Effect of β-Casomorphin-7 on DNA Synthesis in Cell Populations of Newborn Albino Rats

N. V. Maslennikova, E. N. Sazonova, and S. S. Timoshin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 2, pp. 170-172, February, 2008
Original article submitted July 2, 2007

We studied the effect of β-casomorphin-7 on DNA synthesis in cell populations of newborn albino rats. Intraperitoneal administration of a β-casein fragment heptapeptide β-casomorphin-7 (1 mg/kg, 1 or 5 injections) activated proliferative processes in the myocardium and ectodermal and endodermal epithelium of newborn rats.

Key Words: *β-casomorphin-7; exogenous opioids; DNA synthesis; newborn mammals; neonatal period*

Endogenous opioid peptides (OP) and opiate receptors (OR) are widely distributed in tissues of newborn mammals [10]. OR ligands modulate proliferative processes in mammalian tissues during neonatal ontogeny [3,4,6,9].

Nonhydrolyzed bioactive peptides of food products are accumulated in the organism during the neonatal period [2]. Exogenous OP of milk proteins (casomorphins) play a major role in newborn mammals [1]. Published data show that casomorphins modify behavioral reactions and gastrointestinal function in the growing organism [1, 13]. However, the effect of casomorphins on proliferative processes in tissues of newborn mammals remains unknown.

Here we studied the effect of β-casomorphin-7 on DNA synthesis in tissues of newborn albino rats.

MATERIALS AND METHODS

Newborn outbred albino rats received intraperitoneal injections of β-casomorphin-7 (heptapeptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile; Laboratory of Regulatory Peptides, Institute of Molecular Genetics). The dose of this peptide (1 mg/kg) is comparable with the amount of dietary β-casomorphin, which enters the mammalian organism during early postnatal ontogeny [1,12]. The rats were subjected to single (day 4 of life) or 5-fold treatment with test peptide (days 2-6 of life). Control animals received single injection of sterile isotonic NaCl (solvent) in an equivalent volume. Control and treated rats were divided into groups by the method of litter separation to reduce the possible influence of genetic differences.

^{3}H-Thymidine (1 μCi/g) was administered intraperitoneally 23 h after the last injection of β-casomorphin-7 (or 23 h after the last injection in 5-fold treatment with the peptide). The animals were euthanized 1 h after injection of the isotope. The heart, tongue, stomach, and a duodenal fragment (1 cm from the pyloric sphincter) were fixed in Carnoy's fluid. Autoradiographs were prepared by the standard method using KODAK Autoradiography Emulsion NTB (Product code 8895666). Study was performed with 42 animals.

DNA synthesis in the epithelium of the tongue, stomach, and duodenum and various regions of the myocardium (left and right atria, left and right ventricles, and interventricular septum) was studied on autoradiographs stained with Lilly—Mayer hematoxylin. The index of labeled nuclei (ILN) was estimated in each population of cells. ILN (ratio of ^{3}H-thymidine-labeled nuclei) was determined after examination of 2500-3000 nuclei and reflected the

Central Research Laboratory, Far-Eastern State Medical University, Khabarovsk. ***Address for correspondence:*** timoshinss@yandex.ru. S. S. Timoshin

0007-4888/08/1452-0210

proliferative pool of tissues. The labeling intensity (mean number of tracks above labeled nuclei) was evaluated after examination of at least 50 nuclei in each population of cells. This parameter indirectly reflects the rate of DNA synthesis.

The results were analyzed by Statistica 5.0 software. The differences between control and treated animals were statistically significant at $p<0.05$.

RESULTS

Injection of β-casomorphin-7 was followed by the activation of DNA synthesis in all cell populations of newborn rats (Tables 1 and 2). This effect was observed in 5- and 7-day-old animals after single and 5-fold treatment with the peptide, respectively.

β-Casomorphin-7 significantly stimulated DNA synthesis in all regions of the myocardium, which was manifested in a significant increase in the labeling intensity (Table 1). The increase in the labeling intensity in the right atrium and right ventricle was accompanied by a significant rise in the number of DNA-synthesizing nuclei. Fivefold treatment with the peptide had a less pronounced effect. DNA synthesis significantly increased in 4 of 5 myocardial regions.

TABLE 1. DNA Synthesis in the Myocardium of Newborn Albino Rats under Different Regimens of Treatment with β-Casomorphin-7 (*M*±*m*)

Regimen of treatment, myocardial region	Control		Experiment	
	ILN, %	LI	ILN, %	LI
Single injection				
LA	7.46±0.61	17.62±0.61	10.06±1.00	24.18±1.23* (*p*=0.001)
RA	7.17±0.44	18.18±0.54	10.15±1.01* (*p*=0.033)	24.29±1.48* (*p*=0.006)
LV	8.97±0.57	18.12±0.70	10.94±0.90	22.94±0.73* (*p*=0.0009)
IVS	8.20±0.91	18.76±1.11	10.98±0.87	23.91±0.77* (*p*=0.002)
RV	7.48±0.62	18.00±0.72	10.66±0.81* (*p*=0.018)	23.85±0.85* (*p*=0.0005)
Fivefold injection				
LA	7.70±0.46	19.42±1.11	7.94±0.39	22.49±1.03
RA	8.13±0.63	18.34±1.02	9.83±0.29* (*p*=0.03)	23.12±1.01* (*p*=0.004)
LV	7.73±0.35	18.92±1.10	8.17±0.37	22.66±1.00* (*p*=0.006)
IVS	8.51±0.32	19.25±1.11	8.79±0.56	22.61±0.63* (*p*=0.01)
RV	8.05±0.53	18.83±0.93	9.05±0.40	22.79±0.84* (*p*=0.005)

Note. LI, labeling intensity; LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle; IVS, interventricular septum. Here and in Table 2: *significant differences compared to the control; *p* is shown in brackets.

