EXPERIMENTAL BIOLOGY

Effect of Atriopeptides on Proliferative Processes in Albino Rat Myocardium at the Early Stage of Postnatal Ontogenesis

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The effect of atriopeptide AP-II and its 6-amino-acid acyclic fragment (7-12) AP-H-6-OH on proliferative processes in the myocardium of 5-day-old albino rats is examined 24 h after a single administration (200 nmol/kg intraperitoneally) by autoradiography with ^{3}H-thymidine and by analysis of mitotic regime. A significant decrease in the amount of DNA-synthesizing cells in the right atrium, right ventricle, and interventricular septum is recorded after administration of AP-II. Changes in DNA synthesis are attended by significant changes in the amount of cells in different phases of mitosis, indicating a decrease in the number of mitotic cells and a metaphasal delay. Administration of AP-H-6-OH causes no significant changes in DNA synthesis in the myocardium at the early stage of postnatal ontogenesis.

Key Words: *atriopeptides; proliferation; myocardium; postnatal ontogenesis*

Atriopeptides participate not only in the regulation of fluid-and-electrolyte balance, but also in the maintenance of structural homeostasis. Proliferative effects of atriopeptides on mesangial [7], endothelial [9], and vascular smooth muscle cells [13] have been demonstrated *in vitro*. Atriopeptides were shown to influence physiological regeneration of renal epithelia and cortex *in vivo* [1].

The presence of atriopeptides in mammalian myocardium at the early stages of heart development [14] and the effect on the proliferation of cultured chick embryo cardiomyocytes [10] suggest that atriopeptides are involved in cardiac morphogenesis.

The aim of this study was to examine the effect of atriopeptides on cell reproduction in albino rat myocardium at the early stages of postnatal development.

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MATERIALS AND METHODS

The following atriopeptides were used:

1) 23-amino acid-long atriopeptide AP-II: H_2N-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-COOH. Structural characteristics and properties of this peptide are the same as those of native atriopeptides.

2) 6-amino acid-long acyclic atriopeptide analog AP-H-6-OH: H_2N-Arg-Ile-Asp-Arg-Ile-Gly-COOH.

It was reported that similar linear analogs of atriopeptides did not bind to specific B receptors for atriopeptides [8].

Experiments were performed on 4-day-old outbred albino rats. In order to diminish genetic differences control and experimental groups were formed by the method of litter split (eight pups/litter). The

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TABLE 1. Effects of Atriopeptides on DNA Synthesis (ILN, %) in Albino Rat Myocardium During Early Postnatal Ontogenesis (*M±m*)

Myocardial zone	AP-II		AP-H-6-OH	
	control	experiment	control	experiment
Left atrium	4.17±0.55	3.27±0.40	5.49±0.56	5.72±0.53
Right atrium	4.68±0.60	2.82±0.58*	6.60±0.89	6.82±0.76
Left ventricle:				
subendocardial layer	7.19±0.71	5.02±0.82	7.61±0.78	8.32±0.40
intramural layer	6.38±0.86	4.38±0.59	7.12±0.68	6.26±0.50
subepicardial layer	4.85±0.68	3.42±1.02	4.55±0.55	4.48±0.39
Interventricular septum:				
subendocardial layer	7.67±0.59	4.91±0.74*	7.00±0.64	7.91±0.41
intramural layer	8.15±0.76	4.66±0.46*	8.46±0.97	9.38±0.63
Right ventricle:				
subendocardial layer	5.59±0.55	3.63±0.47*	6.17±0.69	6.80±0.37
intramural layer	3.98±0.58	2.01±0.25*	5.12±0.46	5.03±0.61
subepicardial layer	3.60±0.35	1.69±0.27*	3.69±0.63	4.36±0.54

Note. Here and in Table 2: *$p<0.05$ compared with the control.

atriopeptides AP-II and AP-H-6-OH were synthesized at the Laboratory of Peptide Chemistry (Cardiology Research Center, Russian Academy of Medical Sciences, Moscow). They were administered in a single dose of 200 nmol/kg intraperitoneally. Control rats were injected with an equal volume of sterile normal saline (0.01 ml). Proliferation was studied 24 h after administration of both peptides. Radiolabeled thymidine (specific activity 87 Ci/mmol) was injected intraperitoneally in a dose of 1 μCi/g body weight one hour prior to euthanasia. Histotopographic preparations were processed and autoradiography was performed by the standard method [3]. Proliferation was assessed by the following parameters of DNA synthesis: index of ^{3}H-thymidine-labeled nuclei (ILN, %) and intensity of labeling (mean number of silver grains over the nucleus). Mitotic index (MI, ‰) and percent ratios of mitotic phases were used for analysis of mitotic regime in the myocardium.

