JJBS

Volume 5, Number 2, June 2012
ISSN 1995-6673
Pages 91 - 97

Jordan Journal of Biological Sciences

Acute Effects of Arsenic on the Regulation of Metabolic Activities in Liver of Fresh Water Fishes (Taki) during Cold Acclimation.

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Received on December 6, 2011, Accepted on January 14, 2012

Abstract

A variety of *Channa punctatus* fishes were exposed to low temperature for 30 min, 1h, 2h and 4h respectively, since these species of fishes are highly energetic and survive in the critical situations. The protein content in liver extract was not altered significantly with the extension of time and remains similar to the control. Cold exposure does not significantly affect inorganic phosphate (Pi), however, a significant increased level was observed after a 30 min exposure. The alkaline phosphatase (ALKP) activity was significantly enhanced by time dependency and was optimum at 1h of cold exposure. To clarify whether arsenic impairs the cold-induced metabolic functions, groups of fishes were exposed to 10 mM and 100 mM sodium arsenate (Na_2HAsO_4) as well as in cold. The protein content was significantly enhanced by Na_2HAsO_4, however, the effects were lower in response to 100 mM concentration. Similar increase in Pi level in liver in response to arsenic was observed and reduced in cold exposure. ALKP activity was increased by arsenic treatment; however, the higher dose appeared to reduce the potency by inducing the activity. To find the role of arsenic on liver only, arsenic treated and control livers were excluded completely and their weights recorded. A significant increased liver exposed with 10 mM Na_2HAsO_4 was found; and on the other hand, whenever the fishes were exposed to 100 mM Na_2HAsO_4, reduced liver weight was noted. The results demonstrate that cold exposure is critically involved in the regulation of certain metabolic activities in liver and arsenic might be involved in interaction of cold induced effects.

***Keywords*:** Arsenic exposure, low temperature, liver, metabolic regulation.

1. Introduction

Cold exposure has been recognized as a major environmental sympathetic stimulus (Saito, 1928 ; Leduc, 1961) 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 (Lowel and Spiegelman, 2000). In rodents, shivering, activation of the sympathetic axis (Rayner and Trayhurn, 2001; Spiegelman. and Flier, 2001) with remarkable activity of mitochondrial uncoupling proteins (UCPs) (Boss *et al*., 2000; Golozoboubova *et al*., 2001) were reported as pivotal mechanisms. The greater the UCP concentration, the greater the capacity to uncouple mitochondrial oxidative phosphorylation so that heat is produced. Among the peripheral tissues, liver plays an important role in metabolic regulation. The metabolic activities in liver are modulated by both environmental and chemical stimuli. The enhanced nerve activity in response to cold is involved in regulation of metabolic activities in liver as well as in other peripheral tissues. The increased nerve activity in liver has been involved in changes of degradation of cellular ATP (Kennedy *et al*., 1997; Westfall *et al*.,2000). Moreover, liver glycogen metabolism is influenced in response to cold (Thomas and George, 1975). The deposition and degradation of cellular metabolite are biological processes in liver by which the organism is benefited and survives in the atmosphere. Higher degradation of liver glycogen releases energy available for doing mechanical work and survive in the critical circumstances and environment. The vertebrate organisms also utilize shivering and non-shivering thermogenesis for the survival process during energy deficiency. Higher expression of uncoupling protein (UCP-1) involved in non-shivering thermogenesis in peripheral tissue of rodents was found (Golozoboubova *et al*., 2001). UCP has been regarded to be the thermogenic protein and plays the critical role in the regulation of energy balance and its molecular weight is 32 Kdal. Ablation of UCP-1 results in a cold-sensitive phenotype (Ricquier and Bouillaud, 2000). Therefore, it is presumably assumed that UCP-1 plays a crucial role in the regulation of body temperature in rodents. 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

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process, generation of ATP 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 of the survival process is not clarified. It is assumed that other factors are also involved in the survival process.

Channa punctatus is generally found in fresh waters 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 liver might be involved critically on its regulation of metabolites to survive in the atmosphere. However, to survive in the atmosphere, the critical role of liver of these fish species on adaptive response involving the regulation of metabolic processes is not understood.

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 liver 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 (Abdel-Hameid, 2009). The regulation of metabolic activities in liver in response to changes of temperature is an important aspect in fish and to clarify the role of arsenic in cold-induced liver metabolic functions responsible for the survival of these species of fishes in the environment, the current protocol was designed for investigation.

2. Materials and Methods

2.1. Fishes

Taki fishes (*Channa punctatus*) weighing 50 g to 60 g were used and maintained in normal water with ambient temperature (25.0 ± 1.0^{o}C) . On the day of the experiment, cold exposure of between 4~8^{o}C was given to 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 liver carefully removed and weighed (Chyo, JL-180, China) and kept at –20 ^{o}C. Control fishes were similarly used for tissue sampling except not exposing to cold.

