EFFECT OF DALARGIN, A SYNTHETIC LEU-ENKEPHALIN ANALOG, ON PROTEIN AND NUCLEIC ACID LEVELS IN RAINBOW TROUT MUSCLES

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UDC 612.744.14.014.46:[615.31:547.943:547.95].019:597.553.2].08

KEY WORDS: dalargin; nucleic acids; rainbow trout.

The effect of dalargin (D-Ala^2,Leu^5,Arg^6-enkephalin), a synthetic analog of Leu-enkephalin, on animals and man is under active study at the present time. We know that dalargin has a marked protective and antistressor effect, that it affects the levels of ACTH, cortisone, and hormones of the pituitary-thyroid complex in the blood plasma and the cAMP concentration in the tissues of the adrenal and thymus glands during stress [3], accelerates regeneration of the head end of planarian worms [7], protects the duodenal mucosa against ulcer formation [5], increases ornithine decarboxylase activity in the duodenal mucosa in animals with experimental duodenal ulcer [9], and modifies incorporation of ^{3}H-thymidine into resting and mitogen-stimulated lymphocytes [1].

Treatment of rainbow trout eggs with dalargin has been shown to increase the survival rate of the eggs and the weight and size of the young fish [6]. Assuming that dalargin treatment induces definite biochemical changes in the body, we set out to investigate differences in the nucleic acid concentrations in muscles of fish reared from dalargin-treated eggs.

EXPERIMENTAL METHOD

RNA and DNA were determined quantitatively by a modified Schmidt–Thannhauser method [4] in muscles of rainbow trout aged 1 and 2 years, reared from eggs (group 1) treated with a single dose of dalargin in the swelling stage, and from fry (group 2), treated with a single dose of dalargin in the period of appearance of oocytes in the early prophase of meiosis in the gonads. Group 3 consisted of rainbow trout aged 1 and 2 years, reared after two consecutive treatments, initially of the eggs and later of the fry, with dalargin at the same stages of development. The eggs (about 20,000) were treated by total immersion in 5 liters of a solution of dalargin with a concentration of 1 μg/liter for 1 h at 6°C, whereas the fry (about 5000) were treated by immersion in 20 liters of dalargin solution of the same concentration for 1 h at 20°C. The fry were reared at the "Skhodnya" trout farm.

The fish were killed 1 year (one-year-old) and 1.5 years (two-year-old) after treatment with dalargin and the muscles under the dorsal fin were removed. The minced muscle (1 g) was fixed with boiling ethanol (1:30 by volume). After removal of the alcohol by filtration a on a Büchner funnel under a vacuum, the tissue was ground in liquid nitrogen to a homogeneous state. Lipids were extracted from the resulting preparation first with a mixture of chloroform and acetone (5:1), and then with acetone. Acid-soluble material was removed in the cold by a single treatment of the tissue residue with 0.3 N HCl and triple treatment with 0.2 N HCl. Nucleic acids were hydrolyzed with 0.5 N KOH solution and the optical density of solutions containing ribonucleotides and deoxyribonucleotides was determined on an SF-26 spectrophotometer at 270 and 290 nm. Concentrations of nucleic acids were calculated by Spirin's formula. Parallel with determination of the nucleic acid concentrations, protein was determined by Lowry's method [10] in a tissue extract in 0.5 M KCl solution.

EXPERIMENTAL RESULTS

After a single treatment of the eggs and fry with dalargin the fDNA concentration in the muscle tissue of the yearlings increased by 85% (group 1) and 66% (group 2) compared with the

Department of Organic and Biological Chemistry, V. I. Lenin Moscow Pedagogic Institute. Laboratory of Applied Physiology and Toxicology, All-Union Research Institute of Fisheries and Oceanography. (Presented by Academician of the Academy of Medical Sciences of the USSR I. B. Zbarskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 473-475, April, 1989. Original article submitted June 30, 1987.

0007-4888/89/0004-0539$12.50

TABLE 1. Protein and Nucleic Acid Concentrations in Muscles of One- and Two-Year-Old Rainbow Trout Reared after Treatment of Eggs and Fry with Dalargin (in mg/g weight of air-dried substance; M ± m)

Experimental conditions	Protein		RNA		DNA	
	a	b	a	b	a	b
Control	303±10	284±14	10,9±0,7	2,8±0,2	0,35±0,01	0,25±0,01
Group 1	280±10	376±15	6,9±0,3	2,9±0,2	0,65±0,02	0,30±0,01
Group 2	344±13	324±14	8,4±0,3	2,3±0,2	0,58±0,02	0,31±0,02
Group 3	316±17	345±17	8,2±0,4	3,7±0,2	0,18±0,01	0,29±0,01

Legend. a) One-year-old, b) two-year-old. Control – eggs and fry not treated with dalargin; number of experiments n = 10.

control (Table 1; $p < 0.01$). This increase correlates with observations according to which a single treatment with dalargin increases the biomass of yearling fish by 48% and of second year fish by 9%. Histological observations also have shown that the dimensions of the ovaries and the number of oocytes in them are greater, but the dimensions of the oocytes are smaller, in fish developing from dalargin-treated eggs than in the control. Considering data in the literature on the ability of dalargin to stimulate regeneration in animal tissues it can be concluded that dalargin accelerates cell division and increases the number of cells, with the result that the DNA concentration per unit weight of tissue is increased.

A similar picture was observed also for the two-year-old fish, in whose muscle tissue the increase in the DNA content was 19.7% (group 1) and 23% (group 2, $p < 0.1$). This is evidence that the effect of dalargin treatment, although decaying, nevertheless persists for at least 18 months.

The RNA concentration in the muscle tissue of the year-old fish, reared from dalargin-treated eggs, was reduced by 37% (group 1) and by 23-25% (groups 2 and 3, $p < 0.1$) compared with the control. This fact suggests that dalargin has an influence on RNA metabolism. This decrease in the RNA concentration, which appeared in all three groups of year-old fish, was not so clearly defined in the two-year-old fish.

Comparison of data on the protein concentration showed that treatment with dalargin led to an increase of 4-13% (groups 2 and 3) in the one-year-olds and by 14-32% in the two-year-olds compared with the control ($p < 0.5$).

In the modern view, these long-term effects, so difficult to interpret, as the effect on cell growth and protein synthesis, in the case of, for example, insulin can be explained only on the assumption that the peptide can penetrate into the cell [8]. Since the half-life of dalargin is 2 min and its fragments (tetra- and pentapeptides), which can actively bind with opiate receptors, are stable for several hours [2], it is very difficult to explain the results showing an increase in DNA concentration 12 and 18 months after dalargin treatment. We can only postulate the existence of as yet unknown mechanisms of long-term activation of the genetic apparatus of the cells in response to injection of biologically active peptides in the earliest stages of development of the organism.

Thus a single treatment of the eggs and fry with dalargin gives rise to a marked increase in the DNA content per unit weight of muscle tissue in one- and two-year-old rainbow trout, and to a greater degree in the former than in the latter. Parallel with the increase in DNA concentration there was a decrease in the RNA concentration in the one- and two-year-old fish, and an increase in the concentration of soluble proteins in the two-year-olds. Characteristically, the less differentiated the organism when treated, the more marked the effect of the increase in DNA concentration.

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