis in the Myocardium of Albino Rats during Early Postnatal Ontogeny

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Effect of intraperitoneal injection of endothelin-1 on DNA synthesis in the myocardium of newborn albino rats was studied by ^{3}H-thymidine autoradiography. Endothelin-1 injected in a single dose of 10 μg/kg stimulated proliferative processes: the index of labeled nuclei and labeling intensity increased. Repeated (5 times) administration of endothelin-1 in doses of 1 and 10 μg/kg increased labeling intensity, but did not change the index of labeled nuclei. The data suggest that endothelins are involved in morphogenesis of the myocardium during the early postnatal ontogeny.

Key Words: *endothelin-1; myocardium; DNA synthesis*

Vasoactive regulatory peptide endothelin-1 (ET-1) possesses various biological properties, including mitogenic activity [9,14]. Previous studies showed that ET-1 stimulates *in vitro* proliferation of cardiomyocytes [15]. The ET system is probably involved in the pathogenesis of hypertrophic cardiomyopathies [11,12]. Therefore, medicinal preparations containing ET and specific antagonists hold much promise.

In our previous experiments, we studied the effects of repeated (5 times) administration of 100 μg/kg ET-1 on DNA synthesis in the myocardium of newborn albino rats. 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} [6] 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 [5]. Since prolonged infusion is inconvenient for studying proliferative processes, in our experiments ET-1 was injected repeatedly in comparable doses. However, ET-1 in pharmacological doses did not affect DNA synthesis in the myocardium [4].

Here we studied the effects of ET-1 in near-physiological doses (1 and 10 μg/kg) on DNA synthesis in the myocardium of newborn albino rats.

MATERIALS AND METHODS

In series I, ET-1 in a dose of 10 μg/kg was injected to newborn albino rats on day 5 of life. In series II, ET-1 was injected intraperitoneally in doses of 1 and 10 μg/kg from the 2nd to 6th day of life. Control animals received an equivalent volume of sterile isotonic NaCl. Control and experimental groups were composed by the method of litter separation to decrease the genetically determined differences between litters.

DNA synthesis in the myocardium was determined autoradiographically 24 h postinjection (in series

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TABLE 1. Effect of Single Injection of ET-1 on DNA Synthesis in the Myocardium of 5-Day-Old Albino Rats (*M±m*)

Myocardial zone		Control		Experiment	
		ILN, %	LI	ILN, %	LI
Atrium	left	9.21±1.60	26.06±1.62	9.29±1.40	31.23±1.59*
	right	6.72±0.63	24.79±2.85	10.04±1.25*	30.93±1.33
Ventricle	left	9.48±1.33	23.24±1.67	12.75±1.06	31.85±1.53*
	right	6.55±1.02	20.25±2.49	8.18±1.01	30.72±1.19*
VS		10.37±1.55	28.86±2.14	12.81±1.40	28.93±3.05

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

II, 24 h after the last injection). ^{3}H-Thymidine was injected intraperitoneally in a dose of 1 μCi/g body weight (specific activity 1570 TBq/mol) 1 h before euthanasia. Heart samples were fixed in a formalin:alcohol:glacial acetic acid mixture, treated by routine histological methods, and embedded in paraffin. Histological preparations and autoradiographs were prepared by routine techniques.

Labeled cardiomyocytes were separately counted in the left and right atria and subendocardial layers of the left ventricle, ventricular septum (VS), and right ventricle. The index of labeled nuclei (ILN) reflecting the ratio of DNA-synthesizing nuclei and labeling intensity (LI) showing the number of silver grains over the nucleus were calculated.

We evaluated the effects of ET-1 injected in single doses of 10 and 100 μg/kg on lipid peroxidation (LPO) and antioxidant (AO) defense in myocardial homogenates from newborn rats. H_2O_2-induced luminol-dependent chemiluminescence was measured on an LS 50B chemiluminometer (Perkin Elmer) to study LPO processes [3]. Total chemiluminescence (over 2 min) and flash amplitude were measured at room temperature, calculated per 1 mg lipids, and expressed in arbitrary units. The contents of lipid hydroperoxides, malonic dialdehyde (MDA), and vitamin E were measured as described elsewhere [2].

Experiments were performed on 84 rats. The results were analyzed by Student's *t* test.

RESULTS

ET-1 injected in a single dose of 10 μg/kg changed DNA synthesis in the myocardium of 5-day-old albino rats (Table 1). ILN increased by 49.4 and 34.5% (insignificant) in the right atrium and subendocardial myocardium of the left ventricle, respectively. LI increased in the left atrium (by 19.8%) and left and right ventricles (by 37.0 and 51.7%, respectively); in the right atrium, this parameter tended to increase (by 24.8%). Thus, ET-1 injected in a single dose of 10 μg/kg stimulated DNA synthesis in the myocardium: the number of ^{3}H-thymidine-labeled nuclei and the rate of DNA synthesis increased.

