Effect of a Synthetic Leu-Enkephalin Analog on the Intensity of DNA Synthesis in Insects

T. I. Lapteva, N. O. Min'kova, and Yu. B. Filippovich

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The hexapeptide dalargin (a synthetic analog of leu-enkephalin) injected in the hemolymph of bee moth (*Galleria mellonella*) and of mealworm (*Tenebrio molitor*) causes a dose-dependent increase in the DNA content and an increase in the ^{3}H-thymidine incorporation in DNA. In bee moth, the maximum (43-68%) increase in radioactivity is observed after injection of 10^{-8} to 10^{-10} M of dalargin per insect, in mealworm, the highest radioactivity of DNA (73 and 162% increase) is recorded after 10^{-11} and 10^{-9} M dalargin per insect. Dalargin modifies the nucleic acid metabolism in the insects, which manifests itself as accumulation of extra DNA and enhanced DNA biosynthesis.

Key Words: *dalargin; DNA biosynthesis; insects*

Enkephalins produce various regulatory effects on metabolism. They affect proliferation and differentiation of precursor cells and cell division in the tongue and ocular corneal epithelium of rats [7] by stimulating production of DNA. Thus, opioid peptides play an important role in the regulation of cell division.

Along with the discovery of the functional role of endogenous peptides, the effects of their synthetic analogs have been investigated. Dalargin, a synthetic analog of leu-enkephalin, has been found to accelerate tissue regeneration [3,6] and stimulate cell division in the corneal epithelium of white rats [7]. In the fish, dalargin changes the content of nucleic acids in muscles [9] and stimulates DNA synthesis in trout roe [4].

The similarity of the peptidergic systems of vertebrates and insects, complete lack of data on the functional role of opioid peptides in insects, and use of dalargin in medicine and in fish breeding as a bioregulator of fish growth prompted us to investigate the effect of dalargin on the content of nucleic acids and on the intensity of a labeled precursor incorporation in nucleic acids of insects.

Department of Organic and Biological Chemistry, Moscow State Pedagogical University

MATERIALS AND METHODS

Tenebrio molitor imago and *Galleria mellonella* larvae of the VII age were used in the study.

The insects were injected dalargin in doses from 10^{-8} to 10^{-13} M in normal saline for insects, 1 μl per insect. Controls were injected 1 μl of normal saline for insects.

Three hours (for mealworm) and 24 h (for bee moth) after dalargin injection ^{3}H-thymidine was administered in a dose of 0.25 μCi per insect. After 2 h (for mealworm) and 24 h (for bee moth) the levels of the precursor incorporation in DNA were measured.

For this purpose the insects were homogenized in mortars with quartz sand in 5 ml of 5% trichloroacetic acid on the cold for 20 min. After maturation of DNA sediment (30 min at 3-5°C), the mixture was centrifuged on PC-6 centrifuge at 0°C and 6000 rpm. The resultant sediment was washed three times in 5% trichloroacetic acid and twice in ethanol by centrifugation of the mixture under the same conditions. The quality of the pellet washing from labeled precursor was assessed in a Rack-beta (LKB) liquid scintillator by measuring the radioactivity of the supernatant. The nucleic acid sediment was dissolved in 5 ml of concentrated formic acid, and its radioactivity was measured using a ZhS-8 scintillator

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TABLE 1. Content and Radioactivity of DNA in Mealworm Imago and Bee Moth Larvae After Injection of Dalargin

Object of study and phase of development	Dalargin content, M	DNA content, μg per insect	DNA radioactivity, dpm per insect
Bee moth (larva)	Control	26.74±1.55	41,829±3310
	10^{-8}	35.51±2.09	59,639±1842
	10^{-9}	31.98±2.98	70,272±2075
	10^{-10}	29.63±1.64	65,044±1723
	10^{-11}	28.63±1.72	60,777±1732
	10^{-12}	25.73±2.98	49,400±1518
	10^{-13}	21.26±1.71	47,267±1317
Mealworm (imago)	Control	5.97±1.27	10,163±225
	10^{-8}	7.89±1.12	12,397±308
	10^{-9}	9.54±2.42	26,627±511
	10^{-11}	8.93±1.65	14,417±413
	10^{-13}	8.07±1.36	13,461±313

and an Rack-beta (LKB) liquid scintillation spectrometer.

In parallel with measurement of ^{3}H-thymidine incorporation in DNA, the insects were fixed in boiling ethanol for subsequent measurement of DNA by a modified method of Schmidt—Thannhauser [8].