TABLE 2. Effect of β-Casomorphin-7 on DNA Synthesis in the Epithelium of Newborn Albino Rats (*M*±*m*)

Regimen of treatment	Control		Experiment	
	ILN, %	LI	ILN, %	LI
Tongue epithelium				
single	11.25±0.66	18.52±0.96	13.22±0.72	23.46±0.81* (*p*=0.001)
5-fold	11.05±0.58	16.43±0.86	13.22±0.53* (*p*=0.011)	18.25±0.74
Gastric epithelium				
single	7.59±0.57	29.25±1.91	10.03±0.50* (*p*=0.008)	38.95±1.94* (*p*=0.005)
5-fold	7.11±0.35	24.62±2.25	9.08±0.36* (*p*=0.0007)	27.37±2.82
Duodenal epithelium				
single	15.63±71.65	18.72±0.95	21.15±1.04* (*p*=0.01)	20.53±1.11
5-fold	16.52±1.05	16.59±0.66	20.35±1.54	16.81±0.80

β-Casomorphin-7 is a selective μ-OR agonist [7]. Our previous studies showed that another agonist of μ-OR, peptide A10 (dermorphin analogue), stimulates DNA synthesis in the myocardium of newborn albino rats. Taking into account low number of μ-OR in the mammalian myocardium, we can hypothesize that this effect is related to the inhibition of catecholamine release from adrenergic nerve endings of the heart [4].

β-Casomorphin-7 also stimulated DNA synthesis in the ectodermal (tongue epithelium) and endodermal epithelium (gastric epithelium and duodenal epithelium). Our previous studies showed that μ-OR agonist dermorphin inhibits DNA synthesis in the ectodermal epithelium of albino rats [5]. Probably, the stimulatory effect of β-casomorphin-7 on DNA synthesis in the epithelium is not associated with activation of μ-OR. For example, β-casomorphin-7 significantly stimulates gastrin production, but inhibits somatostatin secretion in the gastric mucosa of rats [14].

Our results indicate that β-casomorphin-7 stimulates proliferative processes in various cell populations of newborn albino rats. Published data show that β-casomorphin produces a strong activating effect on axonal growth in mouse neuroblastoma cells [11]. Casomorphins significantly increase thymidine kinase activity and proliferation of cultured prostate cells from adult and young rats [8]. The mechanisms for action of β-casomorphin-7 on proliferation in newborn mammals require further investigations.

The influence of casomorphins on neonatal changes is of biological significance. Previous observations showed that newborn child receives 20-25 mg exogenous opioids with 2 mg dietary casein [1]. Our study provides experimental support to the potential role of food components in vital activity of the organism.

REFERENCES

1. V. A. Dubynin, Yu. A. Ivleva, and A. A. Kamenskii, *Uspekhi Fiziol. Nauk*, No. 1, 83-101 (2004).
2. K. A. Zufarov, *Byull. Eksp. Biol. Med*., **125**, No. 1, 4-11 (1998).
3. O. A. Lebed'ko and S. S. Timoshin, *Ibid*., **131**, No. 2, 205-207 (2001).
4. N. P. Mel'nikova, S. S. Timoshin, N. B. Murzina, *et al*., *Ibid*., **130**, No. 12, 693-695 (2000).
5. M. I. Radivoz, E. I. Mel'nik, S. S. Timoshin, *et al*., *Ibid*., **112**, No. 8, 162-164 (1991).
6. E. N. Sazonova, E. Yu. Zhivotova, O. A. Sazonov, *et al*., *Ibid*., **127**, No. 6, 651-653 (1999).
7. V. Brantl, A. Pfeiffer, A. Herz, *et al*., *Peptides*, **3**, No. 5, 1903-1909 (1982).
8. G. Gayet, B. Dupont, A. Atfi, *et al*., *C R Seanc. Soc. Biol. Fill*., **186**, Nos. 1-2, 107-111 (1992).
9. P. J. McLaughlin, *Am. J. Physiol*., No. 1, Pt. 2, R122-R129 (1996).
10. A. Pujol, C. De Cabo, M. I. Martin, and M. P. Viveros, *Pharmacol. Biochem. Behav*., **46**, No. 2, 373-376 (1993).
11. M. Sakaguchi, K. Murayama, K. Yabe, *et al*., *Neurosci. Lett*., **251**, No. 2, 97-100 (1998).
12. M. Sakaguchi, M. Koseki, M. Wakamatsu, and E. Matsumura, *Eur. J. Pharmacol*., **530**, Nos. 1-2, 81-87 (2006).
13. A. Trompette, J. Claustre, F. Caillon, *et al*., *J. Nutr*., **133**, No. 11, 3499-3503 (2003).
14. Y. F. Zong, W. H. Chen, Y. S. Zhang, and S. X. Zou, *World J. Gastroenterol.*, **13**, No. 14, 2094-2099 (2007).