BIOPHYSICS AND BIOCHEMISTRY

Changes in DNA Synthesis and Morphometric Indexes of the Myocardium in Albino Rats Treated with Angiotensin II

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Autoradiography with ^{3}H-thymidine showed that angiotensin II injected intraperitoneally to newborn albino rats from the 2nd to 6th day of life stimulates DNA synthesis in the myocardium of the left ventricle and increased the area of cardiomyocytes and number and area of nucleoli. Two weeks after treatment the area of cardiomyocytes and the number of nucleoli increased, while the count of PCNA-positive nuclei remained unchanged. Immunohistochemical assay for vimentin showed that the ratio between the number of cardiomyocytes and connective tissue cells remained unchanged. In 45-day-old rats (38 days after treatment) the intensity of DNA synthesis and morphometric indexes of the myocardium did not differ from the control. Fivefold treatment of adult rats with angiotensin II increased the number and area of nucleoli, but had no effect on the count of connective tissue cells.

Key Words: *angiotensin; DNA synthesis; myocardium; morphogenesis*

Our previous experiments showed that neuropeptides modulate DNA synthesis in the myocardium of newborn rats. Treatment with these peptides produces delayed consequences in adult animals [7].

Angiotensin regulates functional activity of the cardiovascular system and maintains structural homeostasis in various tissues, including the myocardium [3,5].

The effects of angiotensin on the myocardium in newborn animals are poorly understood. Moreover, it remains unclear whether angiotensin treatment produces delayed consequences. Here we studied the intensity of DNA synthesis and morphometric indexes in albino rats treated with angiotensin II.

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MATERIALS AND METHODS

Newborn albino rats received intraperitoneal injections of angiotensin II in a dose of 100 μg/kg from the 2nd to 6th day of life. Angiotensin II was synthesized at the Laboratory of Peptide Synthesis (Research Center for Cardiology, Russian Academy of Medical Sciences). In series I the animals were killed 1 day after the last treatment. In series II and III the rats were decapitated on days 21 and 45 of life, respectively (14 and 38 days after the last treatment, respectively). The rats intraperitoneally received ^{3}H-thymidine in a dose of 1 μCi/g (1570 TBq/mol) 1 h before decapitation. In special series adult male rats received 5 injections of angiotensin II (100 μg/kg) and were euthanized 24 h after the last injection. Control animals received an equivalent volume of 0.9% NaCl. Experiments were performed on 106 rats.

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After decapitation the heart was removed from the thorax, fixed with 10% formalin in phosphate buffer (pH 7.5) for 2 days, washed with flowing water for 1 day, treated with alcohols and toluenes in increasing concentrations, and embedded in paraffin. Histotopographic sections (7 μ) were prepared and mounted on glasses coated with poly-L-lysine (DAKO) to improve adhesion during immunohistochemical assay.

Histoautoradiography with type R photoemulsions (Institute KhIMPhOTO) was performed routinely. We examined no less than 2000 nuclei in cardiomyocytes of the left ventricle to evaluate the index of ^{3}H-thymidine-labeled nuclei (ILN, %). Deparaffinized sections were impregnated with $AgNO_3$ to reveal nucleolar organizer regions [6].

PCNA-positive nuclei were detected immunohistochemically (% of 2000 nuclei). The count of vimentin-expressing cells in the myocardium of the left ventricle was determined by the double streptavidin-biotin method (% of 2000 cells). Visualization was performed with diaminobenzidine (DAKO).

Proliferating cell nuclear antigen (PCNA) is expressed by nuclei in G_1, S, G_2, and M phases of the cell cycle. The expression of PCNA peaks by the end of S phase. It is important that cells with low proliferative activity can be studied only in experiments with PCNA. For example, in the myocardium of 3-week-old rats the index of nuclei primarily labeled with ^{3}H-thymidine does not surpass 0.1% [8].

The cytoplasm of connective tissue cells is stained with antibodies against vimentin, which allows evaluating the ratio between stromal and parenchymal cells in the myocardium. However, antibodies against vimentin are of limited usefulness in newborn animals. In these animals cardiomyocytes contain a significant number of vimentin intermediate filaments [1], which determines their positive staining and makes the differential diagnostics of muscle and connective tissue cells difficult.

