MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Endothelin-1 and Atrial Natriuretic Peptide-II on Heart Morphogenesis in Albino Rats

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Computer morphometry and histochemical analysis were used to study the delayed effects induced by endothelin-1 and atrial natriuretic peptide-II (single doses 50 and 100 μg/kg, respectively) in neonatal albino rats subjected to 5 repeated intraperitoneal injections of the drugs during postpartum days 2-6. Injections of endothelin-1 to 21-day-old neonatal rats promoted activation of the nucleolus organizer regions by increasing the number and area of nucleoli and the number of DOT (optically dense regions corresponding to the number of silver grains determined visually). The area and protein content in cardiomyocytes also increased. Protein content in cardiomyocytes remained high in 45-day-old rats. Repeated injections of atrial natriuretic peptide-II to neonatal rats decreased the area of nucleoli in 21-day-old rats, but produced no significant changes in morphometric parameters of nucleoli and cardiomyocytes in 45-day-old rats. The number of nuclei expressing proliferating cell nuclear antigen (PCNA) did not change significantly after administration of endothelin-1 and atrial natriuretic peptide-II in both age groups. These data suggest on delayed opposite effects of the examined neuropeptides on heart morphogenesis.

Key Words: *endothelin; atrial natriuretic peptide; heart ontogeny*

Morphogenesis of the heart at the early terms of postnatal period is a unique program of proliferation and differentiation of all tissue components. The endogenous peptidergic system actively participates in the development of the myocardium. We previously found that injection of endothelin-1 (ET-1) to newborn rats stimulates DNA synthesis in the myocardium [7]. The opposite effect is produced by atrial natriuretic peptide-II (ANP-II), which inhibits the DNA-dependent synthetic processes [2]. Moreover, some peptides injected to newborn animals produce delayed effects on the development of the heart [5]. Our aim was to reveal possible delayed effects of vasoactive peptides and to examine whether the effects of ET-1 and ANP-II on heart morphogenesis are still opposite at latter terms.

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MATERIALS AND METHODS

Albino rats (n=72) received daily intraperitoneal injections of ET-1 (50 μg/kg) and ANP-II (100 μg/kg) on postnatal days 2-6. The peptides were synthesized at the Laboratory of Peptide Synthesis of Russian Cardiology Research-and-Production Complex, Ministry of Health, Moscow. The controls received an equivalent volume of 0.9% NaCl.

In series I, the rats were sacrificed on postnatal day 21 (14 days after the final injection), in series II the rats were killed on postnatal day 45. The hearts were removed and fixed in 10% neutral formalin. Paraffin sections (7 μ) were routinely prepared. For visualization of mitotic nuclei, the sections were treated

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with antibodies to proliferating cell nuclear antigen (PCNA, DAKO) followed by indirect streptavidin–biotin-diaminobenzidine visualization and hematoxylin poststaining. Index of labeled nuclei was calculated as the percent of total number of cardiomyocyte (CMC) nuclei in the left ventricle (no less than 3000 nuclei were examined). For visualization of the nucleolus organizer region, the preparations were stained with 50% $AgNO_3$ [4]. A fragment of the myocardium from the left ventricle was dissociated in 50% KOH and smears of isolated CMC were stained with amido black B [1]. The morphometric indices of the nucleolus organizer and isolated CMC (area and optical density) were determined using a MEKOS-C computer-assisted image analysis system.

The data were analyzed statistically using Statistica 5.0 software and Student's t test at $p<0.05$.