2.2. Arsenic Treatment

To examine the role of arsenic on the regulation of metabolic activities in liver, groups of fishes were exposed with different concentrations of arsenic compound (10 mM and 100 mM Na_2HAsO_4. $7H_2O$, BDH Chemical Ltd.) in cold for 1 h. Respective groups of fishes were treated with only 10 mM and 100 mM of arsenic compound (Na_2HAsO_4) for 1 h in the ambient temperature. The tissues were sampled after the treatment similarly as mentioned above.

2.3. *Assay of Tissue Metabolite*

Liver was homogenized with pre-cooled water and centrifuged at 8000 rpm for 10 min. The supernatant was used as crude extract for assay of protein, inorganic phosphate and alkaline phosphatase activity. Protein was determined by Lowry method (Lowry *et al*., 1951) using 50 μL crude extract. Inorganic phosphate (Pi) and alkaline phosphatase activity were determined by using 100~200 μL liver extract. For Pi estimation, 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 glass tube and kept on ice. 1 mL molybdate reagent (10 g of ammonium molybdate in 100 mL water was taken and 100 mL of 5N H_2SO_4 was added to prepare 200 mL solution) was added and mixed. The solution was mixed with 0.4 mL aminonaptholsulphonic acid reagent. 3.6 mL water was added and mixed. The tube was kept standing for 10 min for the complete development of color. For the 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 a standard KH_2PO_4 solution. The enzyme activity was determined by the procedure of Ramnik (1999). 0.25 mL of *p*-nitrophenyl phosphate (PNPP) (1.2 mg/mL in glycine-NaOH buffer, pH 10.0) was added to 0.5 mL glycine-NaOH buffer (pH 10.0) and incubated for 5 min at 37^{o}C. 100~200 μL of tissue extract was taken to the solution and for blank the same volume of buffer was used in place of tissue extract and incubated for 30 min. After incubation, it was made up to 4 mL with 0.1N NaOH solution and absorbance was taken at 410 nm. The amount of PNP (*p*-nitrophenol) produced after hydrolysis of PNPP by the enzyme was measured from the standard PNP solution (500 μmol/L in buffer, pH 10.0). The enzyme activity is expressed as μmol of PNP/min/mg of tissue.

2.4. 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 paired t-test using SPSS software.

3. Results

3.1. Time Course Effect of Low Temperature on the Regulation of Protein Content in Liver

As shown in Fig. 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 ± 0.57 g, 10.20 ± 0.55 g, 10.15 ± 1.85 g and 10.41 ± 0.94 g respectively while for the control fish, the value was

10.63 ± 0.72 g/100 g of tissue weight. No significant changes in protein content in liver were recorded up to 4 h of cold exposure and almost similar to the control fishes. The results demonstrate that cold exposure is involved in the regulation of metabolic functions in the liver without alteration of tissue protein content.

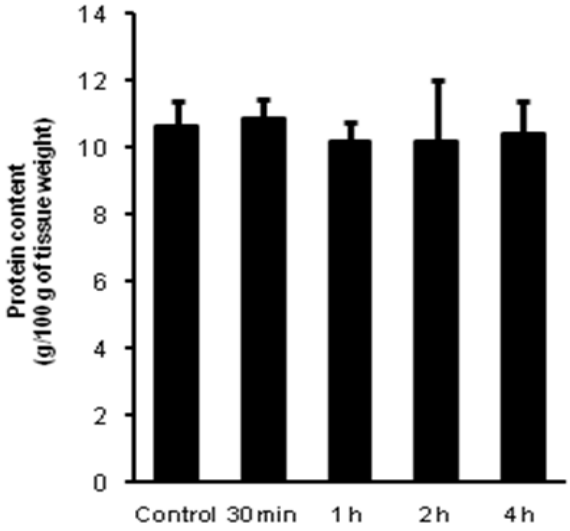

Figure 1. Changes of protein content in liver of fishes exposed to cold. The fishes were kept for 30 min, 1 h, 2 h and 4 h in the cold chamber. Control fishes were similarly used for sampling of tissue except giving cold exposure. The data are ± SEM for 4 fishes in each group.

3.2. Time Course Effect of Low Temperature on the Regulation of Inorganic Phosphate (Pi) in Liver

To examine whether cold exposure is involved in the regulation of liver Pi, groups of fishes were exposed to cold. The amount of inorganic phosphate (Pi) in liver of fishes for 30 min, 1 h, 2 h and 4 h in cold were 3.22 ± 0.18 mg, 2.31 ± 0.33 mg, 2.27 ± 0.17 mg and 1.93 ± 0.27 mg respectively while for the control fish, Pi content was 2.68 ± 0.19 mg/100 g of tissue weight. Although the Pi values decreased by 13.8%, 15.3% and 27.9% for 1 h, 2 h and 4 h respectively, at 30 min cold exposure, a significant (P<0.05) 20.14 % increased Pi was observed compared to the control (Fig. 2).

