

STRESS—INDUCED DYNAMICS OF FISH BRAIN
ACETYLCHOLINESTERASE ACTIVITY

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Introduction

Stress in fish has been studied quite intensively. A number of typical responses to stress induced by a variety of factors have been revealed (increase in catecholamines, cortisol and glucose, changes in blood constituents, etc). However, some aspects of stress in fish remain unclear. One of them is the responses to stress of the cholinergic system. Previous studies on this issue gave, in many cases, contradictory results. For example, both immobilisation and thermal shock resulting in development of typical stress response in common carp led to the activation of the cholinergic system as revealed by the increase in activity of its marker enzyme, the acetylcholinesterase (AChE) (Jurkowski et al., 1979). As opposite, in silver carp (*Hypophthalmichthys molitrix*) and in perch (*Perca fluviatilis*) handling and hypoxia resulted in the brain AChE activity decrease, while in the carp heart and liver the activity fluctuated markedly (Krepis et al., 1989). Exposure of the common carp (*Cyprinus carpio*) to non-cholinergic toxicants, lead and phenol, resulted in the increase the AChE activity, while cyanobacterial toxins caused a sharp decrease in moribund perch (Malyarevskaya, 1979; Tishinova-Nanova and Muleshkova, 1984; Kozlovskaya and Martemyanov, 1991). It must be noted that the comparison of the results of these works is rather incorrect because different experimental protocols, variety of fish species and stressors were used.

The main goal of this paper is to summarize original data on the dynamics of the fish brain AChE in response to stress, caused by changes of water salinity, pH, dissolved oxygen and by exposure to cadmium and naphthalene .

Materials and Methods

In order to obtain comparable data, standardized experimental protocols were strictly followed in all trials. All fish were pre-adapted to experimental conditions for at least 10 days. Perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) were raised from the eggs of parent fish caught in the wild. The fry were then grown in the ponds during summer, transferred to laboratory tanks in the fall and used in experiments during winter. Mozambique tilapia (*Oreochromis mossambicus* Peters) were

obtained from the parent fish kept in laboratory conditions for several generations. No mortality occurred either before the experiments or in control. Well water (pH 7.6-8.0, Ca⁺⁺ content about 40 mg/L, oxygen near the saturation level) was used for experiments and control. The optimal water temperature was maintained for the studied fish (17-19°C for roach and perch, 24-26°C for tilapia). In the salinity experiments, the fish were transferred from the fresh water to the sodium chloride solutions. In the acidity study, well water was acidified with sulphuric acid and a constant pH of 4.5 was kept using an automated titration unit. To induce hypoxic conditions, the artificial aeration was ceased, after which the dissolved oxygen level in experimental aquaria decreased quite fast (from 8-8.5 mg/L to 2.5-3.5 mg./L for 2-3 h). For short- and long-term toxicity experiments, static-replacement and flow-through protocols, respectively were used. Naphthalene (predissolved in acetone) and cadmium sulfate were introduced in the aquarium water directly or via the original diluter system. AChE activity was measured in mid-brain homogenates according to technique by Ellman (Ellman et al., 1961). Either Student's t-test or ANOVA procedure was used to evaluate the differences between assays.

Results and Discussion

It was shown earlier that changes in water salinity, pH and oxygen concentration lead to the development of stress response in different fish species. In this study it was revealed that these stressors induced alterations of fish brain AChE activity.

The effect of stress induced by the change in water salinity has been studied in euryhaline Mozambique tilapia and in stenohaline perch. Direct transfer of tilapia from the fresh water to the 20 and 30 g/L salt water and of perch to the 12 g/L resulted in 100% fish mortality during 2 to 3 days. Such acute stress resulted in a sharp decrease of brain AChE in moribund fish in both species (up to 75% inhibition in tilapia and up to 70.8% in perch). Another pattern of the AChE dynamics was exhibited in fish transferred to the lower salinity water (Fig. 1). In both species the stress caused a significant increase in the enzyme activity followed by the recovery to the control level after 15 days of adaptation. Transfer of the acclimated fish back to fresh water induced an AChE activity change of a similar character. A sharp decrease in water pH (from 8.1 to 4.5) resulted in about 10% mortality in tilapia and 4% in perch. In the surviving fish, acidic stress caused significant enzyme activation (up to 21% in perch and 33% in tilapia) followed by the return to

