Ion Transport and Its Adrenergic Regulation in Erythrocyte Membranes of the Bream *Abramis brama* Injected with a Mixture of Polychlorinated Biphenyls

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In the first 10 days after the injection of Sovol-54 (a mixture of chlorinated biphenyls) into the bream *Abramis brama*, considerable alterations in the hormonal regulation of cotransport were observed for erythrocytes of this fish, along with an increase in the passive permeability of their membranes. No significant changes were recorded in Na^+,K^+-ATPase activity or its adrenergic regulation.

Key Words: *polychlorinated biphenyls; fish erythrocytes; ion transport; adrenergic regulation*

Polychlorinated biphenyls (PCB) are among the more hazardous pollutants of aquatic ecosystems. They can induce carcinogenesis and mutagenesis in a variety of organisms, apparently not only by acting on genetic material, but also by modifying cell membrane properties such as those responsible for ion transport and signal transduction via transmembrane signaling systems [1,7]. In an earlier study we found that Na^+,K^+-ATPase activation was inhibited by norepinephrine in erythrocytes of a carp injected with a mixture of Russian-made PCB [2], but did not establish whether this resulted from a "breakdown" of β-receptors or was associated with a reduction in the activity of adenylate cyclase and protein kinases or in the basal level of cAMP in the cells. In the study described here we examined ion transport and its adrenergic regulation in erythrocytes of the bream *Abramis brama L.* injected with Sovol-54 (a mixture of PCB). In addition to testing the β-receptor agonist norepinephrine for its effect on Na^+,K^+-ATPase, we evaluated the impact on this agonist of the adenylate cyclase activator forskolin and of the protein kinase A activator dibutyryl cAMP.

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MATERIALS AND METHODS

Bream were caught with a sweep net in the Rybinsk Reservoir near Moscow, transferred to ponds, and allowed to adapt to the new environment for 3 weeks. A total of 20 bream aged 6-9 years from one spawning stock were used in the experiment. Test fish were injected with Sovol-54 intraperitoneally in a single dose of 150 mg/kg. Sovol-54 was dissolved in olive oil before use. Control fish were injected with pure olive oil. The injection volume did not exceed 1 ml.

On days 5 and 10 postinjection, the tail was cut off and blood was collected into test tubes moistened with a phosphate-buffered heparin solution, pH 7.4 (1000 units/ml). Blood samples were stored for 12 to 48 h at 3-5°C.

The operation of ion-transporting systems was evaluated after the incubation of isolated erythrocytes in a medium balanced for ionic composition and pH and containing 0.8-1.2 μCi/ml of a radioactive potassium analog and inhibitors of ion-transporting systems ouabain and furosemide. Na^+,K^+-ATPase

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TABLE 1. ^{86}Rb Entry (mmol/liter×h) into Bream Erythrocytes and Its Adrenergic Regulation 5 Days after Sovol−54 Injection ($M \pm m$)

Ion-transporting system and inhibitor	Regulation	Control group (n=5)	Sovol-treated group, 150 mg/kg (n=5)
Na^+,K^+−ATPase, 0.2 mM ouabain	−	2.728±1.53	3.127±1.73
	Norepinephrine, 10^{-6} M	3.915±0.85 (44)	4.272±0.91 (37)
	Forskolin, 10^{-4} M	4.011±0.48 (47)	4.112±0.52 (31)
	Dibutyryl cAMP, 10^{-4} M	2.550±0.32 (0)	3.005±0.41 (0)
Cotransport, 0.5 mM furosemide	−	0.538±0.39	0.376±0.25
	Norepinephrine, 10^{-6} M	0.826±0.74 (54)	0.764±0.3 (103)
"Passive" membrane permeability	−	0.515±0.22	0.447±0.18
	Norepinephrine, 10^{-6} M	1.106±0.67 (115)	1.724±0.5 (286)

Note. Here and in Table 2: figures in parentheses are percentage increments of ^{86}Rb entry.

activity was assessed by the magnitude of the ouabain-inhibited component of ^{86}Rb entry into cells, while the activity of Na^+,K^+,Cl^- cotransport and the furosemide-inhibited part of Na^+,Cl^- and K^+,Cl^- cotransport (designated below by the general term "cotransport") was assessed by the magnitude of the furosemide-inhibited component of ^{86}Rb entry. Since passive diffusion constitutes the basis of the ouabain- and furosemide-inhibitable ^{86}Rb entry, we evaluated the "passive" cell membrane permeability for cations by recording the magnitude of this flow [4].

In order to restore ionic homeostasis in the cells and ensure normal functioning of the ion-transporting systems after the storage of blood samples, erythrocytes were preincubated in the medium without ^{86}Rb for 60 min at 20°C. The procedures used for isolating, incubating, and fixation the erythrocytes, for determining their radioactivity, and for calculating the rates at which the ion-transporting systems operated were described previously [4]; the incubation medium had a temperature of 20°C and a pH of 7.4. In addition, the activity of ion-transporting systems was measured during exposure to the β-receptor agonist norepinephrine, the adenylate cyclase activator forskolin, and the protein kinase A activator dibutyryl cAMP. All inhibitors and activators were used in concentrations (shown in the tables) at which the expected effects were maximal [4]. Because of the small sample size, we considered as statistically significant more than 50% differences of the means from control values if the standard deviations were comparable.