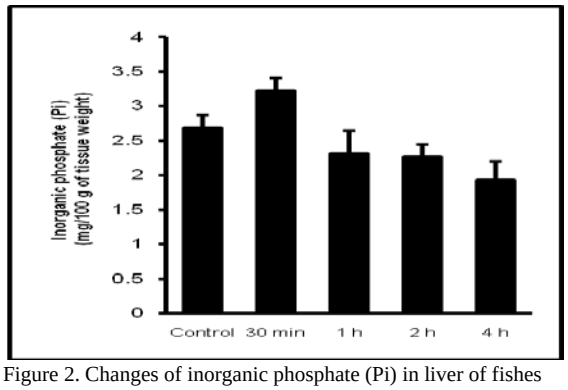

Figure 2. Changes of inorganic phosphate (Pi) in liver of fishes exposed to acute cold exposure. The fishes were exposed to cold for 30 min, 1 h, 2 h and 4 h in the cold chamber. Control fishes were similarly used for sampling of tissue except giving cold exposure. The data are ± SEM for 4~5 fishes in each group.

The results demonstrate that low temperature does not significantly affect Pi in liver however a higher value was found whenever the fishes were exposed to cold for 30 min. It could be interpreted as that after 30 min of cold exposure, the production of the inorganic phosphate is higher than the consumption, while after 1 h of exposure the consumption overcomes the production.

3.3. Time Course Effect of Low Temperature on the Regulation of Alkaline Phosphatase (ALKP) Activity in Liver

To clarify whether cold exposure is involved in the regulation of ALKP activity in liver, fishes were exposed to cold and the average ALKP activities in the extract of liver of fishes for 30 min, 1 h, 2 h and 4 h were 0.61 ± 0.02 μmol, 0.80 ± 0.07 μmol, 0.77 ± 0.06 μmol and 0.76 ± 0.10 μmol respectively, while for the control fish, the enzyme activity was 0.35 ± 0.08 μmol/min/g of tissue weight. A significance of 74.2% (P<0.001) and 128.5% (P<0.005) enhanced ALKP activities in liver were found after 30 min and 1 h respectively (Fig. 3) and 120.0% (P<0.005) and 117.1% (P<0.05) increased activities after 2 h and 4 h were found respectively compared to the liver of control fishes.

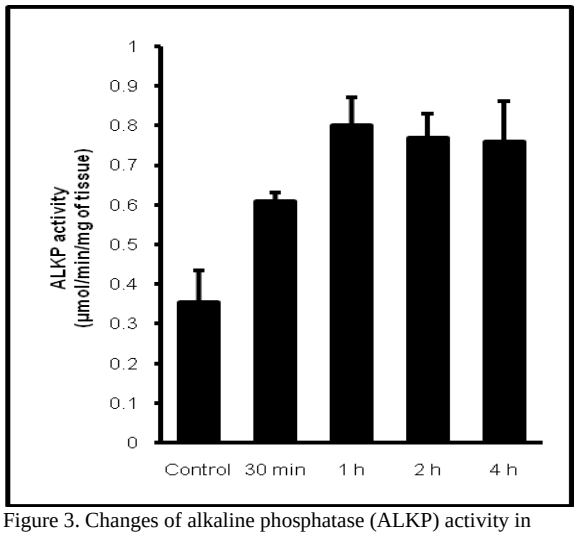

Figure 3. Changes of alkaline phosphatase (ALKP) activity in liver of fishes exposed to acute cold exposure. The fishes were exposed to cold for 30 min, 1 h, 2 h and 4 h in the cold chamber. Control fishes were similarly used for sampling of tissue except giving cold exposure. The data are ± SEM for 4~5 fishes in each group.

Higher alkaline activity was observed after 1 h of cold. Cold exposure stimulates enzyme activity time dependents up to 1 h. ALKP activity is proportional to the duration of the cold exposure until it does not exceed 1 hour. At 1 h of the cold exposure this stress response reaches its maximum and does not increase after further exposures, for 2 and 4 hours. The changes of liver ALKP in response to cold might be involved in the regulation of liver metabolic functions. The alteration of ALKP in liver is an index for the characterization of the sensitivity to the environmental temperature.

3.4. Role of 10 mM and 100 mM Na_2HAsO_4 on Protein Content in Liver

Groups of fishes were used to examine the role of arsenic on the changes of protein in liver. As shown in Fig. 4, the amount of protein in liver in response to arsenic (10 mM) was significantly increased. Protein content of

arsenic-treated fishes for 1 h was 31.2 ± 1.22 g where as for control fishes, was 10.63 ± 0.72 g/100 g of tissue weight. A significance of 193.5% (P<0.001) increased protein in liver was observed after 1 h when compared with control liver. Groups of fishes were exposed to cold with arsenic solution and the amount of protein in liver for 1 h was 20.04 ± 1.16 g. The results indicated that 88.5% (P<0.001) increased liver protein was found in response to 10 mM Na_2HAsO_4 in cold when compared to the control fishes and the value was reduced significantly (P<0.001) in cold exposure even with arsenic solution compared to the respective control.

