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Acute Cold Exposure Triggers Dynamic Metabolic Alteration in Certain Peripheral Tissues of Fresh Water Fishes (*Channa punctata*)

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Abstract: To characterize the sensitivity of environmental low temperature on peripheral tissues of *Channa punctatus*, species of fishes which are energetic and survive in the critical environment, were exposed to cold (4~8^{0}C) for 30 min, 1 h, 2 h and 4 h. Among the peripheral tissues, liver, heart, skeletal muscle and gastro intestinal tract (GIT) of fishes were examined after given cold acclimation. Total protein contents in liver were not significantly changed after 30 min, 1 h, 2 h and 4 h of cold exposure while a gradual increase in protein content in GIT was observed up to 4 h. Cold acclimation did not produce any significant effect in heart up to 2 h, however, an impaired protein synthesis was observed after 4 h of the treatment. A bi-phasial change in protein level in skeletal muscle was noted when compared to the control fishes. The results appear to indicate that low temperature induces a metabolic change involving cellular protein content tissue specifically and preferentially in skeletal muscle and GIT without alteration in liver and heart. The increased protein in these tissues might be involved in the survival process during cold environment.

Key words: Peripheral tissues, low temperature, survival process, taki fish and metabolic regulation.

INTRODUCTION

Taki fish (*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 are the characteristics of metabolic processes. 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[13,7] 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 expenditure, heat production and dissipation, physical activity and appetite[8]. In rodents, shivering, activation of the sympathetic axis[11, 14] with remarkable activity of mitochondrial uncoupling proteins (UCPs)[2,3] were reported as pivotal mechanisms. The greater the UCP concentration, the greater the capacity to uncouple mitochondrial oxidative phosphorylation so that heat is produced. 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 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 mechanism involving the adaptive response for this species is not clarified. Therefore, the present study involves the regulation of peripheral tissue metabolism preferentially the role of cold exposure on the regulation of tissue protein when the species of fishes are exposed to the environmental low temperature.

MATERIALS AND METHODS

Fishes: Taki fish (Channa punctatus) weighing 50 g to 60 g were used. They are maintained in normal water. In the day of experiment, cold exposure (4~8^{0}C) was given to the different groups of fishes in the cold

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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 -20^0C. Control fishes were similarly used for sampling of tissues except giving cold exposure.

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 mL extract. The protein content in tissue was determined by the procedure of Lowry *et al*.[9]. Briefly, alkaline solution was prepared by mixing 50 mL of alkaline Na_2CO_3 solution (2% Na_2CO_3 in 0.1N 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). 50 mL of tissue extract was taken to the test tube and made up to 1 mL with distilled water. For blank, 1 mL water was used in place of tissue extract. 5 mL 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).

Statistical Analysis: Results of the experiments were expressed as mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by pared t-test with SPSS software.

RESULTS AND DISCUSSION

Results: To examine the role of cold exposure on the regulation of tissue protein, the fishes were exposed to cold for 30 min, 1 h, 2 h and 4 h in the cold chamber. For control fish kept in ambient temperature, protein content in skeletal muscle was 4.04 ± 0.71 g/100 g of tissue weight. After 30 min and 1 h exposure of cold, the values were 5.13 ± 0.67g and 6.16 ± 1.19 g/100 of tissue weight respectively. Protein contents were increased significantly by 26.9% (P<0.05) and 52.5% (P<0.05) respectively (Fig. 1). Fishes exposed to cold for 2 h and 4 h had 9.42 ± 1.02 g and 3.28 ± 0.35g protein respectively in their tissues. Cold exposure stimulates the synthesis of protein significantly by 133.2% (P<0.01) after 2 h while the value was reduced non significantly by 18.8% after 4 h when compared to the tissues of control fishes (Fig. 1). The increased protein might be involved in the survival process for this species of fishes.

