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Effect of Synthetic Dermorphin Analogues on Tissue Homeostasis in the Myocardium of Newborn Albino Rats

S. Yu. Kriganovskaya, O. A. Lebed'ko*, E. N. Sazonova, S. S. Timoshin, and L. E. Molokanova*

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DNA synthesis and state of the nucleolar apparatus in myocardial cells of newborn albino rats receiving intraperitoneal injection of sedatin, a synthetic dermorphin analogue, were studied by means of autoradiography and silver impregnation. Labeling intensity significantly increased, while the number of nucleoli in nuclei decreased. Chemiluminescence study showed that the concentration of reactive oxygen metabolites significantly decreased in the myocardium of treated animals after sedatin administration. Non-arginine analogue of sedatin had little effect on tissue homeostasis in the myocardium.

Key Words: *opioid peptides; myocardium; proliferative activity; nucleolar apparatus; reactive oxygen metabolites*

Mammalian myocardium has its own opioidergic system [7]. The concentration of enkephalin derivatives in the fetal and neonatal myocardium of mammals significantly exceeds the amount of opioid peptides (OP) in the heart of adult specimens [14]. The ability of OP to modulate proliferative processes [15] and high concentration of these compounds in the myocardium of newborn mammals suggest that OP play an important role in the postnatal development of structural homeostasis in the heart.

Previous studies showed that dalargin, ligand of μ- and δ-opiate receptors (OR), affects proliferative processes in the myocardium of newborn albino rats [4].

Peptide sedatin synthesized at the Research-and-Production Company Peptos, is a dermorphin analogue and acts as an μ/δ-OR agonist. This peptide contains N-terminal arginine residue.

Here we studied the effect of sedatin on tissue homeostasis in the myocardium of albino rats during the early period of postnatal ontogeny.

MATERIALS AND METHODS

Experiments were performed on 76 newborn outbred albino rats. Peptide sedatin (H-Arg-Tyr-D-Ala-Phe-Gly-OH) and its non-arginine analogue (H-Tyr-D-Ala-Phe-Gly-OH) were injected intraperitoneally (100 μg/kg, 5 injections) to animals of the treatment group on days 2-6 of life. Control animals received an equivalent volume of the solvent (isotonic NaCl). DNA synthesis, state of the nucleolar apparatus in cardiomyocytes, and intensity of free radical oxidation in the myocardium of rats were studied 24 h after the last injection.

For autoradiography, ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 84 TBq/mol) was administered to animals 1 h before euthanasia. Autoradio-

Central Research Laboratory, Far-Eastern State Medical University; *Institute of Maternity and Child Welfare, Khabarovsk Branch, Far-Eastern Research Center for Physiology and Pathology of Respiration, Siberian Division of the Russian Academy of Medical Sciences. ***Address for correspondence:*** timoshinss@yandex.ru**.** S. S. Timoshin

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graphs were prepared by the standard method. The index of labeled nuclei was estimated by counting of 1000 cells in subendocardial layers of the myocardium in each region of the heart (%). Labeling intensity was calculated as the mean number of tracks above 50 nuclei in each region of the myocardium.

The number and area of nucleoli and ratio of nucleus/nucleoli cross-section area were estimated by examining the preparations stained with $AgNO_3$. This study was performed by the method of computer morphometry on a MEKOS-Ts complex image analyzer.

The method of chemiluminescence (CL) was used to study free radical oxidation. CL was measured on a LS-50B luminescence spectrometer (Perkin Elmer). The signal was standardized with Finlab software. Spontaneous and Fe^{2+}-induced CL was estimated as described elsewhere [2]. Total spontaneous CL (S_{SP}) was measured over 1 min and correlated with the intensity of free radical processes. The first flash maximum (H1) of induced CL reflected the content of lipid hydroperoxides. Total CL was recorded over 2 min of the post-flash period ($S1_{IND}$) and reflected the rate of peroxide radical accumulation.

Kinetic parameters of H_2O_2-induced luminol-dependent CL were estimated as described previously [1]. The first flash maximum (H2) reflected the ability of biological object to undergo peroxidation. Total CL was recorded over 2 min ($S2_{IND}$) and depended on activity of the antioxidant and antiradical defense system.

Intergroup differences were significant at $p<0.05$ (Student's t test).

RESULTS

Fivefold injection of sedatin to newborn albino rats was followed by a decrease in DNA synthesis in the myocardium (Table 1). Labeling intensity in myocardial regions decreased by 11-16%. Significant changes were revealed in the myocardium of the right and left ventricle. Our results indicate that sedatin decelerates DNA synthesis in myocardial cells of newborn animals. The decrease in labeling intensity was not accompanied by variations in the index of labeled nuclei.