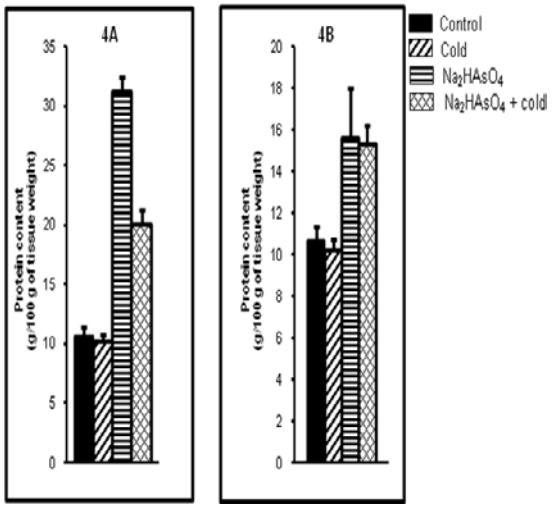

Figure 4. Effects of arsenic on protein content in liver of fishes. The groups of fishes were treated with arsenic solution and kept for 1 h in cold. The respective controls were treated with arsenic only while other fishes were exposed to cold for 1 h. After the treatment, the fishes were immediately decapitated and sampling of tissue was performed. Control fishes were similarly used except giving cold exposure. 4A and 4B represent 10 mM and 100 mM Na_2HAsO_4 respectively. The data are means ± SE for 4~5 fishes in each group.

Fishes exposed to 100 mM Na_2HAsO_4 for 1 h had 15.57 ± 2.42 g protein in their livers while for control; the value was 10.63 ± 0.72 g. The results show that 100 mM Na_2HAsO_4 also causes the increase (46.5%, P<0.1) liver protein compared to that of the control fishes. However, 10 mM Na_2HAsO_4 induces higher protein content than 100 mM concentration (Fig. 4A, Fig. 4B). In response to arsenic in cold, the amount of protein was 15.32 ± 0.90 g while for the respective control exposed to cold only, the value was 10.20 ± 0.55 g. The protein content was increased by 50.2% (P<0.001) and 44.1% (P<0.001) compared to that of cold exposed and control fishes respectively. The results also demonstrate that arsenic causes synthesis of protein in adverse environmental conditions.

3.5. Role of 10 mM and 100 mM Na_2HAsO_4 on Pi Level in Liver

To examine the role of arsenic on Pi level in liver, the fishes were exposed to 10 mM Na_2HAsO_4 solution for 1 h and the respective group was induced by cold exposure. As shown in Fig. 5, Pi content in liver of fishes exposed to arsenic was 9.26 ± 0.78 mg while for the control liver, the value was 2.68 ± 0.19 mg/100 g of tissue and for cold exposed liver, the value was 2.31 ± 0.33 mg per 100 g of tissue. The Pi content in livers of arsenic-treated fish was increased (245.5%) significantly (P<0.005) than in livers of control fish and also of cold exposed fishes (300.8%). Fishes exposed to cold with arsenic had 2.71 ± 0.27 mg Pi in their livers. The results indicate that the increased Pi in response to arsenic was reduced significantly (P<0.001) by cold treatment.

In separate examinations, groups of fishes exposed with 100 mM Na_2HAsO_4 had 3.59 ± 0.59 mg Pi after 1 h and 4.44 ± 0.98 mg in cold with arsenic while 2.31 ± 0.33 mg for cold only. The Pi content in liver was increased similarly in response to 100 mM Na_2HAsO_4 as well as in cold exposure compared to the respective cold-exposed fishes and also to the control. However, the release in Pi in response to higher concentration of arsenic was lower than that of the previous low dose (Fig. 5A, Fig. 5B). The results suggest that the increased Pi in liver might be due to the higher activity of some enzymes responsible for the degradation of the cellular organic compounds and could be considered as the survival factors for this species in critical environment.

