Effect of Sedatin (Synthetic Dermorphin Analog) on the Development of *Acipenser schrenckii* Young Fish

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We studied delayed effects of sedatin (arginine-containing μ/δ-opioid receptor agonist) and its arginine-free analog on the ontogenetic development of fish. Fertilized eggs of the Amur sturgeon (*Acipenser schrenckii*) were treated with the peptides. The results indicate that sedatin increased activities of the antiradical and antioxidant defense systems, stimulated fry development by increasing body weight and length, and increased the number of nucleoli in the myocardium and liver of young fish.

Key Words: *synthetic dermorphin analog; sedatin; fish ontogenesis; fish breeding optimization; Acipenser schrenckii*

Arginine-containing opioid peptide dalargin (leu-enkephalin analog) stimulates proliferation and morphogenesis in different cell populations [10]. Dalargin treatment of trout spawn improved embryo survival, increased fry weight and size, increased DNA and protein content in fish muscle tissue [5, 8]. Sedatin (synthetic dermorphin analog) similarly as dalargin stimulates proliferation in different cell populations and exhibits morphogenetic activity in a wide dose range [9,11,12]. In dalargin (H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH) arginine is present in the C-terminal position, while sedatin contains N-terminal arginine (H-Arg-Tyr-D-Ala-Phe-Gly-OH).

The aim of the present study was to evaluate the effect of sedatin treatment of *Acipenser schrenckii* fertilized eggs on the ontogenetic development of fish and to decipher the mechanisms underlying these effects.

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MATERIALS AND METHODS

The study was carried out on larvae and young sturgeons grown in experimental production department of the Novoamurskoe company. Sedatin (dermorphin analog; H-Arg-Tyr-D-Ala-Phe-Gly-OH) was synthesized in Peptos Research-and-Production company. Sedatin is an agonist of μ/δ-opioid receptors and is close to dalargin by affinity for these receptors. After peptide treatment the container with spawn was placed in Osetr incubator. The spawn was treated during the swelling stage. In order to minimize genetic differences, the control and experimental samples of spawn were collected from the same female. The density of larvae and small fish was maintained at the same levels in control and experimental groups throughout the experiment. The efficiency of embryonic development was evaluated by embryo survival (percent of pre-larvae hatched from spawn), the number of pre-larvae, and time course of their hatching. Somatometric parameters of larvae and small fish were measured over the course of development. Common status, behavioral reactions, and somatometric parameters of young fish were monitored over 2 months (from hatching to transfer into natural environment).

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Integral evaluation of free radical oxidation processes was carried out by chemiluminescence (CL) registered on an LS-50B Perkin Elmer fluorescent spectrometer. The signal was standardized by a FinLab input software. Spontaneous and Fe^{2+}-induced CL were studied as described previously [2]. The following parameters were determined: total yield of spontaneous CL (S_{sp}) over 1 min (reflects the intensity of free-radical processes); fast flash maximum (H1) of induced CL (reflects the content of lipid hydroperoxides); total yield ($S1_{ind}$) over 4 min after fast flash (reflecting the rate of peroxide radical generation).

The kinetics of H_2O_2-induced CL in the presence of luminol [1,13,14] was analyzed by two parameters: fast flash maximum (H2) reflecting the intensity of radical production in Fenton-like reactions, and total yield ($S2_{ind}$) over 4 min, depending on activity of antioxidant and antiradical defense systems.

For histomorphological analysis, young fish was fixed in 10% neutral formalin (pH 7.5), treated routinely, and cross-sections (7 μ) at the level of the liver and heart ventricles were prepared. The nucleolar organizer regions (NOR) were detected as described previously [6]. NOR were analyzed in hepatic and myocardial tissues of 45-day-old fish. This term is chosen because all organs are completely formed in young fish by this age and it gains standard weight to be transferred into natural environment [4]. The nucleoli were counted in 100 nuclei of hepatic and myocardial cells of each young fish. The nucleolar organizer was analyzed using MEKOS-S computer morphometry.

The results were processed statistically by Student's *t* test using Statistica 5.0 software.

RESULTS

Incubation of *Acipenser schrenckii* fertilized eggs with sedatin more than 2-fold increased the percent of hatching (Table 1).

Another important indicator of the positive effect of sedatin was higher rate of hatching. The procedure of spawn collection, artificial fertilization, and incubation are stress factors. Delayed hatching of pre-larvae is considered as a manifestation of embryonic development pathology. Sedatin treatment significantly accelerated hatching, particularly during the first 24 h (Fig. 1). This can be regarded as attenuation of the negative aftereffects of artificial fertilization and incubation by sedatin.

