Decrease in Activity of the Serotoninergic System during Mutagenesis

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We studied changes in the content of serotonin-modulated anticonsolidation protein in the liver of goldfishes and gobies caused by oil and industrial pollution. The concentration of serotonin-modulated anticonsolidation protein in fish liver increased after short-term exposure to oil-contaminated water (100 mg/liter), but decreased under long-term effect of industrial wastes. We hypothesize that serotonin plays a role in antimutagenic protection of the organism and maintains the differentiated state of mature cells.

Key Words: *serotonin-modulated anticonsolidation protein; oil and industrial pollution; mutagenesis*

The degree of anthropogenic environmental changes in industrial areas significantly increased in recent years. The role of adverse environmental factors in the development of pathological disorders in living organisms remains unclear. It is important to evaluate whether tissue changes play a role in the pathogenesis of various diseases under these conditions.

Serotonin is an endogenous compound that respond to stress of different nature. Serotonin not only has neurotransmitter function, but also regulates cell proliferation and differentiation [1,10,14].

Serotonin-producing cells [3] and serotonin receptors [12] appear at the very early stages of embryogenesis [6]. Neurotransmitter properties of serotonin are not realized during this period due to low degree of cell differentiation in the central nervous system. The early appearance of serotonin-producing cells and serotonin receptors indicates that these cells are involved in the regulation of cell proliferation and differentiation in embryonic tissues. This type of regulation is probably maintained in the adult organism. Oil and bottom sediments of industrial waste include considerable amounts of polyaromatic hydrocarbons. These compounds play a role in the induction of mutagenesis [8] and proliferation of transformed cells (carcinogenesis) [5].

Here we studied activity of the serotoninergic system in the liver of fishes under conditions of oil and industrial pollution. Function of serotonin in cells is realized via selective regulation of several genes and synthesis of serotonin-modulated proteins [7]. Activity of the serotoninergic system was estimated from the concentration of serotonin-modulated anticonsolidation protein (SMAP), which depends on serotonin content [2]. It could not be excluded that polyaromatic hydrocarbons have high affinity for serotonin receptors. In that case the measurement of tissue serotonin content is uninformative and does not reflect the actual intracellular serotonin signal.

MATERIALS AND METHODS

Experiments were performed on goldfishes (*Carassius auratus*) weighing 14-18 g. Treated fishes (n=6) were placed in a bath with fresh water. Oil samples from the Chirag oilfield were added to water (100 mg/liter). Control animals (n=10) were maintained in pure fresh water. The time of exposure

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was 5 days. Blood samples were taken from the caudal vein. Blood smears were put in glasses and stained with methylene blue and eosin (Hema-Tek Stain Pak, Bayer). The number of micronuclei per 2000 erythrocytes was estimated in each blood sample. The fishes were killed. The liver was isolated. Total proteins were extracted with 0.05 M phosphate buffered saline (pH 7.2) containing 0.3 M NaCl, 0.1% Triton X-100, and 5 mM EDTA. SMAP concentration was measured by the ELISA using polystyrene plates (Nunc). The concentration of extracted proteins was brought to 10 μg/ml using 0.1 M Tris-HCl buffer (pH 8.6). They served as the antigens for ELISA. Protein concentration was measured by the method of Bradford [4]. Wells were washed 4 times after 20 h. Rabbit anti-SMAP immunoglobulins were diluted in 0.01 M phosphate buffered saline for antibodies (pH 7.2, 1:30). The solution contained 0.15 M NaCl, 1% bovine serum albumin, and 0.05% Tween 20. The wells were washed 4 times after 24 h. Horseradish peroxidase-conjugated goat immunoglobulins against rabbit antibodies were diluted in the buffer for antibodies (1:6000) and added to wells. The wells were repeatedly washed after 3 h. Horseradish peroxidase substrate *o*-phenylenediamine (0.5 mg/ml) dissolved in 0.5 M citrate-phosphate buffer (pH 4.5) with 0.4 μl/ml 30% H_2O_2 was added to wells. The reaction was stopped by adding 50 μl 3 M NaOH after 20 min. The results were recorded on a StatFax photometer at 492 nm. SMAP concentration was expressed in optical density units.

SMAP concentration in Caspian sand gobies (*Neogobius fluviatilis*) was measured to evaluate the influence of long-term exposure to industrial waste pollution in water. They were fished out in 2 areas of relative pure water and water polluted by iron, zinc, and polyaromatic hydrocarbons (7 specimens from each area).

RESULTS

The exposure of fishes to oil-polluted water (100 mg/liter) was followed by a significant increase in liver SMAP concentration (0.237±0.004 *vs*. 0.152± 0.008 optical density units in the control, $p<0.001$). These data show that serotonin content increases in the liver of treated fishes. The micronucleus test revealed no differences between control and treated fishes by the number of micronuclei in erythrocytes (2.0±0.2 and 2.00±0.32 per 1000 cells, respectively).

Liver SMAP concentration in gobies from highly polluted area was much lower compared to fishes from pure areas (0.044±0.001 and 0.122± 0.002 optical density units, respectively, $p<0.001$). The micronucleus test showed that the number of micronuclei in erythrocytes of fishes from polluted areas is much higher compared to those from pure areas (24.08±0.63 and 5.57±0.11, respectively, $p<0.001$).

Our results indicate that SMAP concentration increases in the early stage of exposure to oil-polluted water. The long-term exposure is followed by depletion of the serotoninergic system and decrease in SMAP concentration. The decrease in SMAP concentration and serotonin content is accompanied by an increase in the number of micronuclei in fish erythrocytes. A negative correlation is found between SMAP concentration and number of micronuclei, which illustrates antimutagenic activity of the serotoninergic system. Previous studies showed changes in activity of the serotoninergic system in the brain of animals under the influence of chlorophenoxy herbicides [11].

The increase in the number of micronuclei reflects the early mutagenic changes and is used as a biological index of carcinogenesis [13,15]. Adverse environmental factors induce the decrease in serotonin content in tissues and body fluids to the level observed during embryogenesis [1]. Function of serotonin in cells is realized via the regulation of certain genes [7]. The decrease in serotonin content results in activation of genes that function actively during embryogenesis, but are suppressed in adult organisms. These changes are followed by differentiation of cells that gain the properties of embryonic cells (*e.g.*, high proliferative activity). Previous experiments showed that reversin treatment is followed by dedifferentiation of mouse myoblasts, loss of specific myogenic markers (MyoD and myosin), and suppression of the next stage of differentiation (formation of muscular tubes) [9]. Newly formed stem cells were characterized by pluripotency and ability to differentiate into bone and fat cells under these conditions.

We conclude that the serotoninergic system provides antimutagenic protection of cells and, probably, maintains the differentiated state of mature cells in the adult organism. The impairment of protective activity under the influence of adverse environmental factors is followed by pronounced mutagenic changes in cells.

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