All parameters were determined in the left and right atrium, subendocardial, intramural, and subepicardial zones of left and right ventricles, and in the subendocardial and intramural zones of intraventricular septum, taking into account morphological differences between muscle and nonmuscle cells [4]. Nuclei of questionable tissue specificity were not counted.

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

RESULTS

The myocardium of 4-day-old albino rats was at the stage of maturation, when its proliferative potential is limited by progressive differentiation of cardiomyocytes [6]. The parameters of DNA synthesis in the myocardium of control animals were consistent with the published data on ^{3}H-thymidine incorporation in cardiomyocytes of young albino rats [5].

Administration of 200 nmol/kg AP-II led to a significant decrease in the number of DNA-synthesizing myocardiocytes in the right atrium, subendocardial, intramural, and subepicardial layers of the right ventricle, and subendocardial and intramural layers of the interventricular septum (Table 1). The decrease in the ILN in the left atrium myocardium, subendocardial, intramural, and subepicardial layers of the left ventricle was statistically insignificant. There were no differences in the intensity of labeling, which indirectly characterizes the rate of DNA synthesis, in all zones of the myocardium between control and experimental animals.

The MI in the subendocardial and intramural layers of the septum did not differ significantly in experimental and control groups (Table 2). However, considerable differences were revealed in the number of cells in different phases of mitosis: the number of prophases significantly decreased, the number of metaphases significantly increased, and, consequently, the prophase-metaphase coefficient dropped. Presumably, in the myocardium of experimental animals the decrease in the number of mitotic cells was associated with metaphasal delay, which simulated a stable mitotic activity of the myocardium.

Thus, systemic influence of atriopeptide AP-II in a dose of 200 nmol/kg markedly (1.6- to 2.1-fold) lowered the proliferative activity of cardiomyocytes

TABLE 2. Effect of Atriopeptide AP-II on the Mitotic Regime in Albino Rat Myocardium During Early Postnatal Ontogenesis (*M±m*)

Parameter	Control	Experiment
Mitotic index, ‰		
subendocardial layer of the interventricular septum	5.60±0.64	4.10±0.55
intramural layer of the interventricular septum	3.00±0.28	2.60±0.13
Number of cells in different phases of mitosis, %		
prophase	33.56±2.24	24.84±1.24*
metaphase	46.00±1.79	54.23±2.56*
anaphase+telophase	17.16±1.71	18.87±2.05
Prophase-metaphase coefficient	0.748±0.067	0.469±0.041*

in the right heart and septum. This is confirmed by a decrease in the number of DNA-synthesizing and mitotic cells as well as by mitoses delayed at metaphase. These changes in cellular reproduction may be indicative of both suppressed physiological regeneration of the myocardium and accelerated maturation and differentiation of cardiomyocytes [5].

It was reported that atriopeptide stimulates proliferation of cultured chick embryo cardiomyocytes with a simultaneous activation of myosin and tropomyosin synthesis [10]. The formation of specific contractile proteins reflects differentiation of cardiomyocytes, a process antagonistic to their proliferation [5]. Presumably, the accelerating effect of atriopeptides on the maturation of cardiomyocytes predominates during postnatal development.

Organic topography of the effect may be associated with the pharmacokinetics of the peptide. An intraperitoneally injected compound reaches the left heart via the small circulation. The lungs are known to play an important role in the elimination of atriopeptides from the bloodstream [2,11]. In addition, the localization of changes may be associated with different contents of atriopeptide receptors in the left and right heart.

The specific nature of the AP-II effect is confirmed by experiments with the acyclic peptide AP-H-6-OH, which does not bind to B receptors (atriopeptide receptors coupled to guanylate cyclase) [8]. Administration of 200 nmol/kg AP-H-6-OH caused no significant changes in DNA synthesis in all studied zones of the myocardium: there were no differences in ILN (Table 1) and labeling intensity between experimental and control rats.

Previously, we showed that AP-II and AP-H-6-OH produce a similar effect on proliferative processes in corneal, esophageal, skin, and intestinal epithelium [1]. The responses of the adrenal zona glomerulosa to AP-II and AP-H-6-OH were absolutely different: the acyclic atriopeptide had no effect on the proliferation of adrenocorticocytes [1]. Consequently, the relationship between structure and activity of atriopeptides toward the myocardium of young animals is similar to that established in experiments with the typical target of atriopeptides: zona glomerulosa of the adrenals.

Our results suggest that atriopeptides are involved in the morphogenesis of mammalian heart. Presumably, only atriopeptides with preserved cyclic structure modulate the reproduction of cardiomyocytes.

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