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Effects of DDVP, Naphthalene, and Cadmium on Intestinal Proteolytic Activity in Mozambique Tilapia (*Oreochromis mossambicus* Peters)

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Widespread pollution of inland waterbodies stimulates intensive studies on effect of pollutants on different systems of hydrobionts. One of the poorly studied problem concerns the toxic effects upon digestive function in fish. Potentially, any toxic substance present in the environment may negatively influence any stage of the process of exotrophy, including the stage of digestion. The latter depends largely on the normal functioning of digestive enzymes. Direct or indirect effects of range of toxicants upon fish digestive enzymes have been reported (Gupta, Sastry 1981, Gill et al. 1991, Kuz'mina, Golovanova 1992, Golovanova et al. 1994,). Previous study on chronic effects of organophosphorous insecticide DDVP, polycyclic hydrocarbon naphthalene and heavy metal cadmium has shown that cadmium inhibited intestinal total amylolytic activity in Mozambique tilapia (*Oreochromis mossambicus*) (Golovanova et al. 1994). The main goal of the present study was to examine the chronic effects of the same toxicants on intestinal total proteolytic activity in tilapia.

MATERIALS AND METHODS

Six-months old Mozambique tilapias (average weight 6.2 g at the beginning of the experiment), obtained from the stock population raised in laboratory for several generations, were used in the study. The fish studied here were the same as in the experiment which protocol was described in more details earlier (Golovanova et al. 1994). Briefly, the separate groups of fish were exposed flow-through for 60 days to 0.46 mg/L DDVP [0,0-dimethyl-0-[2,2-dichlorovinyl] phosphate, technical grade, 80% of active ingredient), 1.5 mg/L naphthalene (96% pure technical grade) and 5.0 mg/L cadmium (cadmium sulphate, chemical grade). The fourth group kept in the flowing well water served as control. Tested concentrations were approximately equal to 1/15 of LC_{50} for tilapia (Frumin et al. 1992). The concentrations of tested toxicants were maintained in the flowing aquaria water at constant level using original diluter system (Pavlov, *unpublished*). Since we followed single pass flow-through protocol with the water exchange rate of about 4 volumes per day no repeat tests to account for the toxicant loss due to absorption on the tank walls, food and feces

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were ran. The exposure to toxicants was followed by 60-day recovery period in clean water. During the experiment the fish were fed *ad libitum* commercial trout chow containing 46 g crude protein, 20 g fat, 18 g carbohydrates, and 16 g ash /100 g.

The fish (N=6), sampled on days 15, 30, 45 and 60 of exposure and recovery periods, were decapitated, their intestines were removed, placed on ice and weighed. After this the thyme was also removed and weighed. According to the recommendation by Ugolev and co-workers (1969), in order to prevent contamination of the thyme by microvillar membrane attached proteolytic enzymes the thyme was removed from the incised intestine using very soft plastic spatula. All preparation procedures were performed at 0-4°C. Total proteolytic activity (activities of trypsin EC 3.4.21.4, chymotrypsin EC 3.4.21.1, and range of dipeptidases 3.4.13.1 - 3.4.13.11) was determined in homogenated intestinal tissues (consisting of mucosa, muscles and serose) and thyme by the modified method of Anson (1938). Casein (1%, chemical grade, NPO “Biolar” Latvia) was used as a substrate according to condition of the Anson’s technique. Substrate and homogenates were prepared on Ringer’s solution. The homogenates were incubated with substrate at 37°C for 30 min, and the reaction products were stained with Folin phenol reagent and read at 597 nm (spectrophotometer SF-46, LOMO, St. Petersburg, Russia). The protein content was determined using Lowry technique (Lowry et al. 1951). Specific total proteolytic activity (activity per 1 g of protein) and summary proteolytic activity (sum of the total proteolytic activities in the intestinal tissues and thyme) were calculated. Specific activity was calculated only for intestinal tissues since protein content in thyme is largely dependent on protein content in food. All trials were carried out in 2 replicates. The results of the study were statistically analyzed using ANOVA procedure, followed by Tukey’s Honestly Significant Difference Test for difference of means (Sokal and Rohlf 1995), at a 5% level of significance.