RESULTS

Preliminary experiments showed that the kinetics of ^{3}H-thymidine incorporation in the DNA of mealworm imago during 2 h and in the DNA of bee moth larvae during 24 h is almost linear; therefore, these time parameters were used to assess DNA production in experimental insects. The effect of dalargin on the intensity of DNA synthesis depended on the duration of exposure to the peptide. The highest incorporation of ^{3}H-thymidine in DNA was observed 3 h after injection of dalargin in mealworms and after 24 h in bee moths. The difference between the time of linear incorporation of the precursor and in the time of optimal effect of dalargin on the insect may be due to different intensity of metabolism, which is determined by the phase of development and is species-specific.

At the above time parameters dalargin enhanced the incorporation of the labeled precursor in the mealworm and bee moth DNA (Table 1) in comparison with the control. The effect of dalargin was dose-dependent. A similar dose dependence is known for virtually all peptides described.

In bee moths, the greatest (43-68%) increase in radioactivity was observed after injection of 10^{-8} to 10^{-11} M dalargin per insect. In mealworms, the highest radioactivity of DNA (increased by 73.3 and 162.0%) was observed after injection of 10^{-11}-10^{-9} M dalargin per insect. These findings are consistent with published reports on the effect of dalargin on the rate of labeled precursor incorporation in the DNA of mitogen-stimulated human lymphocytes [2] and in rat corneal epithelium [7]. In the trout, dalargin increased the number of chromosomes and satellite elements [1]. Since dalargin accelerates tissue regeneration [3,6], these findings can be explained by accelerated cell division. However, the correlation between an increase in DNA radioactivity and the mitotic index is not always clearly seen [5]. Regeneration which takes place in disease or regeneration of tissues which are characterized by a high rate of cell restoration has been documented. Exposure of a normal organism to the peptide presumably does not stimulate cell division. The mechanism of direct or indirect effect on the genome remains unknown.

Dalargin caused a dose-dependent increase in the DNA content in both insect species examined in comparison with the control (Table 1). In the bee moth, the DNA content increased 33 and 20% after injection of 10^{-8} and 10^{-9} M dalargin, respectively, in mealworms the content of DNA is 59.8-35% increased after injection of 10^{-9}-10^{-13} M dalargin in comparison with the control. The effect of dalargin on the DNA content in trout muscles was virtually the same [4]. It is noteworthy that dalargin induced identical changes in nucleic acid metabolism in the representatives of different taxonomic groups. It is additional evidence that the peptidergic system of lower animals, specifically, insects, is as complex as that of vertebrates.

Thus, the synthetic analog of leu-enkephalin dalargin stimulates the nucleic acid metabolism in insects, which manifests itself as accumulation of DNA and a higher level of DNA biosynthesis.

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Correction of Biotransformation of Xenobiotics by α-Tocopherol in Combination with Nicotinamide and Methionine in the Liver Damaged by Ultrasound

I. V. Zverinskii, M. I. Bushma, L. F. Legon'kova, P. I. Lukienko, and K. A. Eismont

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Six day after rat liver sonication, the content of cytochrome P-450, rate of NADPH oxidation, activity of NADPH—cytochrome P-450 reductase, and rate of aniline hydroxylation in the microsomal fraction decrease. After 12 days, the rate of ethylmorphine N-demethylation also decreases. Intragastral administration of methionine, nicotinamide, and vitamin E for 6 and 12 days activates these enzymes and uridine 5'-diphosphate glucuronyl transferase.

Key Words: *ultrasound damage to the liver; biotransformation of xenobiotics; α-tocopherol; nicotinamide; methionine*

"Loosening" of the lipoprotein complex of cellular and subcellular membranes caused by ultrasound leads to an increase in membrane permeability and inhibition of membrane-bound enzymes, as shown in experiments with mitochondria. This effect has been related to cavitation and free-radical processes [5,6]. In the present study the effect of ultrasound on the activity of monoxidases and glutathione and glucuronyl transferases and the protective effect of the membrane-stabilizing complex consisting of α-tocopherol, nicotinamide, and methionine were examined.

Laboratory of Biochemical Pharmacology, Institute of Biochemistry, Byelorussian Academy of Sciences; Department of Pathological Physiology, Medical Institute, Grodno

MATERIALS AND METHODS

Experiments were performed on 32 outbred male rats weighing 180-200 g. The liver was sonicated (2 W/cm^2) after laparotomy under ether anesthesia. α-Tocopherol (50 mg/kg) in combination with nicotinamide (50 mg/kg) and methionine (200 mg/kg) was administered intragastrally in starch gel for 6 and 12 days. Control rats (laparotomy) were given the same volume of starch gel. The contents of cytochromes P-450 and b_5, activities of NADPH—cytochrome P-450

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