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FULL LENGTH ARTICLE

Regulatory mechanism on enhancing protein synthesis in skeletal muscles of cold exposed fresh water fish (*Channa punctata*)

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Abstract *Channa punctata* varieties of fish are energetic and survive in critical environment although the molecular mechanism is not known. They were exposed to cold (4–8 °C) for 30 min, 1 h, 2 h and 4 h and the total protein contents in the liver were not significantly changed up to 4 h of cold exposure while a significantly increased protein level in the skeletal muscle was noted and maximal at 2 h. Groups of fish were exposed to Na_2HAsO_4 to examine its role on cold-induced protein synthesis in the skeletal muscle and the increased protein in the skeletal muscle was reduced significantly. The results appear to indicate that cold acclimation induces a metabolic change involving cellular protein content tissue specifically and arsenic might be involved in impairment of the cold-induced effect. To clarify the molecular mechanism, groups of fish exposed to cold for 1 h and 2 h had significantly increased RNA in the skeletal muscle compared to control fish, however, a higher level was found after 2 h of treatment and the enhanced RNA induced by cold was almost completely prevented by Na_2HAsO_4. Our findings will give a new insight into the survival process of this species while toxic arsenic prevents this cellular bioprocess.

1. Introduction

Temperature fluctuation is a common phenomenon of the atmosphere and is involved in changes of various metabolic functions. For example, low temperature has been recognized as a major environmental sympathetic stimulus and 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

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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) (Boss et al., 2000; Golozoboubova et al., 2001) was reported as a pivotal mechanism. The greater the UCP concentration, the greater the capacity to uncouple mitochondrial oxidative phosphorylation so that heat is produced.

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. Peripheral tissue metabolism is affected by both environmental and chemical stimuli; however, endogenous auto regulation of metabolic processes of all species is a common biological process. Degradation of biomolecules as well as biosynthesis is the characteristics of metabolic processes. Among the peripheral tissues, the skeletal muscle and the liver play a great role in metabolic regulation. The metabolic functions in these tissues are influenced by both environmental and chemical stimuli. Liver glycogenolysis is a metabolic process yielding energy for doing mechanical work and the process is enhanced upon activation of the sympathetic nervous system. The skeletal muscle comprises both oxidative and glycolytic fibers and is therefore, metabolically important. Both adrenergic and nor-adrenergic nerves fibers are predominant in this tissue. Therefore, it is speculated that cold exposure would have effect in the regulation of metabolic functions through activation of these nerves. Although fish 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 ATP rather than UCP is predominant and hydrolysis of ATP yields energy useful for doing mechanical work and for living in the atmosphere. However, the molecular mechanism involving the adaptive response for this species is not clarified.

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, fish 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 fish 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). Both cold and toxic arsenic make a critical environment where the fish survive, however, the mechanism underlying the survival process is not clarified.

2. Materials and methods

2.1. Fish

C. punctatus weighing 50–60 g were used and maintained in normal water with ambient temperature (25.0 ± 1 °C). On the day of experiment, different groups of fish were exposed to cold (4–8 °C) in the cold chamber for 30 min, 1 h, 2 h and 4 h period with full aeration and with free access to water. After cold exposure treatment, fish were quickly decapitated and the peripheral tissues including the skeletal muscle from the dorsal part and the liver were sampled carefully and weighed by a digital balance (Chyo, JL-180, China) and kept at −20 °C. Control fish were similarly used for sampling of tissues except cold exposure.

2.2. Arsenic treatment

To examine the role of arsenic on the regulation of metabolic activity involving the amount of protein and RNA in the skeletal muscle, groups of fish were exposed with arsenic compound (100 mM Na_2HAsO_4. $7H_2O$, BDH Chemical Ltd.) in cold for 1 h and 2 h. The respective other group of fish was treated with only 100 mM of Na_2HAsO_4 for 1 h in ambient temperature for determination of protein only. The tissues were sampled after the treatment similarly as mentioned above.

2.3. Assay of tissue protein content

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 protein by using 50 μL extract. The protein content in tissue was determined by the procedure of Lowry et al. (1951). Briefly, an alkaline solution was prepared by mixing 50 mL of alkaline Na_2CO_3 solution (2% Na_2CO_3 in 0.1 N NaOH) and 1.0 mL of copper–sodium potassium tartarate solution (1 g sodium potassium tartarate and 0.5 g $CuSO_4$. $5H_2O$ were dissolved in 100 mL distilled water). Fifty microliters of tissue extract was taken in the test tube and made up to 1 mL with distilled water. For blank, 1 mL water was used in place of tissue extract. Five milliliters of alkaline solution was added to each tube and mixed well. The tubes were allowed to stand for 10 min at room temperature and 0.5 mL of diluted FCR (Commercial FCR was diluted with equal volume of water) was added and mixed well. After 30 min, the absorbance was taken at 650 nm against the blank. The protein content in each tissue was calculated from the standard graph of bovine albumin (1 mg/mL) and is expressed as g/100 g of tissue weight.

2.4. Estimation of RNA content

The RNA of skeletal muscle was estimated by the phenol–chloroform extraction method (Joseph and David, 2001). Briefly, equal volume of phenol:chloroform (10 mL:10 mL) was added to homogenized skeletal muscle in a glass tube with plastic cap and the contents mixed vigorously until an emulsion forms. The mixture was centrifuged at 5000 rpm for 5 min and the lower aqueous phase was transferred to another