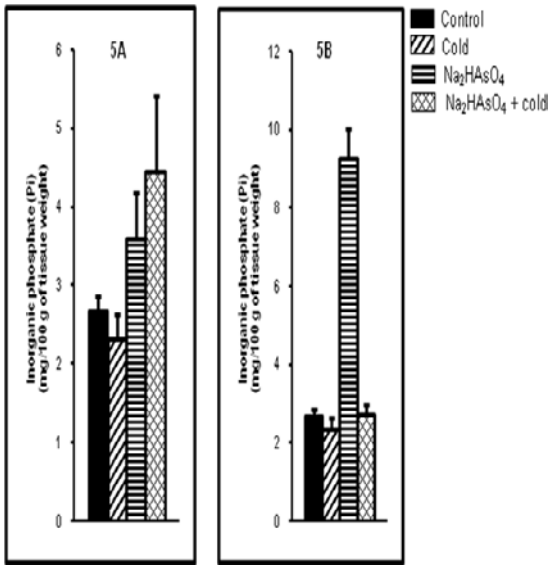

Figure 5. Effects of arsenic on inorganic phosphate (Pi) in liver of fishes. The fishes were exposed to cold with arsenic solution for 1 h. Other groups of fishes were treated with arsenic and exposed to cold for 1 h respectively. After the treatment, the fishes were immediately decapitated and sampling of tissue was performed. Control fishes were similarly used except giving cold exposure. 5A and 5B represent 10 mM and 100 mM Na_2HAsO_4 respectively. The data are means ± SE for 4 fishes in each group.

3.6. Role of 10 mM and 100 mM Na_2HAsO_4 on ALKP Activity in Liver

Liver ALKP is sensitive to toxic response and releases Pi from PNPP (p-nitro phenyl phosphate). To examine the role of arsenic on ALKP activity in liver, groups of fishes were treated with 10 mM and 100 mM Na_2HAsO_4 in cold for 1 h and the respective control livers were also examined with Na_2HAsO_4 only (Fig. 6A and Fig. 6B). The ALKP activity in response to 10 mM Na_2HAsO_4 were 1.28 ± 0.04 μmol and for arsenic treated in cold was 0.65 ± 0.02 μmol whereas for control and the cold exposed fishes, the

activities were 0.35 ± 0.08 µmol and 0.80 ± 0.07 µmol/min/g of tissue respectively. The results demonstrate that ALKP activities were significantly stimulated (265.7%, $P<0.05$) by arsenic compared to control, however, the activity was reduced ($P<0.001$) in cold when compared to arsenic treated group alone. The ALKP activities in livers of groups of fishes in response to 100 mM Na_2HAsO_4 and in cold were 0.93 ± 0.12 µmol and 1.01 ± 0.06 µmol respectively while for control and cold exposed livers, the activities were 0.35 ± 0.08 µmol and 0.80 ± 0.07 µmol respectively.

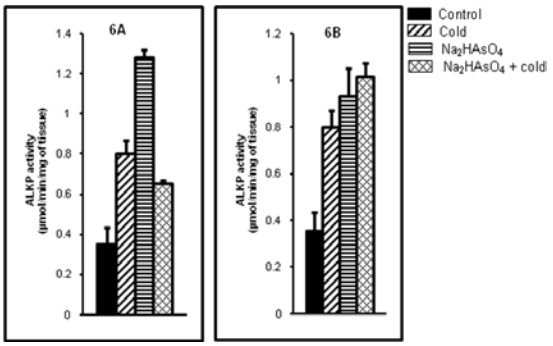

Figure 6. Effects of arsenic on ALKP activity in liver of fishes. The fishes were exposed to cold with arsenic solution for 1 h. Other groups of fishes were treated with arsenic and exposed to cold for 1 h respectively. After the treatment, the fishes were immediately decapitated and sampling of tissue was performed. Control fishes were similarly used except giving cold exposure. 6A and 6B represent 10 mM and 100 mM Na_2HAsO_4 respectively. The data are means ± SE for 4 fishes in each group.

A significant increased response on ALKP activity was observed for fishes exposed to arsenic (165.7%, $P<0.05$) and also even in cold (189.1%, $P<0.05$) when the groups were compared to control. The results would suggest that cold exposure did not overcome the stimulatory effect of higher concentration of arsenic compared to control fishes. The results also demonstrate that higher concentration of arsenic causes stimulation of ALKP activity in liver and the effects were lower than those of 10 mM Na_2HAsO_4.

3.7. Effect of 10 mM Na_2HAsO_4 on Weight of Fish Liver

Groups of fishes were used to examine the role of arsenic on the changes of liver weight. As shown in Table 1, the average liver weight in response to arsenic was significantly increased.

Table 1. Effects of arsenic (10 mM Na_2HAsO_4) on liver weight of respective groups of fishes. The fishes were exposed to cold with arsenic solution for 1 h and 2 h in the cold chamber. The respective controls were treated with arsenic only. After the treatment, the fishes were immediately decapitated and sampling of tissue was performed. Control fishes were similarly used except giving cold exposure.

	Control (n=6)	Na_2HAsO_4 (1 h) (n=5)	Na_2HAsO_4 + cold (1 h) (n=6)	Na_2HAsO_4 (2 h) (n=5)	Na_2HAsO_4 + cold (2 h) (n=6)
Liver weight (g)	0.407 ± 0.04	0.796 ± 0.04^A	0.515 ± 0.04^A	0.474 ± 0.04^B	0.447 ± 0.03^C

The data are means ± SE for 5~6 fishes in each group. $^AP<0.001$ versus control. $^BP<0.05$ versus control. $^CP<0.1$ versus control.