As shown in table 1, the average protein content in liver of fishes exposed to cold for 30 min, 1 h, 2 h and 4 h were 10.87 ± 0.57g, 10.20 ± 0.55g, 10.15 ± 1.85g and 10.41 ± 0.94g respectively while for the control fish, the value was 10.63 ± 0.72 g/100g of tissue weight. No significant changes of protein content in liver were found up to 4 h of cold exposure and were almost similar to the control fishes. The results demonstrate that cold exposure is involved in the regulation of metabolic function in the liver without alteration of tissue protein content in this species of fishes.

Groups of fishes were used to examine the role of low temperature on the changes of protein in heart. As shown in table 2, the amount of protein in heart in response to cold for 30 min and 1 h were 9.87 ± 1.50g and 8.09 ± 1.60g respectively while for 2 h and 4 h in cold, the values were 9.89 ± 1.82g and 6.39 ± 0.15g respectively. For control fishes, the amount of protein was 9.94 ± 1.23g/100g tissue weight. No significant changes of protein in this tissue were observed up to 2 h of cold, however, the value was reduced significantly (P<0.05) after 4 h of the treatment when compared to the control.

To clarify whether cold exposure is involved in the regulation of tissue protein in GIT, fishes were exposed to cold and the amount of protein in the extract of tissue of fishes for 30 min, 1 h, 2 h and 4 h were 7.25 ± 1.63g, 10.93 ± 1.28g, 14.06 ± 1.23g and 7.18 ± 0.41g/100g of tissue respectively while for the control fish, the value was 5.30 ± 0.57g/100g of tissue weight. A significant 36.79% (P<0.05) and 106.22% (P<0.005) enhanced protein in GIT were found after 30 min and 1 h respectively (Fig. 2) and 165.28% (P<0.005) and 35.47% (P<0.05) increased protein after 2 h and 4 h were found respectively compared to the tissue of control fishes. However, maximal response in the synthesis of protein was observed after 2 h of cold. Cold exposure stimulates the synthesis of protein in this tissue time dependently up to 2 h. The changes of protein in response to cold might be involved in the regulation of GIT metabolic functions. The alteration of tissue protein is an index for the characterization of the sensitively to the environmental temperature.

Discussion: Proteins are essential in all living organisms, performing roles ranging from structural to catalytic. The synthesis and degradation of proteins is therefore a fundamental physiological process and an

