plasma, which forms IR^+ complexes on dissociated R receptors in the tissues utilizing the hormone.

2. Insulin initiates intracellular metabolism and degrades in IR^+ complexes, the I^- products of its degradation forming transport complexes I^-R° with dissociated R° erythrocyte receptors.

3. Erythrocytic insulin-receptor complexes are dissociated into R° and I^- in the pancreas; the degraded hormone is repaired with the formation of active I^+ insulin in addition to the produced hormone.

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Effect of Tetrapeptide A10, an Agonist of μ-Opioid Receptors, on DNA Synthesis in the Myocardium and Liver of Albino Rats in Early Postnatal Ontogeny

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 2, pp. 212-215, February, 1997
Original article submitted December 22, 1995

The DNA synthesis in the myocardium and liver of 5-7-day-old albino rats after intraperitoneal injection of selective antagonist of μ-opioid receptors A10 (H-Tyr-D-Orn-Phe-Gly-OH) are studied using ^{3}H-thymidine autography. In the myocardium, both single (70 μg/kg) and 5-fold (100 μg/kg) administration of A10 significantly increases the intensity of labeling. The 5-fold administration of A10 induces an increase in absolute and relative mass of the heart. In the liver, the number of DNA-synthesizing hepatocytes rises in all experimental series. These data attest to a stimulating effect of μ-agonist on physiological regeneration of the myocardium and liver of albino rats in early postnatal ontogeny.

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

Opioid peptides contribute to the maintenance of structural homeostasis in the organism [14]. The effect of opioids on cell proliferation in the nervous tissue [13], surface epithelium [6-8], and lymphoid [12] and hemopoietic [1] tissues is well established. However, little is known about the effect of opioid peptides on cell reproduction in such vital organs as the heart and liver. Since cardiomyocytes and hepatocytes of mature mammals exhibit no proliferative activity under physiological conditions [5], the processes of cell proliferation in these populations may be evaluated *in vivo* only during early ontogeny.

The aim of the present study was to analyze the effect of A10, an agonist of opioid receptors (OR), on DNA synthesis in the myocardium and liver of albino rats during early ontogeny.

MATERIALS AND METHODS

Structural analog of dermorphin tetrapeptide A10 (H-Tyr-D-Orn-Phe-Gly-OH) was used. The affinity of A10 for μ-OR is higher than that of DAGO, a usual μ-OR agonist [9]. A10 synthesized at the La-

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0007-4888/97/0002-0184$18.00

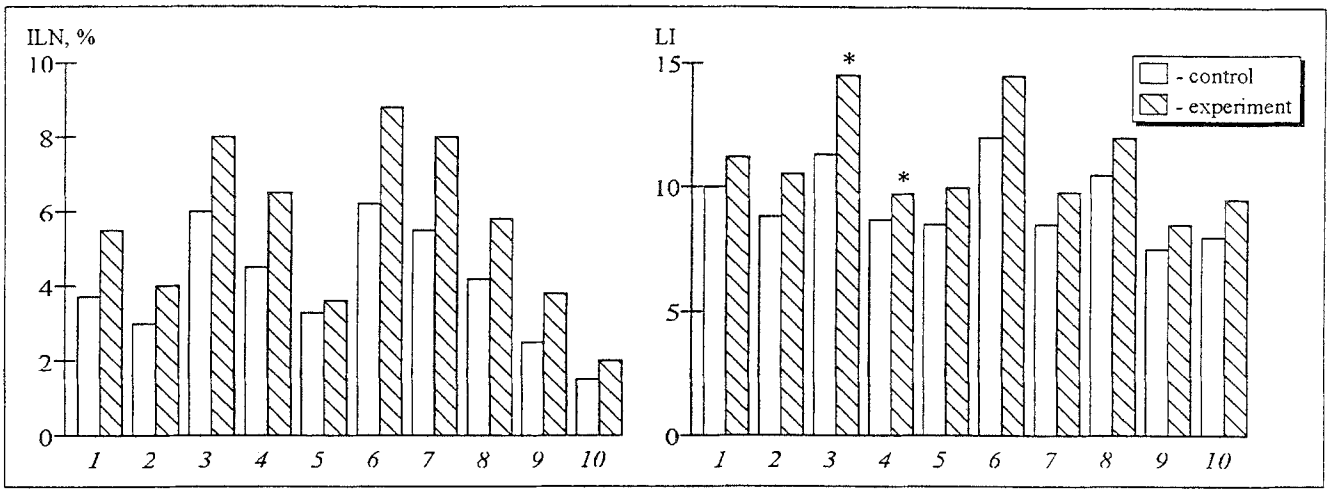

Fig. 1. Effect of the dermorphin analog A10 (70 μg/kg) on DNA synthesis in the myocardium of albino rats during the early postnatal ontogeny. Here and in Fig. 2: myocardium of the left (*1*) and right (*2*) atriums; subendocardial (*3*), intramural (*4*), and subepicardial (*5*) layers of the left ventricles; subendocardial (*6*) and intramural (*7*) layers of the interventricular septum; subendocardial (*8*), intramural (*9*), and subepicardial (*10*) layers of the right ventricles. *$p<0.05$ compared with the control.

