ACTA BIOL. SZEGED. 39, pp. 113-118. (1993)

Thesis of dissertation for academic doctor's degree

RESEARCH INTO THE BIOCHEMICAL EFFECTS OF XENOBIOTICS ON FISH

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Preliminaries

Due to the tremendous development of industry and agriculture, the biosphere has become perceptibly damaged in several respects in recent years. The deleterious processes and their effects on living organisms were first examined by scientists working in certain traditional fields of biology: zoology, botany and ecology. In the 1960 s, chemists, toxicologists and biochemists in the industrially developed countries joined in these scientific investigations by participating in both fundamental and applied research. They introduced the most up to date experimental methods of that time, such as isotope technology, separation technology and the biochemistry of nucleic acids and enzymes. As a result, from the beginning of the 1970s, environment; protecting biochemistry appeared as a virtually new branch of learning in the USA, Canada, the Scandinavian countries and other industrially developed countries (REICHENBACH; KLINKE, 1972; KRISTOFERSSON et al., 1974; OZRETICH et al., 1983; HODSON and HILTON, 1983; LOCK et al., 1983; LOYIWOLA et al., 1983). The fundamental research in this field deals with environment; damaging effects with regard to enzymatic and molecular structures. The applied research utilizes those biochemical processes which, according to the basic research, react in a particular way, or in certain cases specifically as far as the given environment; polluting substances are concerned (biomonitoring).

In modern industrial and agricultural technologies, the use of different chemicals is unavoidable. Various of these compounds can pass into the natural waters during or after their use, and can thereby cause considerable damage to living organisms. The living organisms damaged are mainly those subjects to the accumulation of environment; pollution substances (MCKIM et al., 1970; REICHENBACH; KLINKE, 1972; HORVÁTH and STAMMER, 1979; FERRI and MACHA, 1980; ROJIK et al., 1983; BENEDECZKY et al., 1984) Tissue necrosis is one of the most usual signs of damaged organisms. In the event of tissue necrosis, the enzymes transaminase and lactate dehydrogenase (LDH) pass into the circulation of the damaged organ, and their increased activities in the blood indicate the degree of tissue damage (SCHMIDT and SCHMIDT, 1976; KRISTOFERSSON et al., 1974; NEMCSÓK et al., 1981; NEMCSÓK and BOROSS, 1982). Transaminase (EC 2.6.1.1, EC 2.6.1.2), glutamate dehydrogenase (EC 1.4.1.3), and LDH (EC 1.1.1.27) indicate tissue necrosis and have long been used, mainly to reveal tissue necrosis in human diagnostics (WROBELSKI and LA DUE, 1985;

KRISTOFERSSON et al., 1974; ASZTALOS and NEMCSÓK, 1985). The activity of LDH in the blood plasma indicates the degree of general tissue damage , but its different izoenzyme units allow identification of the target organ (ASZTALOS and NEMCSÓK, 1985). For the identification of necrotized tissue, LDH izoenzyimes are utilized most often (as common general substances) in human diagnostics. In mammals, we can distinguish two enzymes: the M unit, which is characteristic of the skeletal muscle, and the H unit, characteristic of the myocardium. In fish there is an additional unit: the C unit, characteristic of the liver (MARKERT and FAULHABER, 1965; SHAKLE et al., 1973; GOLDBERG, 1965) An alteration in blood sugar concentration is suitable for the measurement of stress in the different organs (WEDEMYER, 1970). The environment; polluting materials cause appreciable damage in the nervous system of fish, e.g. through the cholinergic system. The inhibitor of acetylcholine esterase (AChE; EC 3.1.1.7) is extraordinarily dangerous because this can cause the mass death of fish as a consequence of spastic rigor (NEMCSÓK et al., 1981). Research relating to this enzyme in fish was begun later than in warm-blooded animals, and therefore fewer data are available. The majority of the references concern AChE in the electric organ of the electric eel and the ray. This enzyme has been purified, and its molecular structure and kinetic parameters have been determined (AUGUSTISSON, 1948, 1959 a, b; GAAL et al., 1980).

During their metabolism in the organism, certain compounds result in the production of free radicals. Physicians and chemists played an important role in the recognition of oxygen radicals and in the early studies into their role. Their biochemical significance was recognized in 1969 by MCCORD and FRIDOVICH, who demonstrated the role of superoxide dismutase (SOD) in dismuting superoxide. The enzymes involved in free radical reactions allow accommodation to the aerobic environment, the significance being similar to that of the immune system. SOD (EC 1.15.1.1), catalase (EC 1.11.1.6) and glutathione peroxidase (GP-ase; 1.11.1.9) play central role in the prevention of the damaging effects of free radicals.