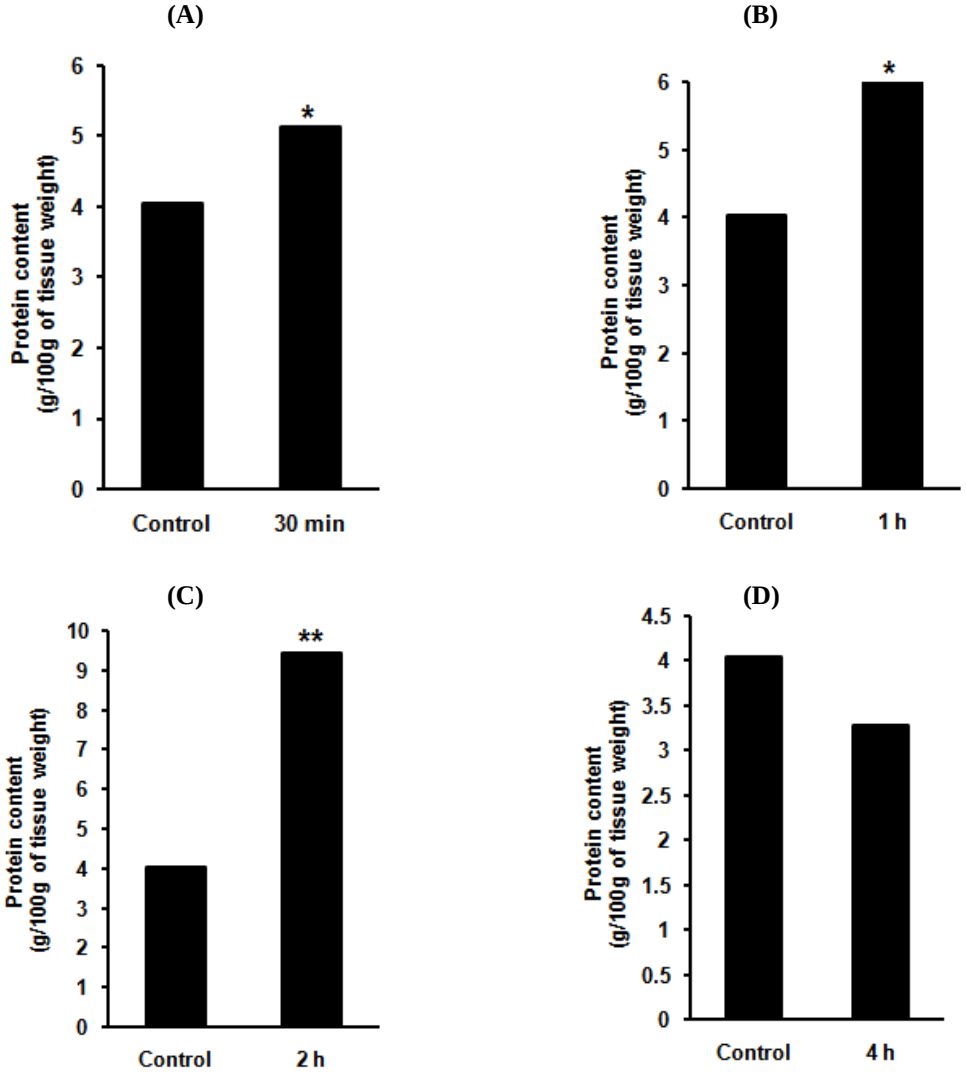

Fig. 1: Changes of protein content in skeletal muscle of fishes exposed to acute cold exposure. The fishes were exposed to cold for 30 min (A), 1 h (B), 2 h (C) and 4 h (D) in the cold chamber. After cold exposure, they were immediately decapitated and the skeletal from dorsal area was separated. Control fishes were similarly used for sampling of tissue except giving cold exposure. Protein content was estimated in cold-exposed and control fishes. The data are ± SEM for 4~5 fishes in each group. *indicates significance of difference when $P<0.05$ and ** indicates significance of difference when $P<0.01$ compared to control.

animal's protein pool is in a continual state of flux, with new proteins entering the pool via protein synthesis and being removed via protein degradation. Protein synthesis is energetically expensive, accounting for 11-42% of basal metabolism in a range of ecto- and endotherms and is therefore a major component of overall animal energetics[4]. The continual synthesis and degradation of proteins is not only vital for tissue maintenance and animal growth but is also important in allowing animals to adapt to changing environmental conditions, to replace denatured or damaged proteins, to mobilize amino acids and to allow metabolic regulation[5].

The fishes exposed to cold had increased protein in their tissues particularly skeletal muscles and GIT. However, this might be mediated likewise by the sympathetic nervous system, since skeletal muscles have been recognized to be supplied with noradrenergic sympathetic axons that are distributed to the muscle spindles and extrafusal muscle fibres[1]. Moreover, the skeletal muscles are thermogenic in nature. Recent investigation reveals that the thermogenesis in skeletal muscle is caused by the higher expression of UCP-3 protein[10]. Cold exposure is the major sympathetic

