Dynorphin Administered to Newborn Rats Modulates Morphogenesis of the Heart

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Proliferation, activity of the nucleolar organizer region, and protein content in cardiomyocytes were studied in 21-day-old rats intraperitoneally treated with κ-opioid receptor agonist dynorphin A_{1-13} in a dose of 100 μg/kg on postnatal days 2-6. The content of catecholamines in the heart and proliferative activity of cardiomyocytes remained unaffected, but the number of nucleoli in cardiomyocyte nuclei and the integral optical density of cardiomyocytes stained with amido black B (protein content) increased. The data suggest that administration of dynorphin to newborn rats considerably modulates morphogenesis of the heart at later terms.

Key Words: *myocardium; cardiomyocytes; protein synthesis; nucleolar organizer region*

Among numerous biological effects of opioid peptides, of special interest is their ability to regulate proliferative activity of various cell populations [1,10], in particular, in the early postnatal ontogeny [13,14]. Our previous studies showed that agonists of various opioid receptors (OR) activate DNA synthesis in the myocardium or newborn rats [2,7]. The most potent effect was produced by selective κ-OR agonist dynorphin A_{1-13}. This work was designed to study the reversibility of structural and functional shifts 14 days after 5 injections of dynorphin to newborn rats (*i.e.*, in 3-week-old rats). This period is characterized by minimum DNA replication and intensive synthesis of contractile proteins in the myocardium [9].

MATERIALS AND METHODS

Dynorphin A_{1-13}, a synthetic selective κ-OR agonist, synthesized at the Laboratory of Peptide Synthesis, Russian Cardiology Research-and-Production Complex, was intraperitoneally injected in a dose of 100 μg/kg to mongrel albino rats on postnatal days 2-6. Control rats were injected with an equal volume of 0.9% NaCl. Pups of the same litter were randomly assigned to either test or control groups. The rats were decapitated on postnatal day 21 (14 days after the last injection). The heart was removed and fixed in 10% neutral formalin for 2 days (for histological sections) or 2 weeks (for cytological examination). Smears of isolated left ventricular cardiomyocytes prepared after alkaline dissociation [4] were stained with amido black B [3]. Histological preparations of the myocardium were routinely dehydrated and embedded in paraffin. Nucleolar organizer regions were visualized by staining 7-μ sections with 50% $AgNO_3$ [6]. Cytophotometric and morphometric examinations of isolated cardiomyocytes and nucleolar apparatus were performed using a MECOS-C computer image analyzer. The areas of nuclei and nucleoli, their number, the area of cardiomyocytes and their integral and mean optical densities were measured. DNA-synthesizing nuclei were revealed by the avidin-biotin technique with antibodies to proliferating cell nuclear antigen (PCNA, DAKO) followed by visualization with diaminobenzidine. The index of labeled nuclei (ILN) was calculated in percents by counting no less than 1000 nuclei in 5 myocardial zones: left and right ventricles, left

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TABLE 1. Effects of Five Injections of Dynorphin A_{11-13} to Newborn Rats on Catecholamine Content in the Heart and Function of Nucleolar Apparatus and Protein Content in Cardiomyocytes of 21-Day-Old Rats (*M*±*m*)

Index	Control	Dynorphin
Catecholamines, μg/g		
epinephrine	0.021±0.004	0.017±0.005
norepinephrine	0.170±0.036	0.19±0.03
DOPA	0.43±0.15	0.33±0.13
Number of nucleoli	1.90±0.05	2.15±0.07*
Protein, g%	6.98±1.96	10.15±2.02
Area of nucleoli, μ^2	1.88±0.12	2.04±0.10
Area of cardiomyocytes, μ^2	1311.4±146.6	1476.6±91.2
Optical density of cardiomyocytes, arb. units		
mean	0.050±0.006	0.053±0.010
integral	68.4±5.7	85.7±4.4*

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

and right atria, and interventricular septum. The content of catecholamines in the heart was evaluated fluorometrically [8].

The data were processed statistically using Student's *t* test.

RESULTS

Two weeks after 5 injections of dynorphin, no significant changes in catecholamine content were found in the heart (Table 1).

Immunohistochemical determination of proliferative activity with anti-PCNA antibodies revealed no significant differences between the test and control groups 14 days after the last injection. The absence of differences in PCNA expression can be explained by stabilization of cardiomyocyte proliferation after dynorphin administration. However, we cannot exclude the possibility that some differences in DNA synthesis can be revealed by autoradiography. Another important feature was the response of the nucleolar apparatus. There were no significant changes in nucleolar activity 24 h after the last injection of dynorphin to newborn rats [7].Two weeks after the 5th injection of dynorphin the number of nucleoli significantly ($p<0.05$) increased (Table 1), but their cross-sectional area and activity of the nucleolar organizer region (nucleolar/nuclear ratio and number and density of dots, *i.e.*, optically dense regions corresponding to silver grains in the nucleoli) remained unchanged.

Changes in nucleolar activity reflect the intensity of plastic processes, in particular, protein synthesis [5]. Biuret reaction revealed a tendency toward an increase in protein content in the heart after dynorphin treatment (Table 1). More accurate method, cytophotometry of isolated amido black B-stained cardiomyocytes, showed an insignificant increase in the mean optical density reflecting protein concentration and in cardiomyocyte area (Table 1). However, the integral optical density derived from these two indices significantly increased.

Thus, activation of DNA synthesis 24 after dynorphin A_{1-13} treatment was followed by intensification of plastic processes, which was manifested in activation of the nucleolar apparatus and protein synthesis in cardiomyocytes. The mechanisms of this metamorphosis require further investigation.

An increase in cardiomyocyte ploidy accompanied by an increase in their protein content cannot also be excluded [11,12]. In addition, dynorphin can induce amplification of genes responsible for protein synthesis. This assumption also requires further studies.

Our findings are consistent with published data on morphogenetic changes in adult animals induced by opioid peptides administered at early terms after birth. Thus, treatment of rainbow trout eggs and fry with δ-OR receptor agonist dalargin increased protein contents in muscles of adult animals [5].

These data suggest that administration of opioid peptides to newborn animals causes considerable changes in morphogenesis of the myocardium at later terms of development.

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