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ACETYLCHOLINESTERASE ACTIVITY MEASUREMENTS AS A TOOL FOR DEMONSTRATING THE POSSIBLE CAUSE OF FISH DECAY

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Abstract

Mass decay occurred in November 1982 in the fish ponds of the 'Eastern Light' Agricultural Co-operative at Pálmonostora, located in the area of the Water Conservancy Directorate of the Lower Tisza Region (Southern Hungary). The temperature of the water was 8 °C, the solved 0_2 content was 4.2 mg/l. Biochemical studies on the organs of 6—700 g carps (*Cyprinus carpio* L.) brought in from the scene of the occurrence demonstrated that one of the possible causes of the mass fish decay might be due to the inhibition of acetylcholinesterase (AChE, EC 3.1.1.7.) by some kind of water pollution. The degree of inhibition in the heart and muscle was 50—60% and 80—90% in the brain and heart respectively.

Key words: fish, acetylcholinesterase, water pollution.

Introduction

Measurements on the changes in enzyme activity of acetylcholinesterase (AChE, EC 3.1.1.7.) have been used for a long time determining the characteristic toxic effect of insecticides containing organic phosphate and of carbamate type (GAGE, 1955; O'BRIEN, 1960; HEAT, 1961; GAGE, 1967). It has also been demonstrated in the case of fish that water contamination may also cause damage in the nervous system through the inhibition of acetylcholinesterase (HOLLAND et al., 1967; COPPAGE et al., 1975; COPPAGE and BRAIDECH, 1976; KLAVERKAMP et al., 1977; KLAVERKAMP and HOBDEN, 1980; HANKE et al., 1983; NEMCSÓK et al., 1984).

Taking the above into cinsideration studies were carried out on still living fish collected from the site of mass fish decay occurring in November, 1982. The purpose of the studies was to determine possible tissue damage, and injury of the nervous system, respectively, in the fish regarding the changes in the enzyme activity of aminotransferase (glutamic acid-oxalacetic acid-transaminase, GOT: EC 2.6.1.1.; glutamic acid-pyruvic acid-transaminase, GPT: EC 2.6.1.2.) in the serum as well as of acetylcholinesterase in the various organs.

Materials and Methods

1. THE SPOT AND CONDITIONS OF THE FISH DECAY

The fish decay was observed on November 4, 1982 in the fish ponds of the 'Eastern Light' Agricultural Co-operative at Pálmonostora. Arriving on the spot, the specialist of the Water Conservancy Directorate of the Lower Tisza Region found a large number of perished, just living fish

in the so-called „winter fish ponds". The temperature of the water was 8 °C, the solved O_2 content was 4.2 mg/l at dawn. Three 6—700 g carps from the still living individuals were taken to the Department of Biochemistry Attila József University for studies. Blood was taken from the tail vessels of the fish, centrifuged and the activity of the GOT and GPT enzymes as well as of the AChE enzyme was measured from the blood plasma. The adequate data of healthy carps kept at identical water temperature and of the same weights were used as controls.

2. Measurements applied for the biochemical studies

2.1. *Determination of the GOT and GPT activities*

One part of the oxalacetic acid formed during the course of the catalysed reaction by these transaminases spontaneously transformed into pyruvic acid, which latter formed coloured complex with 2.4-dinitrophenyl-hydrasine in alkalinic medium, the light-absorption of which was measured.

a) *Reaction mixture used for determining GOT*

0.25 ml 0.1 M phosphate buffer (pH=7.4) containing 0.1 M L-asparagine acid and 2 mM alpha-ketoglutaric acid +0.05 ml blood serum. (The blank sample contained the same amount of destilled water).

b) *Reaction mixture used for determining GPT*

0.25 ml 0.1 M phosphate buffer (pH=7.4) containing 0.2 M DL-alanine and 2 mM alpha-ketoglutaric acid +0.05 ml blood serum. (The blank sample contained 0.05 ml destilled water After incubation at 37° C for 60 min/for 30 min. in the case of GPT) 0.25 ml 1 mM 2.4-dinitrophenyl-hydrasine was added to each sample and the reaction mixture was left for 20° C, then after adding 2.5 ml 0.4 M NaOH the extinction was measured at 540 nm. The activity was expressed in U/1 used and accepted in toxicology (1 U = µmol decomposed substrate) (1 min at 25° C).