Liver weight of arsenic-treated fishes for 1 h and 2 h were 0.796 ± 0.04 g and 0.474 ± 0.04 g respectively whereas for control fishes, the average liver weight was 0.407 ± 0.04 g. A significant 95.5% ($P<0.001$) and 16.4% ($P<0.05$) increased liver weight were observed after 1 h and 2 h respectively when compared with control liver. Groups of fishes were exposed to cold with arsenic solution and the average liver weight of fishes for 1 h and 2 h were 0.515 ± 0.04 g and 0.447 ± 0.03 g respectively. The results indicated that 26.6% ($P<0.001$) and 9.8% increased liver weight were found in response to 10 mM Na_2HAsO_4 compared to the control fishes.

3.8. Effect of 100 mM Na_2HAsO_4 on Fish Liver Weight

The average liver weight in response to arsenic treatment for 1 h and 2 h were 0.313 ± 0.04 g and 0.347 ± 0.05 g respectively and for control the value was 0.407 ± 0.04 g. The results show that 100 mM Na_2HAsO_4 causes the reduced liver weight compared to that of the control fishes. However, the effects of higher concentration of arsenic are almost reciprocal to that of the low concentration of arsenic (Table 1 and Table 2). The results (shown in Table 2) demonstrate that the liver weights of fishes in response to arsenic were reduced significantly by 23.1% ($P<0.05$) and 14.7% ($P<0.1$) after 1 h and 2 h respectively compared to the control liver.

Table 2. Effects of arsenic (100 mM Na_2HAsO_4) on liver weight of respective groups of fishes. The fishes were exposed to cold with arsenic solution for 1 h and 2 h in the cold chamber. The respective controls were treated with arsenic only. After the treatment, the fishes were immediately decapitated and sampling of tissue was performed. Control fishes were similarly used except giving cold exposure.

	Control (n=6)	Na_2HAsO_4 (1 h) (n=6)	Na_2HAsO_4 + cold (1 h) (n=6)	Na_2HAsO_4 (2 h) (n=5)	Na_2HAsO_4 + cold (2 h) (n=5)
Liver weight (g)	0.407 ± 0.04	0.313 ± 0.04^A	0.334 ± 0.06^B	0.347 ± 0.05^B	0.328 ± 0.02^A

The data are means ± SE for 5~6 fishes in each group. $^AP<0.05$ versus control. $^BP<0.1$ versus control.

The average liver weight of other groups of fishes exposed to cold with arsenic solution for 1 h and 2 h were 0.334 ± 0.06 g and 0.328 ± 0.02 g respectively. The values indicate that arsenic treatment in this condition also reduced the liver weight by 17.8% ($P<0.1$) and 19.4% ($P<0.05$) compared to the control fishes.

4. Discussion

The results of the present study demonstrate the regulation of metabolic functions in liver of *Channa punctata* in response to environmental temperature and adverse effect of sodium arsenate (Na_2HAsO_4) in cold induced fishes. The fish are generally energetic and survive in critical environments, for instance, in water deficiency. Therefore, it is assumed to be it as a major source for characterization of the regulation of metabolic activities. Although the amount of protein in liver was not

changed up to 4 h of the study in cold, treatment with both 10 mM and 100 mM Na_2HAsO_4 resulted the significant increased protein during cold acclimation. Because cold exposure has been believed to have dynamic effect on cellular activity, therefore, during the first few hours of cold stimulation, the activation of a stress response may directly influence whole body metabolism; however, after adaptation, central input coordinate responses to warrant optimisation of the effects. Although variation of temperature causes the alteration of cell activity, effect of acute and prolonged exposure to cold is used to maintain the homeostasis of the species. The increased protein caused by arsenic exposure has been found in our study and appeared to be reduced in cold. Both 10 mM and 100 mM Na_2HAsO_4 produced the toxic environment where the fishes want to survive. Therefore, it is reasonable that an adaptive response by the species was created and some stress proteins are synthesized. However, cold exposure decreases the ability to respond to low arsenic exposure and is appreciable that increased sympathetic nerve activity induced by cold prevents the toxic effect of arsenic. Cold exposure was unable to reduce the toxic effect of arsenic whenever the fishes were exposed with 100 mM Na_2HAsO_4. The results clarify that this higher arsenic produces a severe effect on liver where the sympathetic nervous system does not play its role. The liver is the organ where most of the biotransformation of inorganic arsenic takes place (Del Razo *et al*., 2001). Up regulation of several genes in arsenic-induced adaptive response has been observed (Verma *et al*., 2002; Chelbi-alix *et al*., 2003). Their findings suggest that arsenic may induce the synthesis of molecules responsible for the survival process.