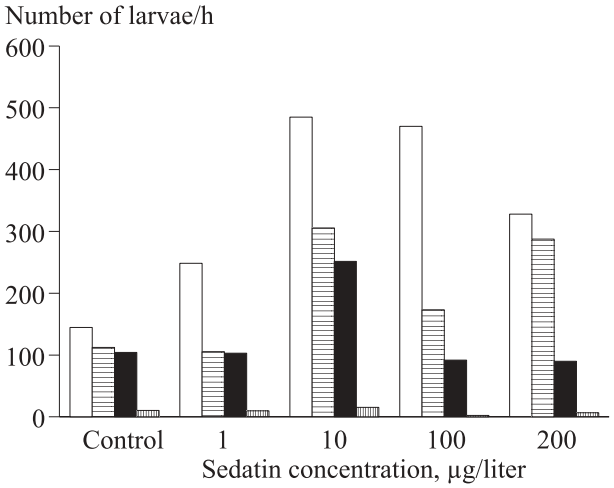

Fig. 1. Time course of hatching of *Acipenser schrenckii* larvae in the control and experimental groups under the effects of sedatin in different concentrations: 1, 10, 100, 200 μg/liter. Light bars: 0-24 h after the beginning of hatching; horizontally hatched bars: 25-31 h; dark bars: 32-38 h; vertically hatched bars: 39-55 h.

Oxidative stress can be a universal mechanism of realization of negative effects on the development of fertilized spawn. Analysis of CL values 18 h after sedatin treatment of spawn explained to a certain measure the positive effect of sedatin (Table 2). Sedatin treatment increased buffer capacity of the antioxidant and antiradical defense systems: $S2_{ind}$ decreased significantly (1.6 times) in comparison with the control. This was paralleled by decreased formation of hydroxyl radicals (H2 reduced 1.2 times) and inhibition of peroxide radical generation ($S1_{ind}$ decreased 1.5 times). Activation of defense systems did not modify the content of lipid hydroperoxides (H1 level was the same as in the control). No changes in the systems generating and detoxifying active oxygen metabolites were observed: S_{sp} virtually did not differ from the con-

TABLE 1. Dynamics of Hatching in Experimental Samples 130 h after Fertilization (*M*±*m*)

Parameter	Control	Sedatin, μg/liter			
		1	10	100	200
Total number of pre-larvae	3899	5198	9749	9541	7254
Hatching, % of fertilized eggs	14.3±0.2	17.1±0.21*	32.1±0.27*	31.4±0.27*	21.7±0.23*

Note. *$p<0.0001$ compared to the control.

TABLE 2. Effects of Sedatin and Its Arginine-Free Analog on CL in *Acipenser schrenckii* Fertilized Spawn

Parameter	Control	Sedatin	Arginine-free sedatin analog
S_{sp}, mV/min	0.012±0.001	0.0098±0.0012	0.0117±0.0010
Fe^{2+}-induced CL			
H1, mV	0.0180±0.0014	0.0150±0.0017	0.0170±0.0019
$S1_{ind}$, mV 4 min	0.0489±0.0039	0.0330±0.0027*	0.0463±0.0040
H_2O_2-induced CL			
H2, mV	0.0210±0.0018	0.0100±0.0007*	0.0190±0.0015
$S2_{ind}$, mV 4 min	0.0455±0.0030	0.0280±0.0023*	0.0406±0.0038

Note. Here and in Table 3: *p<0.05 compared to the control.

TABLE 3. Somatometric Parameters of 62-Day-Old *Acipenser schrenckii* after Treatment with Dermorphin Analogs in a Concentration of 0.1 mg/liter (M±m)

Parameter	Control	Sedatin	Arginine-free sedatin analog
Weight, g	6.663±0.807	9.830±1.044*	7.873±0.859
Length, cm	11.46±0.53	12.98±0.48*	12.10±0.39

trol. In contrast to sedatin, its arginine-free analog did not modify the CL values.

The effect of sedatin on fertilized spawn increased the weight and length of experimental fish in comparison with the control (Table 3). No negative deviations in the somatometric parameters were detected; the Fulton fatness index also virtually did not change. Arginine-free analog caused virtually no changes in the somatometric parameters.

The results of computer morphometry and the number of nucleoli can explain the positive effect of sedatin on the somatometric parameters.

The mean number of nucleoli in hepatocyte nuclei of control specimens was 3.22±0.15 *vs.* 5.43± 0.30. Treatment with arginine-free analog did not appreciably change this parameter (4.01±0.28). Hence, sedatin treatment during the early embryonic period significantly increased the number of NOR in hepatocytes of young fish. The results can be regarded as an evidence of increased functional reserves of the liver in experimental young fish and hence, its inproved resistance to unfavorable environmental conditions [3].

Similar changes were detected in the myocardium of young fish after sedatin treatment. In control and experimental young fish, the mean number of nucleoli in myocardial cells was 3.14±0.14 and 5.03±0.29, respectively (p<0.001). The nucleolus/ nucleus ratio did not change. Hence, single treatment of *Acipenser schrenckii* fertilized spawn with sedatin in a concentration of 0.1 mg/liter significantly increased the number of nucleoli not only in liver cells, but also in the myocardial cells of young fish.

Sedatin capacity to modify the developmental stages after treatment at the early stages of ontogeny is similar to the effect of dalargin on fertilized trout spawn [5]. High biological activity of sedatin can be explained by a combination of its affinity for opioid receptors and the presence of arginine cleaved during the peptide metabolism.

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