Effect of μ-Opiate Receptor Agonist Tetrapeptide A10 on DNA Synthesis and Protein Content in the Myocardium of Albino Rats

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Effect of intraperitoneal injection of tetrapeptide A10 (H-Tyr-D-Orn-Phe-Gly-OH), selective μ-opiate receptor agonist, synthetic analog of dermorphine, in a dose of 100 μg/kg on DNA synthesis and protein content in the myocardium was studied in albino rats. Five injections of tetrapeptide on days 2-6 after birth caused no changes in DNA synthesis 17 days after the last injection, *i. e.* in 24-day rats. The number of nucleoli and their area increased. In adult males long-term (3-week) treatment with tetrapeptide A10 increased the number of nucleoli and the mean and integral optical density of isolated cardiomyocytes stained with amido black B, which probably attested to activation of protein synthesis in the myocardium. Simultaneously, the content of catecholamines in the heart increased. These data are comparable with delayed effects of κ-opiate receptor agonist dinorphine A_{1-13} and indicate that morphogenetic properties of opioid peptides in rat myocardium are realized via the same routes.

Key Words: *opioid peptides; DNA; protein; myocardium; ontogenesis*

Some ligands of opiate receptor (OR) modify DNA synthesis and protein content in the myocardium of albino rats. In newborn animals 5 injections of tetrapeptide A10 (μ-OR agonist, synthetic analog of dermorphine) and κ-OR agonist dinorphine A_{1-13} stimulate DNA synthesis in myocardial cells [3,4]. Fourteen days after the end of experimental treatment with κ-OR agonist dinorphine we observed activation of the nucleolar organizer and an increase in protein content in cardiomyocytes (CMC), but no changes in proliferative processes. Here we investigated possible delayed effect of selective μ-OR agonist tetrapeptide A10 on DNA synthesis, activity of nucleolar organizer, and protein content in rat myocardium. According to published reports, μ-OR ligands are the most potent inducers of heart morphogenesis [10].

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MATERIALS AND METHODS

Random-bred albino rats were intraperitoneally injected with tetrapeptide A10 (100 μg/kg) synthesized at the Laboratory of Peptide Synthesis. Controls were injected with an equivalent volume of 0.9% NaCl. In series I, the peptide was injected on days 2-6 after birth. The rats were decapitated on day 24 of life. ^{3}H-Thymidine in a dose of 1 μCi/g (specific activity 1530 TBq/mol) was injected intraperitoneally 24 and 1 h before sacrifice. Historadioautographs were prepared routinely. The parameters of DNA synthesis, *i. e.* index of ^{3}H-thymidine-labeled nuclei (ILN, %) and labeling intensity (LI, number of silver grains above the nucleus) were estimated separately in CMC and connective tissue cells of the left ventricle. In series II tetrapeptide A10 was injected daily intraperitoneally to adult males (140-170 g) for 21 days. It is known that proliferative processes in the myocardium of adult rats are minimal and cannot be virtually detected by

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autoradiography [9]. Twenty-four hours after the last injection the animals were decapitated and 7-μ paraffin sections were prepared routinely. For visualizing the nucleolar organizer region, the preparations were stained with 50% $AgNO_3$ [7]. The protein content in CMC was evaluated by the mean and integral optical density using MEKOC-C computer image analyzer. A fragment of the myocardium was cut from the left ventricle and after alkaline dissociation [5] smears of isolated CMC were stained with amido black B [2]. In adult animals catecholamine content in the heart was measured fluorometrically [8]. The results were statistically processed using Statistica 5.0 software. The significance of differences between the groups was evaluated using Student's *t* test.

RESULTS

Similarly to experiments with κ-OR agonist dinorphine A, we observed no significant changes in the parameters of DNA synthesis in myocardial parenchymatous and stromal cells of 24-day-old rats, *i. e.* 17 days after treatment with tetrapeptide A10, in comparison with the control (Table 1). A statistically significant increase in ILN of nonmuscular cells in comparison with CMC was observed in both control and experimental groups, which is in line with published data on higher proliferative activity of stromal cells at this stage of postnatal ontogenesis [9]. The number and area of nucleoli were increased in rats treated with opioid peptide A10 in comparison with the control, which indirectly indicates activation of protein synthesis in CMC [7].