The liver is a major target organ of arsenic toxicity in both mice (Waalkes *et al*., 2003) and humans (Chen *et al*., 1992). Arsenic-induced liver injury in humans is a common phenomenon, typically manifesting initially as degenerative lesions with jaundice, progressing to noncirrhotic portal hypertension, fibrosis, cirrhosis, and neoplasia such as hepatocellular carcinoma (Mazumder *et al*.,1998; Lu *et al*., 2001; Centeno *et al*., 2002). Prolonged exposure of higher concentration of arsenic has been involved in liver injury and damage (Taylor *et al*., 1989; Chiou *et al*., 1995). Liver metabolism is a known potential target for the toxic action of chemicals (Hinton *et al*., 2001).

The released Pi in response to cold was enhanced after 30 min which might be due to the effect of degradation of cellular energy rich compound such as ATP. Since Na^+-K^+ ATPase activity has been demonstrated to be increased by cold exposure (Videla *et al*., 1975), therefore, the increassed Pi might be due to the higher activity of the enzyme Na^+-K^+ ATPase. Alkaline phosphatase (ALKP) might also be involved in this respect. Both 10 mM and 100 mM Na_2HasO_4 induce Pi release in liver, however, the increased Pi is significantly reduced in cold. It is assumed that the sympathetic nervous system is induced by cold and is directly involved in reducing the toxic effect of arsenic. Therefore, it is also assumed that the biotransformation of foreign toxic substances might be influenced by activation of the sympathetic nervous system. Allen et al. (2004) found that arsenic impairs the sympathetic nerve activity induced by cold. ALKP is predominantly found in liver and is an index of the characterization for liver pathogenesis. The increased activity was observed by cold and enhanced the time dependently. It is assumed that this enzyme is sensitive to cold or the activation of sympathetic nervous system is involved during cold acclimation. The released Pi might be influenced in response to low dose of arsenic, because ALKP activity is augmented however, the activity was appeared to be reduced in cold. The results would indicate that metabolic function involving higher ALKP activity in liver is sensitive to this dose. Recent investigation reveals that arsenic exposure stimulated ALKP in liver (Sharma *et al*., 2007). The higher activity demonstrates that increased Pi release would be a survival factor during energy deficiency. Liver weights of fishes exposed to higher concentration of arsenic were reduced significantly compared to control while low dose of arsenic significantly increases liver weight suggesting that higher dose of arsenic might be involved in liver damage, on the contrary, low arsenic is assumed to be involved in biosynthesis of lipids (Sharma *et al*., 2007; Chen and Chiout, 2001). 100 mM arsenic can cause liver glycogenolysis because of its severe effects and liver weight is reduced however, cold exposure could not prevent liver damage in presence of higher arsenic level. Up regulation of genes associated with carbohydrate catabolism such as glycolysis and downstream genes associated with generation of precursor metabolites and energy clearly indicate a continuous need for production of precursor metabolites and energy to sustain various homeostatic and adaptive responses in the liver of arsenic-treated fish compared with the liver of control (non-treated) fish.

5. Conclusion

In summary, as a peripheral tissue, liver is metabolically important for energy consumption and energy expenditure. Environmental low temperature is a major stimulus exerting its effect on metabolic changes. However, the regulatory process in response to cold could be prevented or modulated by the toxic effects of arsenic. As a major metabolic site, liver plays the critical role in the biotransformation of foreign toxic substances. The diverse metabolite regulation in response to low temperature is an index for survival of these species and is a useful biological process, however, arsenic probably takes part in modulation of the metabolic process.

Acknowledgement

This study was carried out in the Department of Biochemistry and Molecular Biology, Rajshahi University and was supported by the University Grant Commission (UGC), Bangladesh.

References

Abdel-Hameid NH. 2009. A protective effect of calcium carbonate against arsenic toxicity of the Nile catfish, *Clarias gariepinus*. *Turkish J Fisheries and Aquatic Sci.*, **9**: 191-200.

Allen T. Awasthi A and Rana SVS. 2004. Fish chromatophores as biomarkers of arsenic exposure. *Environ Biol Fishes*,**71(1):** 7-11.

Boss O. Hagen T and Lowell B. 2000. Perspectives in diabetes. Uncoupling proteins 2 and 3, potential regulators of mitochondrial energy metabolism. *Diabetes.* **49**: 143-156.

Centeno JA, Mullick FG, Martinez L, Page NP, Gibb H, Longfellow D, Thompson C and Ladich ER. 2002. Pathology related to chronic arsenic exposure. *Environ Health Perspectives*, **110**: 883-886.

Chelbi-alix MK, Bobe P, Benoit G, Canova A, and Pine R. 2003. Arsenic enhances the activation of Stat1 by interferon gamma leading to synergistic expression of IRF-1. *Oncogene*. **11:** 9121-9130.

Chen CJ, Chen CW, Wu MM, and Kuo TL. 1992. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. *British J of Cancer*. **66**: 888-892.

