REFERENCES

1. L. B. Volodarskii and L. A. Tikhonova, *Khimiya Geterotsikl. Soedinenii (Chemistry of Heterocyclic Compounds)*, **6**, 748-752 (1985).
2. V. P. Miroshnichenko, O. G. Busygina, and I. S. Severina, *Vopr. Med. Khimii*, No. 4, 60-66 (1989).
3. Yu. Yu. Chirkov, I. A. Tyshchuk, and I. S. Severina, *Biokhimiya*, **53**, 1520-1526 (1988).
4. L. J. Ignarro, K. S. Wood, and M. S. Wolin, *Adv. Cycl. Nucl. Protein Phorphor. Res.*, **17**, 267-274 (1984).
5. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
6. D. G. Mazhukin, L. B. Volodarsky, L. A. Tikhonova, and A. Ya. Tikhonov, *Mendeleev Commun.*, **1**, 29-30 (1992).
7. P. I. Morando, E. B. Borghi, L. M. De Scheingart, and M. A. Blesa, *J. Chem. Soc., Dalton Trans.*, 435-440 (1981).
8. R. M. J. Palmer, A. G. Ferrige, and S. Moncada, *Nature*, **327**, 524-526 (1987).
9. I. S. Severina, I. K. Ryaposova, L. B. Volodarsky, *et al.*, *Biochem. Mol. Biol. Intern.*, **30**, No. 2, 357-366 (1993).

Effect of the Leu-Enkephalin Analog Dalargin on DNA Synthesis in the Myocardium and Lingual Epithelium of Rats in Early Postnatal Ontogeny

E. N. Goncharova, S. S. Timoshin, and T. V. Yatsenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 1, pp. 43-45, January, 1997
Original article submitted December 22, 1995

The DNA synthesis is studied in the myocardium and lingual epithelium of 4-5-day-old rats 24 h after a single administration of the leu-enkephalin analog dalargin in a dose of 10 or 100 μg/kg. At 10 μg/kg, dalargin significantly decreases the number of DNA-synthesizing nuclei in the left atrium, while at 100 μg/kg it significantly decreases the number of DNA-synthesizing nuclei in the left atrium and both ventricles. Dalargin does not change the DNA synthesis in the lingual epithelial cells.

Key Words: *dalargin; DNA synthesis; myocardium; epithelium; postnatal ontogeny*

Opioid peptides contribute to the maintenance of structural homeostasis in the body by regulating proliferation and differentiation processes [15]. The presence of endogenous opioid peptides in the mammalian myocardium [10] and the pronounced cardiotropic activity of exogenous opioids [5] suggest that opioids are involved in cardiac morphogenesis.

Our objective was to examine the effect of dalargin, a stable leu-enkephalin analog, on the DNA synthesis in rat myocardium during the early postnatal ontogeny.

Central Research Laboratory, State Medical Institute, Khabarovsk; Institute of Maternal and Child Health, Siberian Division of the Russian Academy of Medical Sciences, Khabarovsk

MATERIALS AND METHODS

Random-bred white rats aged 4-5 days were used. Control and experimental groups were formed by splitting litters so that there were 8 pups per nest. Experimental pups received 10 or 100 μg/kg of the synthetic leu-enkephalin analog dalargin (D-Ala^2-Leu^5-Arg^6-enkephalin) as a single intraperitoneal injection. Control pups were given an equal volume of the solvent (sterile physiological saline). ^{3}H-Thymidine (specific activity 1530 TBq/mol) was injected intraperitoneally in a dose of 1 μCi/g body weight 23 h after dalargin (1 h before euthanasia).

Histotopographic preparations of the heart and tongue were obtained by standard methods [3]. The

0007-4888/97/0001-0036$18.00

TABLE 1. Effect of Dalargin on DNA Synthesis (NLI, %) in Rat Myocardium in Early Postnatal Ontogeny

Myocardial zone	Dalargin doze, μg/kg			
	10		100	
	control	experiment	control	experiment
Left atrium	11.52±0.67	8.95±0.29*	7.86±0.63	5.70±0.55*
Right atrium	9.99±0.68	9.96±0.62	7.48±0.58	5.90±0.56
Left ventricle:				
subendocardial layer	11.50±0.82	11.39±0.60	10.44±0.95	7.61±0.68*
intramural layer	13.10±0.63	11.63±0.71	8.77±0.60	6.26±0.64*
subepicardial layer	11.19±0.70	9.72±0.76	6.37±0.71	5.37±0.94
Interventricular septum:				
subendocardial layer	10.97±0.98	10.59±0.52	10.93±0.87	8.31±1.07
intramural layer	13.37±1.08	13.73±0.80	8.51±0.74	7.26±0.82
Right ventricle:				
subendocardial layer	9.15±0.61	8.58±0.44	9.38±0.73	6.42±0.82*
intramural layer	10.63±0.92	10.34±0.43	7.14±0.52	5.09±0.68*
subepicardial layer	8.96±0.71	8.66±0.62	5.21±0.40	4.09±0.56

Note. P and M emulsion was used, respectively, for autoradiography after administration of 10 μg/kg and 100 μg/kg dalargin. *$p<0.05$ compared with the control.

nuclear labeling index (NLI, expressed in %) and labeling intensity (the mean number of silver grains over the nucleus) were determined.

On the heart autoradiographs, the NLI and labeling intensity were estimated for the left and right atria, subendocardial, intramural, subepicardial layers of the left and right ventricles, and subendocardial and intramural layers of the interventricular septum. Cell types were identified by morphological differences. Cells of doubtful origin were excluded.