boratory of Peptide Chemistry (Cardiology Research Center of the Russian Academy of Medical Sciences) was intraperitoneally injected into 4-day-old rat pups in single doses of 7 and 70 μg/kg (20 and 200 nmol/kg). In an additional experimental series, the animals received 5 injections of 100 μg/kg A10 on postnatal days 2 trough 6. The control animals received an equal volume of the solvent (sterile isotonic NaCl solution, 0.2 ml/g body weight). In order to minimize the genetic differences, the animals of equalized litters were randomly assigned to either control or experimental groups. The intensity of DNA synthesis in the myocardium and liver were assessed by autoradiography 24 h after the challenge (or 24 h after the 5th injection). To this end the animals were injected with ^{3}H-thymidine (specific activity 1530 TBq/mol) in a dose of 1 μCi/g body weight before sacrifice. Radioautographs were routinely prepared as described elsewhere [2].

The index of ^{3}H-thymidine-labeled nuclei (ILN) and label intensity (LI, the mean number of silver granules over the nucleus) were counted on histotopographic preparations of the heart separately for the myocardium of the left and right atriums, subendocardial, intramural, and subepicardial layers of the left and right ventricles, and subendocardial and intramural layers of the interventricular septum. Muscular and nonmuscular cells were identified on the basis of morphological criteria [4]. The nuclei of questionable tissue origin were excluded from the consideration.

In the liver, the number of DNA-synthesizing hepatocytes (ILN, %) was counted, the hemopoietic elements being excluded from the consideration.

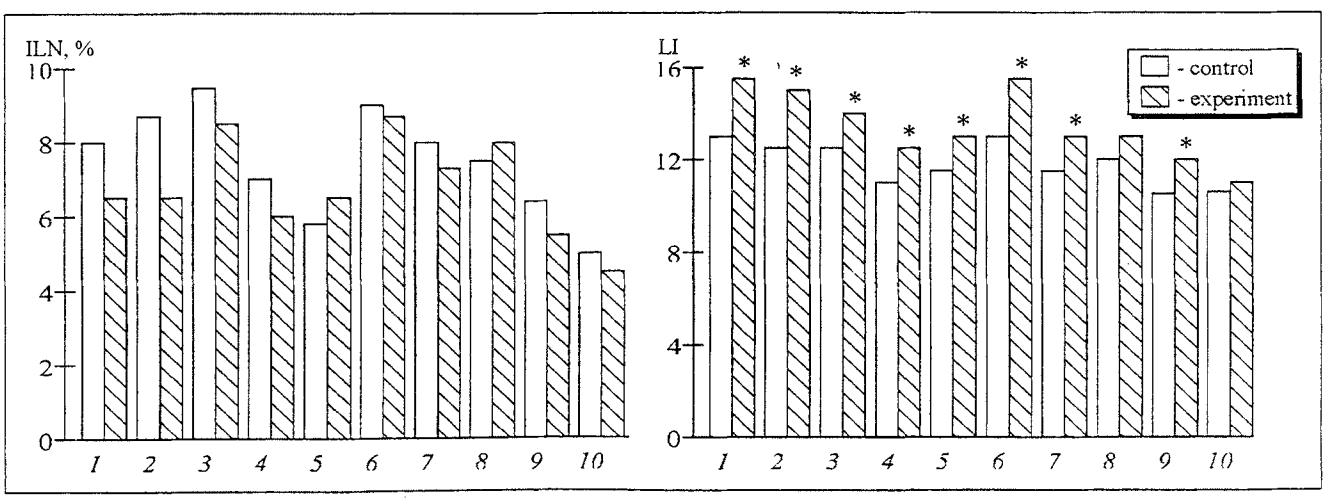

Fig. 2. Effect of 5-fold administration of dermorphin analog A10 (100 μg/kg) on DNA synthesis in the myocardium of albino rats during the early postnatal ontogeny.

TABLE 1. Effect of Dermorphin Analog A10 on DNA Synthesis in the Liver of Albino Rats in Early Postnatal Ontogeny

Dose	ILN, %	
	control	experiment
A10, 7 μg/kg, single injection	4.50±0.43	7.82±0.81*
A10, 70 μg/kg, single injection	4.92±0.18	9.90±0.88*
A10, 100 μg/kg, 5 injections	3.86±0.17	5.40±0.27*

Note. *$p<0.05$ in comparison with the control.

The data were processed statistically using the Student's t test. The differences between the groups were significant at $p<0.05$.

RESULTS

The dermorphin analog A10 in a dose of 7 μg/kg injected into 4-day-old rat pups produced no marked changes in the DNA synthesis in the examined zones of the myocardium.