RESULTS AND DISCUSSION

DDVP and naphthalene exposures did not result in significant changes in total proteolytic activity in tilapia intestinal tissues (Table 1). Exposure to cadmium resulted in gradual decrease in the enzyme activity, statistically significant after 15 and 60 days of exposure (P=0.019 and P=0.0001, respectively). Specific activity decreased significantly (P=0.0008) only after 15 days of exposure to cadmium (Table 1). After termination of the exposure, during all recovery period, the proteolytic activity was insignificantly increased in the fish exposed to DDVP and naphthalene. All fish exposed to cadmium died at the end of recovery period. At the end of experiment specific proteolytic activities both in the control and in the fish exposed to DDVP and naphthalene did not differ significantly from the initial values. Total proteolytic activity in thyme varied considerably both in the control and exposed fish. Significant decrease in this activity was found only in the fish

exposed to cadmium (Table 1). It must be noted, that the fish exposed to this toxicant ceased feeding after first month of exposure and actually did not feed further. During the recovery period thyme total proteolytic activity remained unchanged in the fish exposed to DDVP. In the fish exposed to naphthalene it was slightly increased while in the fish exposed to cadmium it was absent (equal to zero). The changes of the summary proteolytic activity in the intestinal tissues and in thyme exhibited similar patterns. However, the effects of cadmium (decrease in activity) and naphthalene (increase in activity) were more pronounced. Thus, the activity of proteinases was significantly increased ($P=0.0001$) after 60 days of exposure to naphthalene, and significantly decreased after exposure to cadmium for 15, 45, 75 and 90 days ($P<0.00001$, $P=0.0003$, $P<0.00001$ and $P=0.0019$, respectively).

Studied toxicants, under exploited experimental conditions, affected the chains of carbohydrases (Golovanova et al.1994) and proteases by similar ways: DDVP had no effect, naphthalene caused slight increase, and cadmium induced marked decrease in activities. The trends of enzyme activity changes induced by naphthalene and cadmium exposures are especially evident in relation to data on thyme and summary activity of intestinal proteinases. Average thyme proteinase activities in the fish exposed for 60 days to naphthalene are 48.7% higher and to cadmium, 77% lower, comparing to the control. Summary intestinal proteolytic activities differ less: naphthalene exposure resulted in it 18.8% increase, cadmium exposure in 54.4% decrease comparing to control values. Studied toxicants affected the intestinal cavity enzymes to the higher extent than the intestinal tissues enzymes. The enzymatic activities in the intestinal tissues relate mainly to the processes of membrane and intercellular digestion taking place in mucosa. In the thyme, the enzymatic activities relate mainly to the intestinal cavity and cavital digestion. These facts with respect to the results of our study suggest that the enzymes of the pancreatic origin dominating in the intestinal cavity (Ugolev, Kuz'mina 1993) are more susceptible to toxic impacts.

The data presented here are in a good accordance with the data on the chronic negative effects of mercury and cadmium chlorides on the gut trypsin activity in the cattish (*Heteropneustes fossilis*) (Gupta, Sastry 1981). In our *in vitro* study significant decrease in total proteolytic activity in intestinal tissues was observed only at the highest tested cadmium (as cadmium sulphate) concentration of 50 mg/l in pike, while in other 12 species of freshwater teleosts even this concentration caused only statistically insignificant decrease in the enzyme activities (Kuz'mina, *unpublished)*. Consequently, we suggest that in the chronic experiments we observed not a direct molecular effect of cadmium upon the enzymes but most likely the indirect effects through whole fish organism. It was shown earlier that chronic cadmium exposure decreases the intensity of feeding in bream by suppression the motivation to feed (Gerassimov et al. 1991). The cessation of feeding noted in the present study suggests that the absence of substrate (i. e., food) could be the most important factor contributed to observed

Table 1. Effects of exposure to DDVP, naphthalene and cadmium, and of subsequent recovery on proteolytic activity in the intestinal tissues and thyme in Mozambique tilapia **(***Oreochromis mossambicus***).**