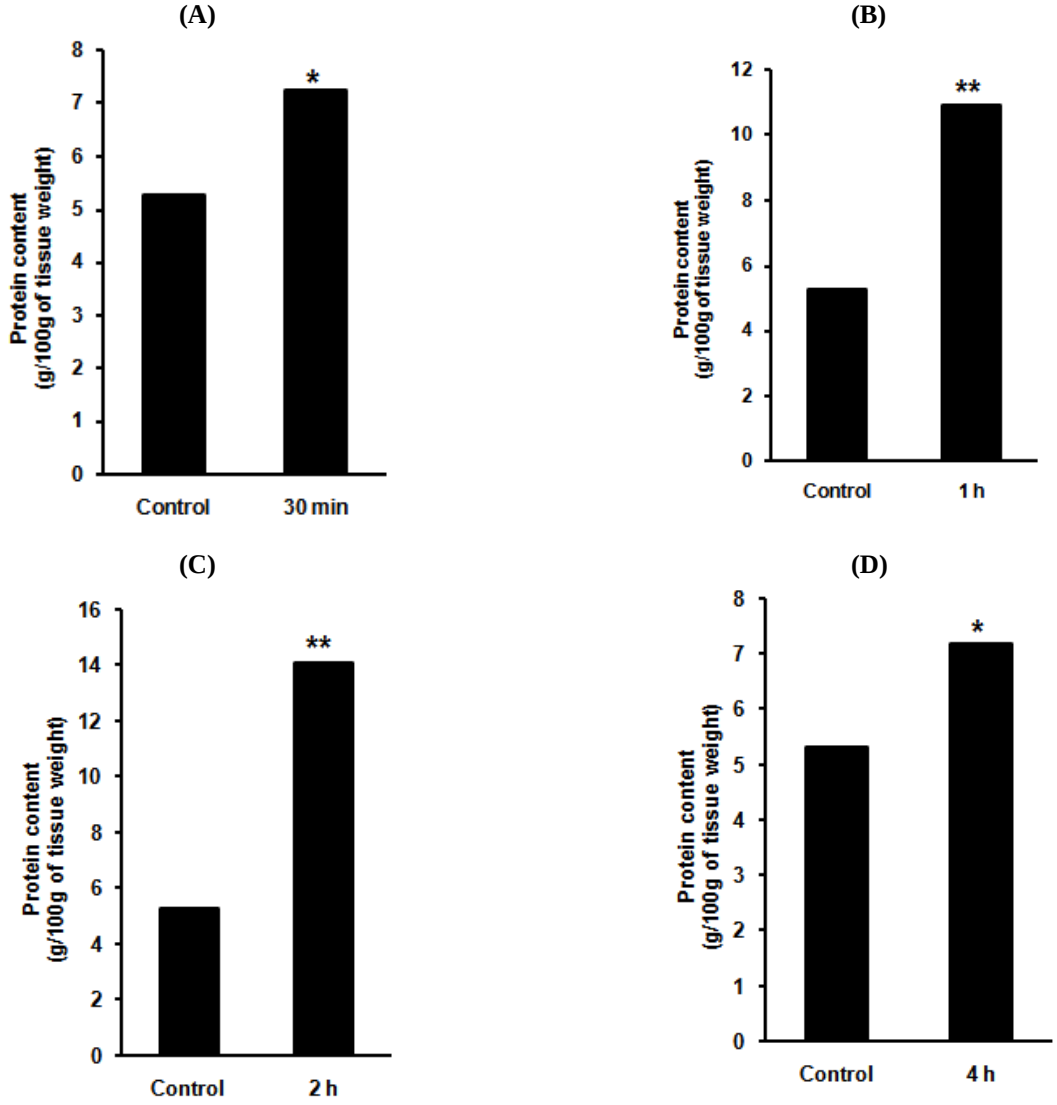

Fig. 2: Changes of protein content in GIT of fishes exposed to acute cold exposure. The fishes were exposed to cold for 30 min (A), 1 h (B), 2 h (C) and 4 h (D) in the cold chamber. After cold exposure, they were immediately decapitated and GIT was separated. Control fishes were similarly used for sampling of tissue except giving cold exposure. Protein content was estimated in cold-exposed and control fishes. The data are ± SEM for 4~5 fishes in each group. *indicates significance of difference when $P<0.05$ and ** indicates significance of difference when $P<0.005$ compared to control.

stimulus regulating metabolic functions. It is known that cold exposure affects the hypothalamus and activates sympathetic nerves releasing noradrenaline which binds to the β-adrenergic receptor of the tissue and activates protein kinase A through cAMP production. The thermogenesis caused by the higher expression of UCP-3 in skeletal muscle particularly in cold environment is referred to as the nonshivering thermogenesis linked to the generation of heat directly. It is assumed that the increased protein in response to cold might be a survival factor for this species during environmental low temperature. The mechanisms that induce thermogenesis in this tissue have been identified. Briefly, the release of NE from the terminal endings of the sympathetic neurons initiates the events leading to the onset of nonshivering thgermogenesis. In cold acclimated fish, the increase in the percent of FABP (fatty acid binding protein) carrying fatty acid which is augmented by oxidation process. FABP from the skeletal muscle has a molecular mass of 14,800 Da[12]. If cold-acclimated striped bass upregulate their ability to use fatty acid fuels in aerobic muscle, they

Table 1: Effect of low temperature on protein content in liver of fishes. The fishes were exposed to cold for 30 min, 1 h, 2 h and 4 h in the cold chamber. After the treatment, the fishes were immediately decapitated and sampling of liver was performed. Control fishes were similarly used except giving cold exposure.