At an early stage of environment pollution, it is usual for the intensity of motion of fish to decrease. They swim close to the water surface, slowly and in an uncoordinated way. The enzymes involved in the intensity of motion may have a central role in this, e.g. the Ca^{2+} ATP-ase in the sarcoplasmatic reticulum (ER) membrane system (EC 3.6.1.18). The main protein component in the ER membrane system is the Ca^{2+} transport ATP-ase with a molecular mass of 105 kD, which comprises 85% of the total membrane protein mass (MARTONOSI and BEELER, 1984). This high local concentration of the enzyme made its visualization technically possible with the use of an unmanipulated membrane preparation. To date, little information is available on ER Ca^{2+} ATP-ase in fish muscle, and especially its sensitivity to environmental pollution.

Aims and experimental approaches

With regard to the above-mentioned facts and problems in our Department of Biochemistry we have formed a group which carries out research relating to biochemistry in environment protection. Our aim is to study mainly enzymatic processes that indicate the damaging effects of environmental pollution on fish. We examine enzymes, and apply our introduce enzymological methods that indicate tissue necrosis and a damaged nervous system. Some of the fundamental research involves examination of the biochemical kinetic and structural characteristics of enzymes that can be used for biomonitoring, and the effects of anthropogenic compounds in changing these features.

We have initially concentrated on five major tasks:

1. Measurement of serum transaminase and LDH activity in order to determine the degree of tissue damage.

2. Study of the cholinergic system in order to detect the damaging processes in the nervous system.

3. Characterization of the antioxidative enzymes of fish in order to clarify their role in the elimination of free radicals induced by environmental pollution.

4. Research into the Ca^{2+} ATP-ase in the ER membrane in order to discover possible damaging effects on the biochemical process in the muscle.

5. Cytopathological research of the damaging effects of selected pesticie types with the help of light and electonmicroscopic methods.

In our experiments we used carp (*Cyprinus carpio*), which is a very widespread species in Hungary. In certain cases we compared the results on carp (mixed alimentation) with those on species with a different mode of life (carnivorous and herbivorous species).

We examined the effects of $CuSO_4$ (a known fungicide), methidation (MD); (a known insecticide) and paraquat (PQ); (a known insecticide) on fish via the above parameters. The effects of these three compounds were examined individually and also in combination. In certain cases, we analyzed the effects of the most important ecological factors (O_2 saturation, acidity and change in water temperature) on the toxicity of the pesticides. The changes in the biochemical parameters of the fish as functions of the chemical parameters of the water were then investigated in in-cage natural water experiments in order to forecast the expectable environmental pollution. The aim of all this work was to explain certain environment - damaging effects by means of molecular biochemical methods.

Summary

With the use of an enzyme - diagnostic method developed in 1979, the species - dependent, specific organ - damaging effects of the examined pesticides can be followed.

1. Our research revealed that the proportions of the LDH isoenzimes in the carp heart, the body muscle and the gill are not equal. Thus, $CuSO_4$ can be stated to cause necrosis of the heart and body muscle, causes necrosis of the gill, and MD causes necrosis of the body muscle. The serum transaminase and LDH activities demonstrated the largest degree of tissue necrosis in *Hypophthalmichthys molitrix* after $CuSO_4$ treatment. The tissue necrosis in the carp was moderate, while that in the catfish (carnivorous) was the least among the examined fish species. In certain cases where the three pesticides were used in combination at a sublethal concentration level ($CuSO_4$+MD, $CuSO_4$+PQ, or PQ+MD) the tissue - damaging effects revealed that the results of these treatments were additive. In vivo and vitro experiments with insecticides provided more evidence that compounds used in agriculture can cause damage to the biochemical processes of the organism in many ways, and thus are dangerous to the balance of the aquatic ecosystems. It has been proved that PQ and MD affect the oxygen-dependent pathways of the metabolism of fish through their ability to promote free radical production. Cu^{2+} similarly promotes free radical reactions, and can also increase the SOD activity if the Cu^{2+} concentration is high. The toxic effects evoked in fish by PQ through free radical production was decreased by selenium as antioxidant.