Study of the nucleolar apparatus in treated animals showed that 5-fold injection of sedatin significantly increases the number of nucleoli (Table 2). State of the nucleolar apparatus is an adequate criterion for anabolic protein-synthetic activity of cardiomyocytes [8]. Previous studies revealed a positive correlation between the intensity of protein

TABLE 1. Effect of 5-Fold Treatment with Dermorphin Analogues on DNA Synthesis in the Myocardium of Newborn Albino Rats ($M\pm m$)

Group	Index of labeled nuclei, %				Labeling intensity			
	left atrium	left ventricle	right atrium	right ventricle	left atrium	left ventricle	right atrium	right ventricle
Control	8.59±0.68	8.57±0.81	9.04±0.44	8.17±0.69	28.54±1.11	29.71±0.76	29.21±1.00	28.25±0.94
Sedatin	8.99±0.43	9.84±0.58	9.18±0.66	9.87±0.57	25.41±1.36	24.95±0.96*	25.35±1.43	24.22±0.84*
Non-arginine sedatin analogue	8.66±0.97	8.89±0.39	9.11±0.66	7.66±0.71	28.00±2.30	28.89±0.97	28.12±1.51	29.46±1.10

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Effect of 5-Fold Treatment with Dermorphin Analogues on the Nucleolar Apparatus in Myocardial Cells of Newborn Albino Rats (*M*±*m*)

Group	Number of nucleoli		Area of nucleoli, μ²		Nucleus/nucleolus area ratio	
	left ventricle	right ventricle	left ventricle	right ventricle	left ventricle	right ventricle
Control	2.29±0.08	2.01±0.12	2.68±0.24	2.55±0.14	20.90±1.77	20.2±0.8
Sedatin	2.59±0.07*	2.54±0.12*	2.99±0.22	2.95±0.17	17.30±0.97	16.90±0.88*
Non-arginine sedatin analogue	2.22±0.19	2.42±0.20	2.88±0.98	2.74±0.18	18.66±0.63	18.90±1.64

synthesis in mammalian myocardium and rate of cardiomyocyte differentiation [11].

The decrease in the rate of DNA synthesis in the myocardium of newborn animals after 5-fold injection of sedatin was accompanied by activation of the nucleolar apparatus. These changes probably reflect acceleration of cardiomyocyte differentiation. Similar changes in tissue homeostasis of the myocardium in newborn albino rats were revealed after single administration of μ/δ-OR agonist dalargin [4]. Similarly to sedatin, dalargin contains arginine, but in C-terminal position.

Similarly to dalargin, sedatin in a wide dose range stimulates proliferative processes in various cell populations [4,9,10].

Dalargin exhibits high antioxidant activity. At the same time, reactive oxygen metabolites play a major role in the regulation of proliferative processes [3]. For evaluation of the role of reactive oxygen metabolites in the morphogenetic effect of sedatin on the neonatal myocardium, we studied the intensity of free radical oxidation in the myocardium of newborn animals receiving 5 injections of sedatin by the method of CL.

Sedatin modified biogenesis of reactive oxygen metabolites. This conclusion was derived from the decrease in the content of lipid hydroperoxides and suppression of peroxide radical accumulation (Table 3).

The mechanisms for the effect of μ/δ-OR agonists on the myocardium of newborn animals are poorly understood. μ-OR were not identified in the myocardium of newborn mammals [12]. The effects of δ-OR in newborn rats are realized only after the 10th day of life [13]. The biological effects of sedatin are mediated by its interaction with OR. It should be emphasized that arginine also plays a role in the morphogenetic response. Previous studies showed that metabolic transformation of dalargin is accompanied by the release of the arginine molecule [5]. Arginine is an important component of the NO—NO synthase system. Blockade of NO synthesis with L-NAME modifies the effect of dalargin on DNA synthesis and free radical oxidation in respiratory organs of newborn albino rats [6].

It can be hypothesized that arginine and NO—NO synthase system mediate the effect of sedatin on the myocardium in newborn albino rats. This hypothesis was indirectly confirmed by the results obtained in experiments with non-arginine sedatin analogue H-Tyr-D-Ala-Phe-Gly-OH. The non-arginine sedatin analogue had no effect on tissue homeostasis in the neonatal myocardium (Tables 1, 2, and 3).

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TABLE 3. Effect of 5-Fold Treatment with Dermorphin Analogues on CL in Myocardial Homogenates from Newborn Albino Rats (rel. units, *M*±*m*)

Group	S_{SP}	H1	$S1_{IND}$	H2	$S2_{IND}$
Control	1.58±0.09	1.39±0.09	3.42±0.25	4.72±0.28	8.01±0.63
Sedatin	1.42±0.07	0.90±0.07*	2.81±0.14*	4.93±0.35	8.37±0.68
Non-arginine sedatin analogue	1.7±0.1	1.55±0.12	3.84±0.21	5.13±0.35	8.94±0.72

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