2.2. *Determination of acetylcholinesterase with the method of Ellman et al. (1961)*

The acetylcholinesterase enzyme hydrolized the acetylcholine-iodide to thiocholine and acetic acid. The —SH group of thiocholine gave colour reaction with dithio-bis-nitro-benzoic acid (DTNB). The reaction mixture was: 2 ml 52 mM phosphate buffer (pH=7.2) containing 0.26 mM DTNB). 0.05 ml acetylthiocholine-iodide of 82.4 mM+0.01 ml blood serum. Reaction was started by adding blood serum which was registrated for 3 min at 412 nm by light absorption. (The change was linear within this time). Activity was expressed in U/1.

Results and Discussion

In the collected carps the blood vessels were well observable at the lower part of the abdomen, the movement of their opercule was irregular, their posture was numb, jerky. The aminotransferase enzyme activities measured from the blood plasma were of similar values as the control, however, the AChE activities of the blood plasma showed considerable decrease compared to the values of the control individuals (Table 1). The AChE activity also significantly decreased in the organs of the fish

Table 1. Values of serum transaminase activity in control carps and carps originating from polluted water at the time of the fish decay observed in Csongrád county.

Sample	GOT (U/1)	GPT (U/1)
Still living carps collected from the area of the fish decay (3 individuals)	28.3±3.5	1.27±0.31
Control carps (10 individuals)	32.4±18.8	1.38±0.63

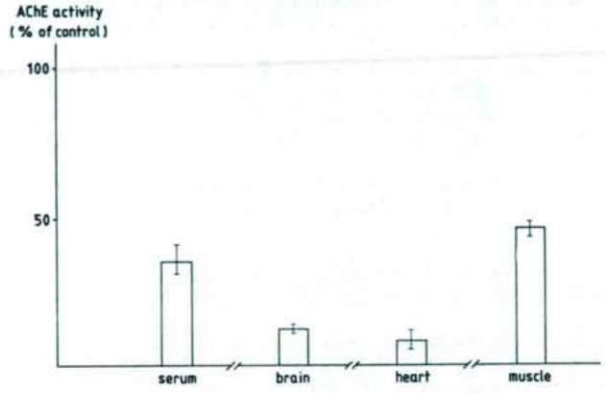

Fig. 1. The acetylcholinesterase activity in different organs of carp. Fish were collected from pesticide polluted water. Values are expressed in the percentage of „healthy" fishes, collected from non polluted water.

(Fig. 1.). The inhibition of acetylcholinesterase in the various organs of fish is harmful from several points of view.

1. It inhibits the normal nerve function and the various vital behaviour functions which are essential in obtaining food as well as in the defensive, escaping reactions (ABOU—DONIA and MENZEL, 1967; BASLOW and NIGRELLI, 1961; COPPAGE, 1971).

2. The inhibition of acetylcholinesterase is especially dangerous in the heart, since the cholinergic system has decisive role in the innervation of the hearts in fish (PENNEC and LA BRAS, 1984): inhibition of acetylcholinesterase may lead to the increase of vagus effect, which may cause severe disturbances in the metabolism-process related to the circulation. This is so, since the inhibition of the heart function has harmful effect on the O_2 uptake and CO_2 release, thus it may cause anoxia at the tissue level. On the basis of our results, the acetylcholinesterase inhibition might also have played role in the fish decay. The cause of this was presumably the insecticide or other type of chemicals used in the environment of the fish ponds which may cause acetylcholinesterase inhibition of significant degree even at rather low concentrations (COPPAGE, 1971, 1972; COPPAGE and MATTHEWS, 1975; COPPAGE et al., 1975; COPPAGE and BRAIDECH, 1976; KLAVERKAMP et al., 1977; DUANGSAWASDI and KLAVERKAMP, 1979; KLAVERKAMP and HOBDEN, 1980; NEMCSÓK et al., 1984).

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