Chen KL and Chiout PW. 2001. Oral treatment of mule ducks with arsenicals for inducing fatty liver. *Poultry Sci*., **80(3):** 295-301.

Chiou HY, Hsueh YM, and Liaw KF. 1995. Incidence of internal cancers and ingested inorganic arsenic: a seven year follow up studies in Taiwan. *Cancer Res*., **55**: 1296-300.

Del Razo LM, Quintanilla-Vega B, Brambila-Colombres E, Calderon-Aranda ES, Manno M, and Albores A. 2001. Stress proteins induced by arsenic. *Toxicol and Applied Pharmacol*., **177**: 132-148.

Golozoboubova V, Hohtola E, Matthias A, Jacobsson A, Cannon B and Nedergaard J. 2001. Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *The FASEB J*., **11**: 2048-2050.

Hinton DE, Segner H and Braunbeck T. 2001. Toxic responses of the liver. In: **Target Organ Toxicity in Marine and Freshwater Teleosts–Organs**, edited by Schlenk D and Benson WH. London: Taylor and Francis, vol. 1, p. 224-268.

Hughes MF. 2002. Arsenic toxicity and potential mechanisms of action. *Toxicol Letters.* **133**: 1-16.

Kennedy C,Todorov LD, Todorov SM and Sneddon P. 1997. Release of soluble nucleotidases: a novel mechanism for neurotransmitter inactivation. *Trends in Pharmacol Sci*., **18**: 263-266.

Leduc J. 1961. Excretion of catecholamines in rats exposed to cold. *Acta Physiol Scandinavica.* **51**: 94-100.

Lowel BB and Spiegelman BM. 2000. Towards a molecular understanding of adaptive thermogenesis. *Nature*. **404:** 652-660.

Lowry OH, Rosenbrough NJ and Randall RJ. 1951. Protein measurement with the Folin-phenol reagent. *J Biol Chem*., **183:** 265-275.

Lu T, Liu J, LeCluyse EL, Zhou YS, Cheng ML and Waalkes MP. 2001. Application of cDNA microarray to the study of arsenic-induced liver diseases in the population of Guizhou, China. *Toxicol Sci*., **59**: 185-192.

Mazumder DN, Das Gupta J, Santra A, Pal A, Ghose A and Sarkar S. 1998. Chronic arsenic toxicity in west Bengal–the worst calamity in the world. *J Indian Medical Asso.*, **96**: 4-7.

Ramnik S. 1999. **Medical Laboratory Technology: Methods and Interpretations**. Jaypee Brothers, Medical publishers (P) Ltd., New Delhi; pp. 485-487.

Rayner DV and Trayhurn P. 2001. Regulation of leptin production: Sympathetic nervous system interactions. *J Molecular Med*., **79**: 8-20.

Ricquier D and Bouillaud F. 2000. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochemical J.*, **345(Pt 2):** 161-179.

Saito S. 1928. Influence of application of cold or heat to the dog's body on the epinephrine output rate. *Tohoku J. Exp. Med.*, **11**: 544-567.

Sharma A, Sharma MK and Kumar M. 2007. Protective effect of Mentha piperita against arsenic-induced toxicity in liver of Swiss albino mice. *Basic and Clinical Pharmacol and Toxicol.*, **100(4):** 249-57.

Spiegelman B and Flier JS. 2001. Obesity and the regulation of energy balance. *Cell*. **104**: 531-543.

Taylor PR, Qiao YL and Schatzkin A. 1989. Relation of arsenic exposure to lung cancer among tin miners in Yunnan Province, China. *Br. J. Ind. Med*., **46**: 881-6.

Tchounwou PB,Patlolla AK and Centeno JA. 2003. Carcinogenic and systemic health effects associated with arsenic exposure–a critical review. *Toxicol Pathol*, **31**: 575-388.

Thomas VG and George JC. 1975. Changes in plasma, liver and muscle metabolite levels in Japanese quail exposed to different cold stress situations. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **100(4)**: 297-306.

Verma A, Mohindru M, Deb DK, Sassano A, Kambhampati S, Ravandi F, Minucci S, Kalvakolanu DV and Platanias LC. 2002. Activation of Rac1 and the p38 mitogen-activated protein kinase pathway in response to arsenic trioxide. *J Biol Chem.*, **277**: 44988-44995.

Videla L, Flattery KV, Sellers EA and Israel Y. 1975. Ethanol metabolism and liver oxidative capacity in cold acclimation. *J. Pharmacol. Exp. Ther*. **192**: 575-582.

Waalkes MP, Ward JM, Liu J and Diwan BA. 2003. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol *Applied Pharmacol*., **186:** 7-17.

Westfall TD, Sarkar S, Ramphir N, Westfall DP, Sneddon P and Kennedy C. 2000. Characterization of the ATPase released during sympathetic nerve stimulation of the guinea-pig isolated vas deferens. *British J Pharmacol*., **129(8):** 1684-1688.