In 45-day-old and adult animals this diagnostics is simple and can be performed on preparations stained with hematoxylin and eosin [1].

To obtain isolated cardiomyocytes the myocardium of the left ventricle was subjected to alkaline dissociation [4], and smears were stained with amide black B [2]. The area of isolated cardiomyocytes and the number and area of nucleoli were estimated using a MEKOS-Ts video image analyzer.

The results were analyzed by Student's *t* test (Statistica 5.0 software). Intergroup differences were significant at $p<0.05$.

RESULTS

Fivefold treatment of newborn rats with angiotensin II increased ILN, area of cardiomyocytes, and number and area of nucleoli (Table 1). Stimulation of DNA synthesis was accompanied by activation of the nucleolar apparatus, which indirectly reflects protein-synthesizing activity of myocardial cells [6].

The area of cardiomyocytes and number of nucleoli remained high in 3-week-old animals. In these rats the number of nuclei expressing PCNA and count of connective tissue cells did not differ from the control.

Changes produced by 5-fold treatment of newborn rats with angiotensin II were not observed in 45-day-old animals. Morphometric indexes of the myocardium did not differ in experimental and control rats.

Published data show that the sensitivity of rat myocardium to angiotensin depends on animal age [12]. In our experiments repeated treatment with angiotensin II produced similar changes in newborn and

TABLE 1. Effects Angiotensin II (5 Injections from the 2nd to 6th Day of Life) on Morphometric Indexes of Left Ventricular Myocardium in Albino Rats (*M*±*m*)

Parameter	7-day-old		21-day-old		45-day-old	
	control	experiment	control	experiment	control	experiment
Area of cardiomyocytes, m^2	685.75±57.32	871.72±54.098	1655.75±55.56	1866.22±76.04*	2223.65±35.32	2114.6±61.68
Number of nucleoli	2.47±0.05	2.81±0.1*	1.62±0.03	2.01±0.09*	2.21±0.08	2.21±0.06
Area of nucleoli, m^2	2.59±0.09	3.22±0.22*	1.64±0.11	1.75±0.12	2.73±0.11	2.61±0.19
ILN, %	6.95±0.32 (1)	8.53±0.22* (1)	6.11±0.3 (2)	4.98±0.45 (2)	0.11±0.03 (1)	0.18±0.04 (1)
Number of connective tissue cells, %	—	—	48.97±2.44 (3)	53.22±2.65 (3)	60.21±1.2 (4)	62.19±0.46 (4)

Note. *$p<0.05$ compared to the control. ILN after administration of ^{3}H-thymidine (1) and staining with antibodies against PCNA (2) and vimentin (3) or hematoxylin and eosin (4).

TABLE 2. Effects Angiotensin II (5 Injections) on Morphometric Indexes of Left Ventricular Myocardium in Adult Albino Rats (*M*±*m*)

Parameter	Control	Experiment
Area of cardiomyocytes, μ^2	3700.6±168.82	3886.21±179.38
Number of nucleoli	1.86±0.07	2.24±0.05*
Area of nucleoli, μ^2	3.07±0.28	4.04±0.27*
Number of connective tissue cells, %	63.12±1.06	62.79±0,86

Note. *p<0.05 compared to the control.

adult rats. In adult animals the preparation increased the number and area of nucleoli, but had no effect on the ratio between the number of muscle and non-muscle cells (Table 2).

The effect of angiotensin can be realized via its interaction with receptors on cardiomyocytes [11]. It cannot be excluded that the action of this peptide is mediated by changes in activity of adrenergic intra- and extracardiac neurons [9] and hemodynamic load on the heart due to modulation of the vascular tone [10].

Our results are consistent with published data [7] that activation of DNA synthesis in newborn rats produces delayed consequences in 21-day-old animals, which includes stimulation of the nucleolar organizer regions (increase in the number and area of nucleoli). Interestingly, administration of atrial natriuretic peptide to newborn rats inhibits DNA synthesis. Two weeks after treatment the intensity of DNA synthesis remains unchanged, while activity of nucleolar organizer regions is suppressed [7].

Delayed consequences of angiotensin treatment suggest that normal morphogenesis requires a balanced state of the renin-angiotensin system during the early postnatal development.

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