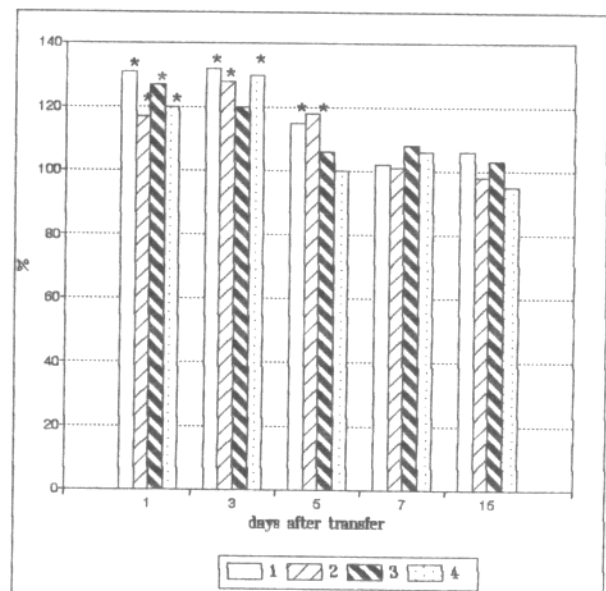


Figure 1. AChE activity (% of control) in tilapia and perch after transfer from fresh to salt water and back.

1. tilapia, transfer to 16 g.l⁻¹ water
2. tilapia, transfer back
3. perch, transfer to 6 g.l⁻¹
4. perch, transfer back

* - significantly different from control

control level after 7 days of acclimation (Fig. 2). Similar phenomenon was observed in the field study carried out in acidified lakes of Darwin National Reserve (Russia). In these lakes, fast acidification (coinciding with the snow melt) has also resulted in the perch brain AChE activation (Pavlov, unpublished). Hypoxic stress affected perch brain AChE activity by a similar manner: significant activity increase (18.1% above control) and following recovery (Fig. 2). Toxic stress in our study was induced by exposure of fish to cadmium, naphthalene and their mixtures. Exposure of fish to these toxicants alone and in mixtures has led to AChE activity fluctuations similar to those induced by other studied stressors. That is, AChE activity peaked at the beginning of the exposure and then turned back to pre-exposure level (Table 1). Similar changes of the enzyme activity have been revealed in carp exposed to lead and phenol, and in rosy barb (*Barbus conchoni*) exposed to lead (Tishinova-Nanova & Muleshkova, 1984; Kozlovskaya & Martemyanov, 1991; Gill et al, 1991).

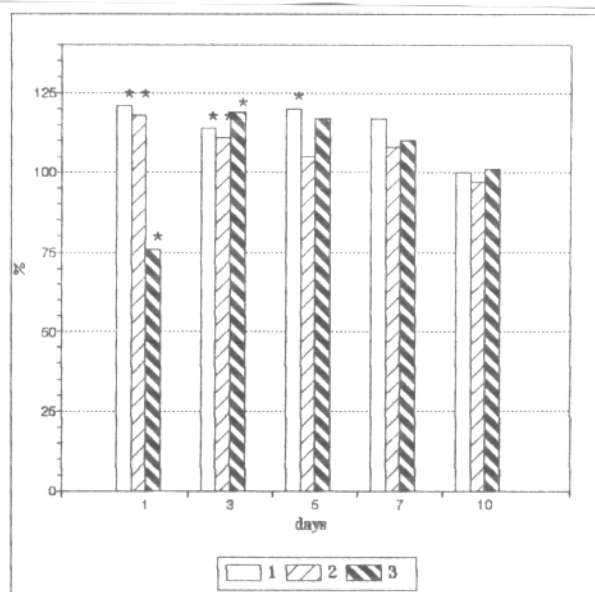


Figure 2. Brain AChE activity (% of control) in perch and roach exposed to low pH and hypoxic stresses.

1. perch, transfer from pH 8.1 to pH 4.5 water

2. perch, hypoxia

3. roach, hypoxia

* - significantly different from control

If the fish were exposed to one toxicant and then another one was added to the experimental tank, this resulted again in transient increase in the enzyme activity. Similar results occurred to the AChE dynamics when fish being chronically exposed to toxicants or their mixtures were transferred back to the clean water. Such transfer was obviously stressful and the enzyme activity fluctuated by a manner similar to that found at the beginning of toxicants exposure. The effect of hypoxic stress has also been studied in combination with simultaneous exposure to the toxicant mixture. The roaches in this experiment were pre-exposed to a cadmium/naphthalene mixture for 15 days. By that time AChE activity in exposed and in control fish did not differ significantly. Sharp decrease of dissolved oxygen content in experimental toxic solution (from 6.8-7.0 to 2.4-3.0 mg/L) caused no mortality. However, 1 day after exposure to hypoxic conditions, brain AChE activity increased (up to 28.8%-34.0% compared to the control) and then (3 to 7 days after) turned back to pre-exposure level.