RESULTS

Data on the activity of ion-transporting systems and their adrenergic regulation in bream erythrocytes 5 days after Sovol-54 injection are presented in Table 1. The test and control groups did not differ significantly in Na^+,K^+-ATPase activity. After the β-receptor agonist norepinephrine and the adenylate cyclase activator forskolin were added to the incubation medium, the work of the carrier increased 1.5 to 2.5 times in both groups; no significant deviations from control values were detected in the Sovol-54-injected group (Table 1). Nor did the addition of the protein kinase A activator dibutyryl cAMP alter Na^+,K^+-ATPase activity significantly.

In the Sovol-54-injected breams, the level of furosemide-inhibited cotransport was 1.43 times lower than in the control fish and rose much more under exposure to norepinephrine (2.03 times *vs.* 1.54 times in the control group) (Table 1).

The two groups did not differ significantly with regard to the "passive" ^{86}Rb diffusion into erythrocytes, but the Sovol-54-treated group showed a much greater increase in ^{86}Rb diffusion (3.86-fold *vs.* 2.15-fold-fold in the control group) when norepinephrine was added to the incubation medium (Table 1).

TABLE 2. ^{86}Rb Entry (mmol/liter×h) into Bream Erythrocytes and Its Adrenergic Regulation 10 days after Sovol−54 Injection ($M \pm m$)

Ion-transporting system and inhibitor	Regulation	Control group (n=5)	Sovol-treated group, 150 mg/kg (n=5)
Na^+,K^+−ATPase, 0.2 mM ouabain	−	1.537±1.31	1.229±0.71
	Norepinephrine, 10^{-6} M	2.464±1.4 (60)	2.026±1.28 (65)
	Forskolin, 10^{-4} M	2.158±1.25 (40)	2.124±1.24 (73)
	Dibutyryl cAMP, 10^{-4} M	1.594±1.36 (0)	1.296±0.58 (0)
Cotransport, 0.5 mM furosemide	−	0.613±0.2	0.607±0.34
	Norepinephrine, 10^{-6} M	0.922±0.5 (50)	1.418±0.56 (134)
"Passive" membrane permeability	−	0.541±0.22	0.740±0.35
	Norepinephrine, 10^{-6} M	1.779±0.3 (229)	1.951±0.47 (164)

Table 2 presents data on the activity of ion-transporting systems and their adrenergic regulation 10 days after Sovol-54 injection. At this time, the intergroup differences were insignificant, both in the presence of norepinephrine and forskolin in the incubation medium and in their absence, but cotransport in the erythrocytes of Sovol-54-injected bream was activated by norepinephrine much more strongly than in those of control fish (2.34-fold *vs.* 1.5-fold), and "passive" erythrocyte membrane permeability was also much higher (by a factor of 1.37) in the test group (Table 2).

The findings indicate that Sovol-54 alters the pattern of ion transport in the first few days after entering the body. The dose used in this study (150 mg/kg) is 3 times as high as the one found to maximize induction of the microsomal oxidation system in carp liver [3] and far exceeds the PCB concentrations capable of eliciting carcinogenesis in fish tissues [5]. However, no depression of the system responsible for adrenergic regulation of Na^+,K^+-ATPase was recorded. This is at variance with the findings from our laboratory obtained 3 weeks after injecting carp with a Sovol-54 dose of 50 mg/kg [2]. The degree to which this system is inhibited appears to be determined by the duration of exposure. It may be that its modification under the influence of PCB is one of the body's nonspecific responses to stressors.

Na^+,K^+-ATPase activity in the erythrocytes of the Sovol-54-injected group did not differ significantly from that in the control group, but the regulation of cotransport by norepinephrine in the test group underwent considerable alteration and, in addition, the passive membrane permeability for ions was increased in this group.

After 5 and 10 days of exposure to Sovol-54, norepinephrine activated the cotransport to erythrocytes much better than it did in the control group. In the vast majority of fish species that have been tested, increases in cotransport rates have been accompanied by adrenergic swelling of erythrocytes [4,6,8]. Sovol-54 probably reduces cell membrane microviscosity and allows erythrocytes to increase their volume during adrenergic swelling. Under these circumstances, the rate of cotransport to these cells may rise.

The "passive" erythrocyte membrane permeability in the Sovol-54-treated group did not differ from that in the control group after 5 days of exposure but had increased 1.66 times by day 10 in the former group while remaining unchanged in the latter.

This study has thus provided evidence for a high sensitivity of ion-transporting systems to PCB such as Sovol-54. The functions of these systems undergo changes just a few days after the xenobiotic enters the organism. The cyclase system of signal transmission from β-receptors to proteins that are responsible for active ion transport is not modified over the 10 days after Sovol-54 injection. Disturbances of the cell membrane properties and functions studied may substantially alter the normal course of cell activity and may be an additional factor contributing to malignant change of the tissue.

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