In an order of magnitude higher dose (70 μg/kg) the preparation significantly increased LI in the subendocardial and intramural layers of the left ventricle (Fig. 1) without significant changes in the number of DNA synthesizing cells. This effect was somewhat more pronounced after repeated administration of A10: a significant rise of LI was noted in 8 of 10 examined zones (Fig. 2). Here again ILN was almost unchanged. Thus, the μ-OR agonist injected in the early ontogeny significantly increases the intensity of labeling in the myocardium of albino rats, which indirectly attests to acceleration of DNA synthesis. A significant rise of both the absolute (94.88±2.78 *vs.* 76.31±3.06 mg, $p<0.001$) and relative (6.75±0.35 *vs.* 5.32±0.24 mg/g body weight, $p<0.001$) mass of the heart in 7-day-old animals injected for 5 days with A10 is also indicative of its stimulating effect on physiological regeneration of the myocardium. The body weight of experimental animals did not differ from the control (14.42±0.65 *vs.* 14.98±0.68 g, $p>0.05$).

An increase in the mass of an organ is usually a result of a considerable activation of proliferative processes. However, the rise of LI was not accompanied by marked changes in the number of DNA-synthesizing myocardiocytes. It can be assumed that stimulation of physiological regeneration in the myocardium of A10-treated rats manifests itself in accelerated passage of the cells through the stages of the mitotic cycle but not in the rise of the tissue proliferative pool. This type of activation of physiological regeneration is typical of certain cell populations [5]. Moreover, possible chronobiological peculiarities of the observed effect should be also taken into account [15].

The heart of mammals has its own opioidergic system. Opioid peptides produce a considerable effect on the functional state of the myocardium [3]. Hence, the observed changes in cell reproduction are probably a secondary phenomena arising from the modulating effect of μ-agonist on the heart function.

The study of DNA synthesis in the liver showed that the A10-induced stimulation of physiological regeneration is typical not only of the myocardium. The peptide markedly increased the number of S-phase hepatocytes in all experimental series (Table 1). A clear-cut tendency toward an increase in the relative mass of the liver was noted in experimental animals given 5 injections of the μ-agonist (31.47± 1.29 *vs.* 28.40±1.06 mg/kg body weight, $p<0.1$).

Thus, systemic administration of the selective agonist of μ-OR stimulates cell reproduction in the myocardium and liver of albino rats during the early postnatal ontogeny. When analyzing the mechanisms of this effect, of special interest are the data on activation of ornithine decarboxylase, a representative marker of anabolic processes, in the heart and liver of 6-day-old rat pups induced by subcutaneous injection of β-endorphin.

The direction of opioid-induced shifts in cell proliferation is determined by the type of OR subpopulation [7,8]. Stimulation of δ-OR usually leads to activation of cell division [6,7]. The effect of μ-agonists depends on the type of target tissue. For instance, in mature albino rats, μ-agonists suppress cell proliferation in the corneal epithelium and stimulate proliferative activity in the gastric epithelium [7]. It seems likely that the opposite effects of μ-agonists are also typical of early ontogeny, since some investigators [11,13] have revealed an inhibiting effect of μ-agonists on the proliferative activity in the developing brain of albino rats.

Our experimental findings suggest that opioid peptides are involved into the ontogenetic development of tissue homeostasis in the liver and heart of mammals, thus substantiating the concept that opioids are an important factor of morphogenesis.

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Cytological Analysis of the Histone Component of Hepatocyte Nuclear Chromatin in Rats with Impaired Vagus Innervation of the Liver

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 2, pp. 216-219, February, 1997
Original article submitted October 15, 1995

The supramolecular arrangement of rat hepatocyte nuclear chromatin is studied by differentiated staining of the histone component after bilateral subdiaphragmal vagotomy in intact and phenobarbital-induced animals. The spectrum of hepatocyte nuclei staining is not altered in denervated liver; however, pronounced shifts in the quantitative ratio of different types of staining occur 1-2 weeks after denervation. One month after the operation, the hepatocyte population is almost the same as in the control regarding the staining pattern and quantitative ratio of its variants. In the phenobarbital group, the spectrum of hepatocyte staining does not change compared with intact control and vagotomized animals at all terms after surgery. This may be due to the restructuring of the nucleohistone—chromatin complex or lack of such restructuring.

Key Words: *chromatin; histones, hepatocyte; vagotomy*

Bilateral subdiaphragmal vagotomy changes the functional activity of hepatocytes. The ultrastructural basis of the initial morphofunctional changes in various cytoplasmic organelles of denervated liver is known [2], while the status of the cell nuclei during the neurodystrophic process is little studied. Changes in the supramolecular structure of hepatocyte nuclei chromatin in a denervated organ are virtually unknown.

Our purpose was to investigate the supramolecular structure of chromatin, which is maintained mainly by histones, by light microscopy using specific staining with ammonium silver (AS).

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MATERIALS AND METHODS

Outbred male albino rats weighing 160 to 180 g were subjected to bilateral subdiaphragmal vagotomy. The animals were divided into 5 groups: 1) intact rats; 2) laparotomized; 3) treated with phenobarbital (PB); 4) vagotomized; and 5) vagotomized and treated with PB at various terms after surgery. Group 4 animals were examined on days 7, 14, and 30 after vagotomy.

0007-4888/97/0002-0187$18.00