It was shown that *in vitro* cadmium inhibits AChE (Olson and Christensen, 1980). However, in our previous study chronic exposure of bream (*Abramis brama*) and tilapia for 30 and 60 days, respectively, did not cause a decrease in the AChE activity (Pavlov et al., 1990; Pavlov, 1994). Moreover, at the beginning of the exposure, an increase in the enzyme activity was noted. In tilapia the comparison of the dynamics of the AChE activity and cadmium accumulation has shown that the patterns of these processes did not coincide: if cadmium body burden gradually increased during

the exposure period, the AChE activity fluctuated and was described by the S-shape curve (Pavlov, 1994). Naphthalene has actually no effect on the AChE activity *in vitro* at all (Pavlov, 1994). That proves that toxicants chosen are non-specific against the AChE and hence their effects upon the enzyme *in vivo* could be attributed to stress.

Table 1. Brain AChE activity in roach exposed to cadmium, naphthalene and their mixtures.

Exposure days	Control	Cadmium, mg/L 8.0	Naphthalene, mg/L 0.6	8.0 mg-L ⁻¹ Cd + 0.6 mg/L Nap	4.0 mg-L ⁻¹ Cd + 0.4 mg/L Nap.
1	1270.7± ^a 53.0	1482.0± ^b 74.8	1497.1± ^b 72.5	1665.4± ^b 42.1	1512.6± ^b 34.8
3	1318.5± 47.7	1507.9± ^b 31.8	1612.1± ^b 81.4	1598.9± ^b 67.0	1298.2± 67.3
5	1295.4± 56.4	1657.8± ^b 48.7	1712.0± ^b 54.7	1321.9± 61.7	1301.0± 46.2
7	1192.8± 154.6	1733.6± ^b 88.1	1654.9± ^b 79.8	1317.6± 87.1	1391.5± 64.7
10	1281.4± 65.9	1532.9± 98.2	1445.1± 99.9	1198.9± 101.3	1239.3± 65.4

^a - activity (umol AChJ/g tissue/h), average± S.E. N=10

^b - significantly different from control

Table 2. Brain AChE activity in roach chronically pre-exposed to cadmium after addition of naphthalene.

Duration, days	Control	Cd 8.0 mg/L added 0.6 mg/L naphthalene	Cd 4.0 mg/L added 0.4 mg/L naphthalene	Cd 2.0 mg/L added 0.2 mg/L naphthalene
1	1284.9±42.9 ^a	1576.6±82.9 ^b	1539.2±136.0 ^b	1483.6±69.1 ^b
3	1199.8±30.8	1709.2±82.7 ^b	1673.6±78.1 ^b	1691.3±63.8 ^b
5	1197.1±33.0	1653.0±93.4 ^b	1200.3±37.2	1117.8±83.2
7	1072.7±64.2	1130.3±87.5	1263.8±44.2	1206.8±38.1

^a - activity (umol AChJ/g tissue/h), average± S.E. N=8

^b - significantly different from control

The data given in the present study show that the patterns of the AChE activity dynamics in response to different stressors were, in general, similar to each other irrespective neither to the nature of a stressor nor to species of fish belonging to different systematic groups. These patterns resemble

Selye's classic S-shaped curve of the developing "General Adaptation Syndrome" (Selye, 1950). Characteristic increase of the AChE activity during the beginning of stress impact reflects Selye's phase of "mobilization," during which the increase in catecholamine levels in blood is the most typical physiological response. Previously we revealed that adrenaline injection simulating the above response causes not only the development of typical stress-related physiological processes in perch (such as transient hyperglycaemia) but AChE activity fluctuations similar to that observed in this study as well (Pavlov et al., 1994). Similar effects have been observed in rats (Maslova & Reznik, 1971). In rats, the AChE activation is attributed to the induction of adenylate and guanylate cyclases by adrenaline. It leads to an increase in protein synthesis, due to activation of secondary messengers, the cAMP and the cGMP (Elayev, 1985). The likeness of these processes in mammals and fish is proved by adrenaline-induced increase in brain protein content and simultaneous AChE activation found in perch (Pavlov et al., 1994). Thus, it could be suggested that stress-induced fluctuations of brain AChE activity in fish are of a non-specific nature and reflect changes in protein synthesis in adapting animals to changed conditions.

As it is shown in Fig. 2 in some cases the patterns of stress-induced AChE activity dynamic were slightly different from others: one day after exposure to stressor the activity decreased, then increased and, finally returned to the pre-exposure level. If stress-induced AChE dynamic does really obey Selye's scheme of developing stress response then it explains decrease of enzyme activity found in these cases: this decrease reflects the "alarm" phase. As it was noted above, revealed general pictures of the AChE responses to stress were common. Peculiarities found in different experiments (time and duration of phases, magnitudes of fluctuations) more likely relate to such factors as differences in fish physiological states, "power" and, perhaps, nature of a stressor.

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