Hence, delayed effects of μ-agonist were similar to those of κ-OR agonist dinorphine A_{1-13}. Tetrapeptide A10 activated DNA synthesis in newborn animals after 5 injections [4], and 17 days after the treatment this reaction was replaced by activation of CMC nucleolar organizer. Similarly to experiments with dinorphine, the parameters of DNA synthesis did not change. Delayed effects of tetrapeptide A10 on the myocardium and their similarity to those produced by κ-OR agonist dinorphine A_{1-13} suggest that opioid peptides of different classes modulate protein synthesis in the myocardium via a universal mechanism.

The pathway of paracrine regulation of CMC growth are different in neonates and adults [14]. Different reactions to μ-agonist in newborn and adult rats can be due to different number and affinity of μ-OR in the rat myocardium in these age groups [11]. Moreover, the ratio of μ-OR subclasses can also change. For example, activation of μ1- and μ2-OR in the heart causes opposite effects on the heart rate [12].

TABLE 1. Effect of 5 Injections of Tetrapeptide A10 to Newborn Rats on Morphometric Parameters of Left-Ventricular Myocardium of 24-Day-Old Rats (*M*±*m*)

Parameter		Control	A10
ILN, %	CMC	1.16±0.15	1.12±0.17
	connective tissue cells	2.84±0.44	2.26±0.58
LI	CMC	21.9±0.9	20.7±1.3
	connective tissue cells	20.00±0.21	19.3±1.0
Number of nucleoli in CMC		1.76±0.05	2.05±0.04*
Area of nucleoli, μ^2		2.4±0.8	2.90±0.13*

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

TABLE 2. Effect of 21 Injections of Tetrapeptide A10 on Morphometric Parameters of Left-Ventricular CMC and Catecholamine Content in the Heart of Adult Rats (*M*±*m*)

Parameter		Control	A10
CMC area, μ^2		3598.5±120.7	3341.10±246.14
Optic density	integral	98.33±9.9	146.8±15.8*
	mean	0.030±0.003	0.044±0.004*
Number of nucleoli in CMC		2.00±0.07	2.42±0.11*
Area of nucleoli, μ^2		3.50±0.28	4.11±0.28
Catecholamine	gepinephrine	0.23±0.02	0.62±0.10*
content, μg/	norepinephrine	0.31±0.05	2.26±0.49*
	DOPA	0.030±0.005	0.24±0.06*

Injections of tetrapeptide A10 to adult animals for 3 weeks significantly increased the number of nucleoli (Table 2). Their area also increased, though negligibly. The mean and integral optical density of CMC stained with amido black B increased, while cell area remained unchanged. Delayed effects of tetrapeptide A10 are similar in newborn and adult rats.

Previously we hypothesized that these effects can be explained by changes in the functional activity of the heart during chronic injections of opioid peptides due to modulation of neurotransmitter release and their content in nerve endings [6]. Catecholamines exert trophic effects on the heart and regulate DNA and protein synthesis in cardiomyocytes [13,15]. Our data on changed catecholamine content in the myocardium of adult rats chronically treated with tetrapeptide A10 (Table 2) confirm this hypothesis: the concentrations of epinephrine, norepinephrine, and DOPA increased 2.7-, 7.3-, and 8-fold, respectively.

These changes can also be explained by the effect of opioid peptides on gene activities or CMC ploidy. Accumulation of genetic material creates prerequisites for increased protein production [1].

Hence, opioid peptides are able to modify heart morphogenesis. Similarity of the delayed effects of μ-OR agonist tetrapeptide A10 and κ-OR agonist dinorphine A_{1-13} indicate that these effects are realized via the same pathways.

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