The NLI for the tongue was estimated in the generative zone of its epithelium (basal and spinous layers) along the superior and lateral surfaces of the middle third.

The results were analyzed using Student's *t* test.

RESULTS

At 10 μg/kg dalargin significantly decreased NLI in the left atrium, but not in any other heart zone (Table 1).

At 100 μg/kg it decreased this index in all studied myocardial zones, the decrease being statistically significant in the left atrium and in the subendocardial and intramural layers of the left and right ventricles (Table 1). A tendency toward a decrease in the NLI ($p<0.1$) was observed in the right atrium and the subendocardial layer of the interventricular septum. There were no intergroup differences in the labeling intensity, an indirect indicator of the rate of DNA synthesis.

Thus, the leu-enkephalin analog dalargin markedly decreases the number of DNA-synthesizing nuclei in the myocardium of rats in the early postnatal ontogeny, as evidenced by low NLI.

Dalargin stimulates cell proliferation in the epithelia [6] and connective tissues [9], promotes recovery from experimental myocardial infarction [1], and activates anabolic processes in the myocardium of adult rats, judging from increased rate of protein synthesis and RNA levels [5].

Presumably, inhibition of physiological regeneration of the myocardium observed after dalargin injection was due to the ontogenic characteristics of cardiovascular responses to opioid peptides in newborn animals. Structural and functional changes occurring in the cardiovascular system of adult mature rats after systemic administration of enkephalins are associated with their antiadrenergic activity [4]. In 4-5-day-old rats, the significance of this mechanism is very low, since sympathetic innervation of the heart is not developed at this stage of ontogeny [2].

Furthermore, the pattern of tissue responses to biologically active substances in the early postnatal period is probably determined by ontogenic features of their specific receptors. Dalargin binds predominantly to δ-opioid receptors (OR) [8]. These receptors were not found on the striatal neurons of rat embryos [14]. In rats, they appear in small amounts in the spinal cord in early postnatal life [11]. It was hypothesized that at this period of ontogeny the functions of OR are other than those in adult organism [12].

Specific reception of OR at the early stage of ontogeny has been confirmed by experiments with dalargin and lingual epithelial cells. At 10 or 100 μg/kg, dalargin practically did not change proliferative activity of lingual epithelial cells in 5-day-old rats: 7.9±0.3% *vs.* 7.1±0.3% in the control and 7.0± 0.4% *vs.* 7.3±0.4% ($p>0.05$), respectively. However, in adult rats the same doses of dalargin markedly increased the number of DNA-synthesizing nuclei in the lingual epithelium [6]. Thus, the effects of dalargin on cell proliferation are age-dependent.

It should be remembered that a decrease in the number of DNA-synthesizing cells in the myocardium of young animals may reflect an accelerated differentiation of cardiomyocytes [7]. This is indirectly supported by the anabolic effect of dalargin [5] that probably stimulates the synthesis of specific myocardial contractile proteins.

Our results indicate that the opioid peptide dalargin induces substantial changes in the reproduction of cardiomyocytes during early postnatal ontogeny.

REFERENCES

1. N. I. Afonskaya, O. B. Il'inskii, V. F. Kondalenko, *et al.*, *Byull. Eksp. Biol. Med.*, **102**, No. 12, 754-757 (1986).
2. G. B. Bol'shakova, *Intertissue Relationships in the Development of Heart* [in Russian], Moscow (1991).
3. O. I. Epifanova and V. V. Terskikh, *Autoradiography in the Study of Cell Cycles* [in Russian], Moscow (1969).
4. Yu. B. Lishmanov, L. I. Maslov, I. G. Khaliulin, and N. A. Barbarash, *Vestn. Ross. Akad. Med. Nauk*, No. 3, 5-8 (1992).
5. L. V. Maslova, Yu. B. Lishmanov, and G. N. Smagin, *Vopr. Med. Khimii*, No. 1, 63-65 (1991).
6. T. D. Pan'kova, "Effects of dalargin, an opiate receptor ligand, on cell division in different types of epithelium," Author's Synopsis of Dissertation [in Russian], Vladivostok (1992).
7. P. P. Rumyantsev, *Cardiomyocytes in Reproduction, Differentiation, and Regeneration Processes* [in Russian], Leningrad (1982).
8. M. I. Titov, V. A. Vinogradov, and Zh. D. Bespalova, *Byull. Vsesoyuz. Kardiol. Nauch. Tsentra* (*Bulletin of the All-Union Cardiology Research Center*), No. 2, 72-76 (1985).
9. A. B. Shekhter, A. I. Solov'eva, S. E. Spevak, and M. I. Titov, *Byull. Eksp. Biol. Med.*, **106**, No. 10, 487-490 (1988).
10. J. Archelos, J. Z. Xiang, M. Reinecke, and R. E. Lang, *J. Cardiovasc. Pharmacol.*, **10**, S45-S50 (1987).
11. B. Attali, D. Saya, and Z. Vogel, *Dev. Brain Res.*, **53**, No. 1, 97-102 (1990).
12. D. A. Gibson and A. Vernadakis, *Dev. Brain Res.*, **4**, No. 1, 23-29 (1982).
13. I. Kitchen, *Neurotoxicology*, **14**, 115-124 (1993).
14. P. J. Vaysse, R. S. Zukin, K. L. Fields, and J. A. Kessler, *J. Neurochem.*, **55**, No. 2, 624-631 (1990).
15. I. S. Zagon, S. R. Goodman, and P. J. McLaughlin, *Brain Res.*, **482**, 297-305 (1989).