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FULL LENGTH ARTICLE

Cross talk between arsenic and cold on the regulation of inorganic phosphate level in peripheral tissues of fresh water fishes (*Channa punctata*)

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Abstract Cold acclimation shows the increased Pi in skeletal muscle of *Channa punctata* variety of fishes after 1 h and 2 h while reduces at prolonged exposure (4 h). Similar stimulatory effects were observed in heart, however, reduced at 30 min and 4 h and in liver it causes prevention of Pi release after 30 min, 1 h, 2 h and 4 h respectively. In gastrointestinal tract, the effects were pronounced whenever the fishes were exposed to cold for 1 h and 2 h, while reduced activity was demonstrated after 4 h of the treatment. To clarify the role of arsenic on cold-induced Pi release, fishes were exposed to Na_2HAsO_4 which reduced the effect in skeletal muscle, gastrointestinal tract and heart effectively and significantly. Whenever the fishes were exposed to cold with arsenic, the amount of Pi was also reduced than the control. In liver of arsenic treated fishes, the increased results were found while in cold, the values were reduced again in presence of arsenic compared to control and cold exposed fishes. Our findings give a new insight for the regulation of adaptive response tissue specifically and differentially and arsenic might be involved in cross talk through impairment of the cold-induced effect.

1. Introduction

Environmental low temperature is believed to be involved in the activation of sympathetic nervous system (Leduc, 1961) and stimulates the higher blood flow (Adán et al., 1994), therefore, metabolism of blood and cellular electrolyte might be influenced. However, auto regulatory process of the biosystem is common to all organisms by which they survive in the atmosphere, moreover, to survive in such low temperature; alteration of metabolic activities is substantial. Cold exposure is a stressful event that elicits different thermogenic adaptive responses in endotherms and exotherms. In mammals, including humans, the physiological responses involve changes in energy expenditure, heat production and dissipation, physical activity and appetite (Lowel and Spiegelman, 2000). In rodents, shivering, activation of the sympathetic axis (Spiegelman and Flier, 2001) with remarkable activity of mitochondrial uncoupling proteins (UCPs) (Golozoboubova et al., 2001) was reported as pivotal mechanisms.

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Channa punctatus is generally found in fresh water of haor, bil, river in Bangladesh. They are much energetic and survive in the critical circumstances for long time. They are the major sources of protein in the diet for human being. It is assumed that the higher energy content of this fish is caused by the increased activity of the sympathetic nerves. During environmental low temperature, it is assumed that the peripheral tissues might be involved critically and coordinately on the regulation of metabolites to survive in the atmosphere. Although fishes are exposed to various environmental stimuli, the species wants to maintain the homeostasis of the body. Adaptive thermogenesis, the dissipation of energy in the form of heat in response to external stimuli, has been implicated in the regulation of energy balance and body temperature. In shivering thermogenesis, because of the higher oxidative process, generation of adenosine triphosphate rather than UCP is predominant and hydrolysis of ATP yields energy for living in the atmosphere. Therefore, it is generally accepted that the organisms survive in the critical environment by different mechanisms and varies species to species. However, the mechanism involving the adaptive response in this species is not clarified. The peripheral tissues play the important roles in metabolic regulation. The enhanced nerve activity in response to cold is involved in regulation of metabolic activities in skeletal muscle, heart, liver, gastrointestinal tract (GIT) as well as in other peripheral tissues. For example, the increased nerve activity in liver has been involved in changes of degradation of cellular ATP (Westfall et al., 2000). Moreover, liver glycogen metabolism is influenced in response to cold. Higher degradation of liver glycogen releases energy available for doing mechanical work and survive in the critical circumstances and environment.

