GENETICS

Effect of Dynorphin A_{1-13} on DNA Synthesis and cAMP Level in the Myocardium of Albino Rats during Early Postnatal Ontogeny

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Effect of intraperitoneally injected dynorphin A_{1-13} on myocardial DNA synthesis in newborn rats was studied by ^{3}H-thymidine autoradiography. Single administration of 100 μg/kg peptide had no effects on DNA synthesis, while repeated (for 5 days) administration of the peptide significantly increased the labeling index of nuclei and labeling intensity in the myocardium 24 h postinjection. Single administration of dynorphin decreased cAMP levels in heart homogenates 4 h postinjection. Dynorphin is probably involved in the establishment of myocardial structural homeostasis. This effect is mediated by the system of cyclic nucleotides.

Key Words: *opioid peptides; DNA synthesis; myocardium; cAMP; ontogeny*

Opioid peptides are involved in the regulation of proliferative activities of various tissues in the organism [4,11]. Agonists of μ- and δ-opioid receptors (OR) are involved in the establishment of myocardial tissue homeostasis [1]. Mammalian heart has its own opioid system [2]. Prodynorphin gene and mRNA were found in cardiomyocytes [10]. κ-OR are prevalent in the heart. It was shown that these receptors appear earlier in ontogeny in comparison with others [9] and probably predominate during the early postnatal development [5]. This period coincides with the final stages of morphogenesis in mammalian heart [3]. Here we studied the involvement of κ-agonist dynorphin A_{1-13} in the establishment of myocardial tissue homeostasis.

MATERIALS AND METHODS

Selective κ-agonist dynorphin A_{1-13} synthesized in the Laboratory of Peptide Synthesis (Russian Cardiology Research-and-Production Complex, Russian Ministry of Health) was used. In series I, 4-day-old albino rats received single intraperitoneal injection of 100 μg/kg dynorphin. In series II, dynorphin was intraperitoneally injected in a daily dose of 100 μg/kg body weight from the 2nd to the 6th day. Control animals were injected with equivalent volume of sterile isotonic NaCl. Control and experimental groups were formed by the method of offspring separation to reduce genetically determined differences. DNA synthesis was studied by autoradiography 24 h postinjection (24 h after the last injection for repeated administration). ^{3}H-Thymidine (1530 TBq/mol) was injected intraperitoneally in a dose of 1 μCi/g body weight 1 h before euthanasia. Autoradiographs were prepared by routine techniques [2].

The labeling index of nuclei (LIN, %) and labeling intensity (LI, mean number of silver grains above the nucleus) were differentially calculated in myocardial tissues of the left and right atria, left and right ventricles, and interventricular septum using histotopographic heart preparations. Epithelial and connective tissue cells were not considered.

In series III, the effect of dynorphin on myocardial cAMP was studied. Four-day-old albino rats re-

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ceived single injection of 100 μg/kg dynorphin. The heart was washed in physiological saline and frozen in liquid nitrogen 4 h postinjection. The content of cAMP in heart homogenates was determined by radioimmunoassay using standard kits (Amersham).

The results were analyzed by Student's *t* test. Intergroup differences were considered to be significant at $p<0.05$.

RESULTS

Single injection of dynorphin to 4-day-old albino rats induced no significant changes in DNA synthesis parameters: LIN and LI in all myocardial regions did not differ from the control (Table 1). Repeated injection of the peptide in the same dose increased LIN in the myocardium by 63.35%. The most pronounced changes in LIN were found in the interventricular septum (by 72.13%), while the minimum changes were observed in the left atrium (by 59.32%).

LI also increased in all myocardial regions by on average 13.17%. This indirectly indicated acceleration of cell passage through the S-phase of the cell cycle. Similarly to changes in LIN, the maximum increase (by 15.5%) in LI was found in the interventricular septum and the minimum changes (by 11.54%) were observed in the left atrium (Table 1).

After repeated injection of dynorphin, the absolute and relative weights of the heart remained unchanged and were 85.13±2.77 mg and 6.77±0.22 mg/g body weight, respectively, *vs.* 84.47±3.39 mg and 6.47± 0.21 mg/g, in the control group, respectively. Our previous experiments showed that repeated administration of μ-agonist A10 (structural analogue of dermorphin) activated DNA synthesis and increased the absolute and relative weights of the heart [1].

Dynorphin-induced stimulation was observed only after repeated administration that was probably due to accumulation of this substance or its effects in the body. Moreover, age-related differences in the reaction of proliferative processes to dynorphin cannot be excluded. P. P. Rumyantsev [3] showed that the intensity of DNA synthesis in the myocardium of 7-day-old rats is lower than in 5-day-old rats. This is in good agreement with our findings (Table 1).

It should be emphasized that administration of κ-OR agonists causes some functional changes in the cardiovascular system of experimental animals [8]. Therefore, the effect observed in our experiments is probably mediated by functional changes.

There are at least two types of dynorphin binding sites in the myocardium: specific κ-OR and nonopioid binding sites [6]. Stimulation of κ-OR is known to decrease cell cAMP level [7]. We studied the effects of dynorphin on cAMP levels in the heart of experi-

TABLE 1. Effect of Dynorphin on DNA Synthesis in the Myocardium of Albino Rats during Early Postnatal Ontogeny

Myocardial region		Single injection				Repeated injection			
		LIN		LI		LIN		LI	
		control	experiment	control	experiment	control	experiment	control	experiment
Atrium	left	7.06±0.57	7.42±0.56	18.12±0.93	18.07±0,74	6.49±0.39	10.34±0.46*	16.74±0.53	19.08±0.49*
	right	7.17±0.78	7.33±0.77	16.95±0.67	17.41±0.68	6.43±0.36	10.47±0.56*	16.73±0.65	18.66±0.39*
Ventricle	left	10.66±0.72	10.35±0.69	21.02±0.85	20.75±0,94	7.98±0.22	12.93±0.49*	17.67±0.58	19.73±0.31*
	right	8.24±0.81	8.99±0.71	19.48±1.04	18.6±0.81	6.8±0.24	10.91±0.35*	16.01±0.43	18.12±0.34*
Interventricular septum		10.74±0.82	10.73±0.79	21.83±0.85	22.48±0.87	7.5±0.27	12.91±0.57*	16.71±0.51	19.3±0.46*

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

mental animals. In young albino rats, administration of dynorphin in a single dose of 100 μg/kg led to a 1.9-fold decrease in myocardial cAMP (34.22±4.3 fmol/mg *vs.* 66.42±11.18 fmol/kg in the control). Taking into account the involvement of cAMP in the regulation of proliferative processes, these data suggest that the decrease in tissue cAMP level stimulates DNA synthesis in the myocardium.

Our findings suggest that the opioid peptide dynorphin is involved in the establishment of myocardial structural homeostasis. This effect is probably mediated by the system of cyclic nucleotides.

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