RESULTS

Two weeks after the 5th injection of ET-1 to newborn rats we observed a significant increase in the number and total area of nucleoli. The number of DOTs (optically dense regions corresponding to the number of silver grains) also increased. These changes attest to activation of plastic function of CMC [4]. Significant increase in the total area and integral optical density of CMC stained with amido black B (Table 1) attests to an increase in the total protein content in these cells. In 45-day-old rats we observed only the increase in the protein content. In the control, the number of PCNA-positive nuclei decreased with age (Table 1), which reflects a tendency to inhibition of CMC proliferation during postnatal ontogeny of the heart [6]. In both age groups treated with ET-1 or ANP-II the number of PCNA-positive nuclei did not significantly differ from the control (Table 1). It should be noted that in newborn rats ET-1 stimulates DNA synthesis 24 h after five repeated injections [7], while similar injections of ANP-II inhibit it [2]. The number of PCNA-positive nuclei was higher than the number of nuclei labeled with ^{3}H-thymidine. This phenomenon can be explained by peculiar expression of PCNA, which is synthesized throughout the mitotic cycle [8]. In some cell populations (including myocardium) this phenomenon can be used for evaluation of the proliferating pool in animals at the age of 1 month and older, when thymidine index decreases below 0.1% [6]. At the same time, parallel decrease in the number of nuclei initially labeled with ^{3}H-thymidine and PCNA-positive nuclei in rat myocardium at the early postnatal stages confirms the adequacy of the use of antibodies against to PCNA for evaluation of the number of cycling nuclei.

Repeated injections of ANP-II on postnatal days 1-6 promoted the decrease of total area of nucleoli in

TABLE 1. Effect of Five Repeated Injections of Vasoactive Peptides to Newborn Rats on Morphological Indices of CMC in Left Ventricle ($M\pm m$)

Index	ET-1				ANP-II			
	21-day-old rats		45-day-old rats		21-day-old rats		45-day-old rats	
	control	test	control	test	control	test	control	test
Area of nucleoli, μ^2	1.64±0.11	2.08±0.16*	2.73±0.11	2.94±0.22	1.69±0.10	1.42±0.06*	2.35±0.13	2.03±0.16
Number of nucleoli	1.62±0.03	2.05±0.05*	2.21±0.08	2.15±0.12	1.81±0.06	1.72±0.08	2.03±0.13	2.01±0.11
DOT number	3.51±0.1	3.83±0.09*	4.15±0.08	4.23±0.10	3.48±0.14	3.42±0.13	3.75±0.13	3.74±0.14
CMC area, μ^2	1655.75±55.56	1942.87±114.21*	2223.65±35.32	2539.15±144.83	1248.00±51.76	1135.49±30.99	2751.27±146.24	2443.37±126.49
Integral optical density of CMC, arb. units	60.39±3.68	81.27±6.97*	138.66±13.41	173.94±8.94*	42.17±8.08	44.62±7.28	161.23±15.95	158.39±11.71
Number of PCNA-positive nuclei, %	6.1±1.2	6.8±0.9	1.50±0.21	1.8±0.17	5.4±0.8	4.9±1.1	1.90±0.14	1.60±0.34

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

21-day-old rats against the background of stable geometric and optical parameters. In these rats the number of PCNA-positive CMC nuclei in the left ventricle did not significantly differ from the control (Table 1). In 45-day-old test and control rats all myocardial indices were similar.

Therefore, ET-1 and ANP-II can produce delayed effects on plastic function of rat CMC. In 3-week-old rats their effects on protein synthesis are similar to their effect on DNA synthesis in newborns. In addition, the effects of ET-1 and ANP-II on protein content and activity of the nucleolus organizer are opposite.

The opposite effects of ET-1 and ANP-II can be explained by their opposite effects on vascular tone. ET-1 induces vasoconstriction via ET_A-receptors [9]. ANP-II decreases Na^+ content in vascular wall thus inducing vasodilation [11]. It is known that hemodynamic load is an important factor controlling synthesis of contractile proteins in the myocardium.

Participation of age-related peculiarities in the realization of the effects of the examined peptides cannot also be excluded. During the early postnatal ontogeny the content of ET-1 in the heart increases [12], while that of ANP-II decreases [10].

In comparison with ET-1, long-term treatment with ANP-II changes fewer morphometric parameters. In 45-day-old rats these changes were absent (Table 1).

Our study confirms the existence of opposite delayed effects of ET-1 and ANP-II on heart morphology in newborn rats.

There is evidence on delayed behavioral changes in rats receiving casomorphin during the early postnatal ontogeny [3]. We conclude that the delayed effects can also manifest in the form of structural changes.

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