Treatments	Amount of protein (g/100 g of tissue)
Control	10.63 ± 0.72
30 min	10.87 ± 0.57
1 h	10.20 ± 0.55
2 h	10.15 ± 1.85
4 h	10.41 ± 0.94

The data are means ± SE for 4 fishes in each group. No significant changes of protein with respect to control were observed.

Table 2: Effect of low temperature on protein content in heart of fishes. The fishes were exposed to cold for 30 min, 1 h, 2 h and 4 h in the cold chamber. After the treatment, the fishes were immediately decapitated and sampling of heart muscle was performed. Control fishes were similarly used except giving cold exposure.

Treatments	Amount of protein (g/100 g of tissue)
Control	9.94 ± 1.23
30 min	9.87 ± 1.50
1 h	8.09 ± 1.60
2 h	9.89 ± 1.82
4 h	6.39 ± 0.15*

The data are means ± SE for 4~5 fishes in each group. *indicates significance of difference when $P<0.05$ compared to control.

may also increase the flux of fatty acids to the site of oxidation (primarily mitochondria in muscle tissues). Mitochondrial uncoupling protein-2 (UCP-2) is widely expressed in various mammalian tissues, although its physiological functions are not well understood. Dietary fish oil has been involved to induce UCP-2 expression in the rat small intestine, in which UCP-2 mRNA levels are higher than in other organs[16]. Therefore, higher expression of UCP-2 in GIT could be modulated by low temperature.

Liver is the major organ involved in metabolic regulation. The stored glycogen in liver is influenced by the activation of the sympathetic nervous system induced by cold exposure[17]. The energy output from the liver responsible for doing mechanical work is caused by the activation of glycogenolysis process. Although UCP-2 is expressed in this tissue, however, cold exposure did not alter protein content in this tissue. It might be possible that the mechanism involving the triggering response to the synthesis of protein is different from other tissues. Kent *et al*.[6] found that there had no change in either total liver DNA content or protein concentration per gram weight, following acclimation of channel catfish to a reduction in temperature. Therefore, their findings made a good illustration to support the result.

Heart muscle slows rhythmic contractions. Heart muscle also contains myosin and actin filaments but differ from skeletal muscle in that it is continuously active in a regular rhythm of contraction and relaxation. Although the heart must sometimes work harder and faster than normally, e.g, when the body's demand for oxygen increases or when the heart is stimulated by adrenaline, it does not have the very large range of work output shown by skeletal muscle. Moreover, the heart has a completely aerobic metabolism at all times, in contrast to skeletal muscle which can function anerobically for short periods. Mitochondria are much more profuse in heart muscle than in skeletal muscles, they make up almost half the volume of the cells. As fuel, the heart uses a mixture of glucose, free fatty acids and ketone bodies arriving from the blood. Up to 2 hours of cold exposure, no significant changes of protein content were observed, however, protein content was significantly reduced after 4 hours at cold. It is assumed that cold exposure might be involved in reducing protein synthesis in heart during prolonged exposure of cold. Recent studies reveal that mitochondrial protein synthesis in rainbow trout heart is impaired by low temperature[18, 15]. Their studies support the present findings.

Conclusions: In summary, these tissues are metabolically important for energy consumption and energy expenditure. Central stimulation by cold exposure regulates peripheral metabolism probably by changing their protein concentration. The diverse metabolite regulation in response to low temperature is

an index for the survive of these species and is a useful biological process. The increased protein synthesized in the tissues probably takes part in the survival process in the critical atmosphre.

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