Repeated (5 times) administration of ET-1 in a dose of 10 μg/kg did not increase the number of cardiomyocytes in S phase of the cell cycle: ILN in all myocardial zones did not differ from the control. LI increased in the right atrium (by 25.7%), left and right ventricles (by 34.7 and 27.6%, respectively), and VS (by 28.1%, Table 2).

Repeated (5 times) administration of ET-1 in a dose of 1 μg/kg produced similar changes in DNA synthesis in the myocardium (Table 2).

TABLE 2. Effect of Repeated (5 Times) Administration of ET-1 on DNA Synthesis in the Myocardium of 7-Day-Old Albino Rats (*M±m*)

Myocardial zone		Control		ET-1, μg/kg			
				1		10	
		ILN, %	LI	ILN, %	LI	ILN, %	LI
Atrium	left	4.8±0.5	11.00±0.58	5.44±0.57	13.88±0.69*	4.05±0.74	13.25±1.31
	right	4.34±0.63	10.53±0.51	4.58±0.68	13.45±0.78*	4.93±0.94	13.24±0.80*
Ventricle	left	7.90±0.54	12.54±0.66	7.43±0.80	15.53±1.02*	6.49±1.03	16.89±1.03*
	right	6.32±0.65	11.52±0.45	6.53±0.68	14.94±0.64*	5.88±1.15	14.69±0.94*
VS		8.04±0.78	14.72±0.68	8.64±0.69	16.68±0.78	7.42±0.86	18.85±1.15*

TABLE 3. Effect of ET-1 on the LPO-AO System in the Myocardium of 7-Day-Old Albino Rats (*M±m*)

Parameter	Control	ET-1, μg/kg	
		10	100
MDA, fluorescence units/mg lipids	11.75±1.19	9.71±0.61	11.13±1.06
Lipid hydroperoxides, optical density units/mg	0.23±0.02	0.15±0.02*	0.18±0.02
Vitamin E, μg/g heart	9.69±1.52	6.89±1.42	6.84±1.02
Total chemiluminescence, arb. units	13.31±3.04	11.81±1.25	31.84±4.73*
Flash amplitude, arb. units	13.12±2.52	15.9±3.67	28.05±4.59*

Thus, repeated (5 times) administration of ET-1 accelerated DNA synthesis in various myocardial zones, but had no effect on proliferative processes. It should be noted that the increase in LI at the constant number of DNA-synthesizing nuclei is a peculiar type of activation of proliferation in various cell populations.

In our experiments ILN remained unchanged, while LI increased by 23-30% in the left and right atria, ventricles ($p<0.05$), and VS ($p<0.1$).

In a dose of 100 μg/kg ET-1 produced no activating effects on DNA synthesis in the myocardium [4]. Similar findings were reported by K. Chung *et al.* (inversion of growth-stimulating effects) [6]. It was shown that ET-1 in doses of 10^{-9}-10^{-7} mol/liter activates proliferation of cultured mesangial cells. However, increasing the dose of ET-1 to 10^{-6} mol/liter leads to inhibition of cell growth.

These differences can be attributed to the effects of ET-1 on the LPO-AO system in the myocardium (Table 3). ET-1 in a dose of 100 μg/kg elevated total chemiluminescence and flash amplitude by 2.3 and 2.2 times, respectively. These changes indicated intensification of LPO and inhibition of the AO system in the myocardium. Cytotoxic effects of LPO products probably counteract mitogenic properties of the peptide. This assumption was confirmed by the fact that decreasing the dose of ET-1 by one order of magnitude reduced the content of lipid hydroperoxides and stimulated DNA synthesis in the myocardium.

Our findings indicate that ET-1 activates proliferative processes in the myocardium of albino rats during the early postnatal ontogeny. These changes are observed under the effect of ET-1 in a dose of 1 μg/kg, which suggests the specificity of its action. The ET system is probably involved in heart morphogenesis both in birds [10] and mammals. Our previous experiments showed that angiotensin II stimulates proliferative processes in the myocardium of newborn albino rats [1]. Thus, the interaction between the ET and angiotensin systems probably determines morphogenesis of the myocardium during the early postnatal ontogeny. These results suggest that the LPO-AO system is involved in the realization of ET-1 effects on cardiomyocyte proliferation. Moreover, tyrosine kinases [12], protein kinase C [14], Ca^{2+} [13], and cAMP [9] systems can also play a role in the mitogenic effect of ET.

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