2. Certain ecological factors, either alone or combination with xenobiotics, can cause much damage to the fish organism, as proved by the molecular changes in enzymes. In trout, we found that the increasing acidity and the low O_2 concentration of the natural waters in Hungary enhance the tissue-damaging effect of $CuSO_4$ at a sublethal concentration, and also increases the stress load. A hypoxic environment increased the free radical production in fish. Free radicals are harmful for the whole body of the fish because of their damaging effects on the cell membrane. Our results indicate that high temperature, the ammonia concentration and a low level of O_2 have decisive (or primary) roles in the development of gill necrosis. The pathology of fish swimming bladder disorders caused by inflammation has not been clarified so far. Our results suggest that the elevated free radical and H_2O_2 production (indicated by the increased activity of antioxidative enzymes and increasing lipid peroxidation (LPO)) at the site of the inflammation may play an important part in the emergence of this illness. We found that MD inhibits AChE activity to different extents, depending on the season-dependent changes in water temperature. This permits a more exact forecast of how much damage the water temperature-dependent accumulation and metabolism of MD can cause to the nervous system of fish.

3. Our results demonstrated a close relation between the quantity of acetylcholine (ACh) and the activity of choline acetyl transferase (ChAT) in the brain of the examined species of fish. There is no connection between the AChE activity, the quantity of

ACh and the ChAT activity. This supports the observation that the ChAT activity is more characteristic of the cholinergic system than is the activity of AChE (CONTESTABILE, 1978). On the basis of the ChAT activities measured in the brain, three different groups can be established. The first group consist of the species with the highest ChAT activity, all these species having excellent visual orientation. They are those carnivorous fish species whose eyesight is of primary importance in the acquisition of food. For the carp, belonging in the second group, eyesight is only of secondary importance. The catfish and Ictalurus nebulosus, which belong in the third group, detect their food by touch. These data reinforce the supposition that the activity of ChAT in the fish brain is close by connected with the state of development of the eyesight, which depends on the mode of life of the fish (carnivorous or herbivorous).The activity of the ChAT in the trunk muscles (primarily involving cholinergic innervation) is highest in carp and trout. This can be explained in that these species move continuously while searching for food. The ChAT activity in the trunk muscle of carnivorous fish is relatively low, which means a reduced cholinergic innervation. The food acquisition of carnivorous fish is characterized by little movement. These fish usually lie in ambush, and the capture of the prey needs only a sudden short movement.

The isoelectric point of carp brain ChAT the Km values demonstrates that ChAT is a conservative enzyme: perhaps because of its important role in the organism, its basic biochemical features have not changed during millions of years, even in species far from each other phylogenetically.

The results of radioligand type binding experiments led to the first description of the predominance of the receptor types in the carp brain, as evidence of the ancient character and phylogenetic inferiority of the carp brain in comparison with the brain of mammals. Further evidence of this is the extraordinarily high proportion of the molecular subform A1, 2 of AChE.

4. There are differences in the distributions of SOD, catalase and GP-ase activity among the various organs, and the degree of LPO is not identical in different species of fish. This signals an existing connection between the extent of free radical reactions and the differences in their modes of life. This is connected with their metabolism, which evokes an accelerated growth in weight. The activities of antioxidative enzymes also increased, which means that the organism is not fully protected against LPO processes, even under normal physiological conditions. Membrane damage of little significance can occur constantly. This reinforces the role of free radicals in aging processes: in old age, not only do the activities of the enzymes of the immune system decline, but the repair processes deteriorate too. Damage to membranes and proteins of vital importance and nucleic acids can therefore cause disorder in the physiological functions.

5. Biochemical examination of SR Ca^{2+} ATP-ase demonstrates significant differences in the functional role of several segments of this enzyme and in their positions in the molecule in comparison with the similar enzymes of mammals. The examined pesticides inhibited the Ca^{2+} ATP-ase of carp specifically. It can therefore be supposed that various pesticides in sublethal concentrations decrease the intensity of motion-dependent reactions in connection with the acquisition of food and escape through inhibition of ER Ca^{2+} ATP-ase.

Results for utilization

1. The chosen biochemical parameters are suitable for the complex examination of the damage caused to fish populations by different chemical substances of agricultural and industrial origin.

2. Our methods are suitable for determination of the degree of danger that can result in the fish body in consequence of pollution.

3. Certain harmful biochemical and histological changes can be detected, as in the case of the mass death of eels in Lake Balaton in 1991.