Arsenic is toxic to the living organisms. Prolonged exposure of arsenic has detrimental effects in tissues. It may impair the glycolysis as well as the oxidative processes (Tchounwou et al., 2003) and causes different types of pathogenic syndromes in rodents, fishes and other organisms. Exposure of higher concentration of arsenic in water may also cause severe effects in fish and might be involved in producing cancer or other cellular effects. However, the mechanism underlying the effects of acute arsenic exposure on the regulation of oxidative and glycolytic processes in tissues of fishes exposed to cold is not known. Arsenic is classified as a human carcinogen based on several epidemiological studies showing an association of arsenic exposure with cancers in lung, bladder, kidney and liver (Hughes, 2002; Tchounwou et al., 2003). Moreover, fish have long been used as sentinels for biomonitoring of aquatic environmental pollutants and are good indicators of arsenic toxicity (Tisler and Zagorc-Koncan, 2002). The regulation of metabolic activities in peripheral tissues in response to the changes of temperature is an important aspect in fish and to clarify the role of arsenic in cold-induced metabolic functions responsible for survive of the species of fishes in the environment, the current protocol was considered.

2. Materials and methods

2.1. Fishes and cold acclimation

Channa punctatus (Taki fish) weighing 50–60 g were used. They were maintained in normal water with ambient temperature (24.5 °C). In the day of experiment, cold exposure (4–8 °C) was given to the different groups of fishes in the cold chamber for 30 min, 1 h, 2 h and 4 h period with full aeration and with free access of water. After cold exposure treatment, fishes were quickly decapitated and the peripheral tissues including skeletal muscle from the dorsal part, liver, heart and gastrointestinal tract (GIT) were sampled carefully and taken weight by digital balance (Chyo, JL-180, China) and kept at −20 °C. Control fishes were similarly used for sampling of tissues except giving cold exposure.

2.2. Arsenic treatment

To examine the role of arsenic on the regulation of metabolic activities in liver, skeletal muscle, heart and gastrointestinal tract (GIT), groups of fishes were exposed with arsenic compound (10 mM Na_2HAsO_4 $7H_2O$, BDH Chemical Ltd.) in cold for 1 h. The respective other groups of fishes were treated with only 10 mM of arsenic compound (Na_2HAsO_4) for 1 h in ambient temperature. The tissues were sampled after the treatment similarly as mentioned above.

2.3. Assay of inorganic phosphate (Pi)

Tissues were homogenized with pre-cooled water and were centrifuged at 8000 rpm for 10 min. The supernatants from each tissue homogenate were used as crude extract for assay of inorganic phosphate (Pi) described by Ramnik (1999). 200 μL tissue extract was diluted to 5 mL with water and was mixed vigorously with 5 mL of 5% TCA (trichloroacetic acid) and centrifuged at 6000 rpm for 10 min. 5 mL supernatant was transferred to another tube and kept on ice. 1 mL molybdate reagent (10 g of ammonium molybdate in 100 mL water was taken and 100 mL of 5 N H_2SO_4 was added to prepare 200 mL solutions) was added and mixed. The solution was mixed with 0.4 mL aminonaptholsulphonic acid reagent. 3.6 mL water was added and after mixing, the tube was kept standing for 10 min for the complete development of color. For blank, 5 mL of 5% TCA and 5 mL water were mixed only. Absorbance was taken at 690 nm against the blank. The Pi in tissue extract was calculated using standard KH_2PO_4 solution.

2.4. Statistical analysis

Results of the experiments were expressed as mean and standard error of different groups. The differences between the

Table 1 Changes of inorganic phosphate in skeletal muscle of fishes exposed to cold for 30 min, 1 h and 2 h and 4 h in the cold chamber.

Treatments	Inorganic phosphate (Pi) (mg/100 g of tissue weight)
Control	3.87 ± 0.37
30 min	2.50 ± 0.19
1 h	9.01 ± 0.40[a]
2 h	4.78 ± 0.20[b]
4 h	1.08 ± 0.07[c]

The data are means ± SE for four fishes in each group.
[a] $P < 0.001$ versus control.
[b] $P < 0.05$ versus control.
[c] $P < 0.01$ versus control.