Toxicant	Day of experiment							
	15	30	45	60	75	90	105	120
Control								
IT	9.95±1.21	6.87±0.46	9.15±0.82	8.68±0.28	8.97±0.81	10.28±0.73	11.23±0.39	10.05±0.48
IS	101.7±8.70	84.00±6.30	76.20±6.80	77.70±2.20	n/d	n/d	80.00±9.40	85.30±4.60
ChT	9.35±0.51	5.55±1.82	7.58±1.59	2.58±1.70	9.77±0.23	6.75±2.27	7.32±2.40	11.03±0.66
Sum	19.30±1.43	12.43±2.10	16.72±2.02	11.27±1.57	18.70±0.92	17.03±2.61	18.57±2.42	21.07±0.75
DDVP								
IT	9.45±0.23	7.45±1.06	9.13±0.41	9.53±0.59	10.20±0.33	11.63±0.86	9.90±0.34	10.23±0.40
IS	92.50±6.5	93.00±15.2	84.70±7.1	95.50±12.0	n/d	n/d	91.80±8.80	90.20±5.20
ChT	9.70±0.20	7.45±2.71	2.97±1.88	4.42±1.99	9.57±0.61	8.27±1.74	10.58±0.68	9.47±0.36
Sum	19.15±0.41	15.25±3.51	12.10±2.05	13.93±2.06	19.78±0.48	17.85±3.65	20.48±0.87	15.70±0.08
Naphthalene								
IT	8.97±0.58	5.82±0.83	9.22±1.17	9.40±0.43	9.35±0.76	10.58±0.61	10.12±0.64	9.77±0.38
IS	76.70±9.8	70.50±10.9	93.20±12.9	86.20±9.70	n/d	n/d	87.00±6.9	82.50±4.20
ChT	9.47±0.26	10.20±0.90	9.40±0.35	8.57±0.84	9.10±0.28	11.68±0.45	5.48±2.64	9.92±0.39
Sum	18.43±0.46	16.00±1.27	18.60±1.31	17.98±1.24[1]	18.47±0.86	22.28±0.61	15.57±3.07	19.68±0.76
Cadmium								
IT	6.43±0.69[1]	5.35±0.99	7.10±0.52	5.43±0.79[1]	6.73±0.87	7.32±1.35	---	---
IS	49.20±6.1[1]	50.8±9.00	61.30±5.20	45.70±6.50	n/d	n/d	---	---
ChT	1.42±1.42[1,2]	1.45[3]	no chyme	no chyme	no chyme	no chyme	---	---
Sum	7.85±1.87[1]	6.80±2.01.	7.10±0.52	5.43±0.79	6.73±0.87[1]	7.32±1.35[1]	---	---

Values are presented as mean (activity in $\mu mol \cdot g^{-1} \cdot min^{-1}$)± SE; N=6. [1]- significantly different from control $P<0.05$; [2]- N=2; [3]- N= 1; n/d - not determined due to technical reasons; --- not determined because all fish were died. *Abbreviations used:* IT - total proteolytic activity in the intestinal tissues; IS - specific total proteolytic activity in the intestinal tissues; ChT - total proteolytic activity in thyme; Sum - summary proteolytic activity in the intestinal tissues and thyme.

enzymatic effects. That is, the "substrate regulation" of enzymatic activity (Gruzdkov et al. 1981) is presumably the main mechanism controlling the proteinase activities under the influence of toxicants tested in our study. As indirect proof of this presumption is the similarity in the effects of cadmium on the intestinal tissues proteolytic and on the specific activities (Table 1). Comparative inertness of the intestinal tissues is more likely due to long life of fish enterocytes and due to less pronounced influence of toxicants on the activity of the enzymes of the proteinase chain having intestinal origin. Considerable tolerance of tilapia digestive proteinases to the effects of toxicants of different natures (especially to DDVP and naphthalene) may contribute to the general tolerance of Mozambique tilapia to toxic impacts. Thus, the study revealed that tested toxicants under present experimental conditions have different effects on the level of total proteolytic activities in tilapia intestine. Cadmium has the most pronounced inhibitory effect, which to a high extent is due to decrease in the intensity of fish feeding.

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