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Original Article

Serum Inorganic Phosphate and Alkaline Phosphatase Activity in Cold-Exposed Rat

Md. Shahidul Haque[1], Zerin Fahmida Shima [2]

Abstract

To characterize serum constituents in blood in response to cold exposure, we measured serum inorganic phosphate (Pi) and alkaline phosphatase (ALP) levels. After 30 min exposure of cold, the serum Pi and alkaline phosphatase levels were 3.4 ± 0.06 mg/l and 72.33 ± 0.45 µmole/l respectively where as the control levels of these constituents are 3.05 ± 0.03 mg/l and 84 µmole/l respectively. Cold exposure for 30 min induces serum Pi concentration by 11.5 % and decreases ALP levels significantly by 13.9 % compared to control rats. Serum Pi for 1 h and 2 h were 3.8 mg/l and 4.17 ± 0.16 mg/l while ALP levels were 57.33 ± 0.88 µmole/l and 91 ± 1.73 µmole/l respectively in different groups of rats. Cold exposure stimulates serum Pi by 24.6 % and 36.7 % and reduces ALP activity by 31.7 % significantly and without significant change for 1 h and 2 h respectively. Another groups of rats exposed to cold for 4 h had Pi and ALP levels 4.66 ± 0.88 mg/l and 85.66 ± 0.88 µmole/l respectively. Cold exposure similarly stimulates Pi content significantly by 52.8 % without affecting ALP activity compared to the control rather reaches to basal level. Our results demonstrate that Pi and ALP levels in cold-exposed rats are regulated in different ways.

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Introduction

Homeostasis of living organism is an important feature. The changes in environmental factor affect the homeostasis of the living cell. However, the organism has the capability to maintain this homeostasis. Cold exposure is the major sympathetic stimuli[2] disrupts the homeostasis of serum constituents. After prolonged exposure of rats to cold, a sustained increase in systolic pressure occurs, giving rise to a cold-induced model of hypertension [3]. Activation of the sympathetic nervous system is thought to contribute to the development of hypertension in cold-exposed rats[5]. Serum inorganic phosphate and alkaline phosphatase (ALP) are important constituent in blood. However, the regulatory mechanism of the molecules in blood is not clearly understood. Serum Pi and ALP are the tools for the proper diagnosis of disease. In the pathological condition, these constituents are changed. The degradation of cellular ATP is a common phenomena either by the environmental stress or by endogenously through hydrolytic enzyme. As a result, the serum Pi is increased. ALP levels are also increased in the pathological condition preferentially in liver disease, kidney disease etc. However, very little is known whether the cold exposure can influence the concentration

[1] Associate Professor, Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205.
[2] Associate Professor, Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205.

of these molecules in the serum. Therefore, we examine the effect of cold stress on the Pi and ALP levels in rats.

Materials and Methods

Animals: Male rats weighing 200 to 260 g were used. They were housed in the cages at ambient temperature and given free access to laboratory foods and water. In the day of experiment, cold exposure (4-8^{o}C) were given to the different groups of rats in the cold chamber for 30 min, 1 h, 2 h and 4 h with full aeration and with free access of water. After cold exposure treatment, the rats were immediately anesthetized with diethyl ether and were quickly decapitated. Blood was drawn from the jugular vein and was centrifuged at 3000 rpm for 10 min. The serum in the supernatant was kept at –20^{o}C.

Estimation of serum inorganic phosphate (Pi) and activity of alkaline phosphatase (ALP) : Serum Pi was estimated by calorimetric method[1] and ALP was estimated according to the prescribed procedure[4]. All data, expressed as means ± SE, were analyzed using analysis of variance (pared t-test) by Stat View soft ware.

Results

Serum inorganic phosphate (Pi) level: Fig. 1 shows serum inorganic phosphate (Pi) for control and cold-exposed rats. For control rats kept in ambient temperature, Pi content was 3.05 ± 0.03 mg/l. After 30 min, fig. 1 (a) exposure of cold, the Pi content was 3.40 ± 0.06 mg/l indicating 11.5 % increase in their concentration (P<0.1). After 1 h of cold exposure, fig. 1 (b), Pi content was 3.8 mg/l in the serum showing 24.6 % significant increase (P<0.05). After 2 h, fig. 1 (c) and 4 h, fig. 1 (d) of cold exposure, Pi contents were 4.17 ± 0.16 mg/l and 4.66 ± 0.88 mg/l demonstrating 36.7 % and 52.8 % increases significantly (P<0.05) in concentration respectively compared to the control rats. Serum Pi was gradually increased significantly up to 4 h of cold exposure.

Serum alkaline phosphatase (ALP) level: As shown in fig. 2, the serum ALP activity in response to cold exposure is significantly regulated. The Pi content of cold-exposed rats for 30 min, fig. 2 (a) and 1 h, fig. 2 (b) were 72.33 ± 0.45 µmole/l and 57.33 ± 0.88 µmole/l respectively where as for control, the ALP level was 84 µmole/l. The results demonstrate that cold exposure significantly (P< 0.05) reduces ALP activity by 13.9 % and 31.7 % after 30 min and 1 h respectively compared to the control rats. However, after 2 h, fig. 2 (c) and 4 h, fig. 2 (d), the ALP levels were 91 ± 1.73 µmole/l and 85.66 ± 0.88 µmole/l respectively indicating no significant increases compared to the control rats rather reaches to the basal level. The results suggest that short-term exposure reduces its concentration and prolonged exposure maintains homeostasis of serum ALP levels.

Table: Serum inorganic phosphate (Pi) and alkaline phosphatase (ALP) level in response to cold exposure: Different groups of rats were used for giving cold exposure. The rats exposed to cold temperature for 30 min, 1 h, 2 h and 4 h were kept in the cold chamber. After giving cold exposure, the rats were immediately anesthetized with di-ethyl ether and blood was taken from the jugular vein to get serum for the analysis of Pi and ALP. Control rats were similarly used for Pi and ALP except giving cold exposure. The data are means ± SE for 3 rats in each group. *and** indicate significance of difference from the control rats when P<0.1 and P<0.05 respectively.

	Control Rat (n=3)	Cold-Exposed Rat			
		30 min (n=3)	1 h (n=3)	2 h (n=3)	4 h (n=3)
Inorganic phosphate (Pi) (mg/l)	3.52 ± 0.03	3.40 ± 0.06*	3.80 ± 0.00**	4.17 ± 0.16**	4.66 ± 0.88**
Alkaline phosphatase level (ALP) (µmole/l)	84.00 ± 0.00	72.33 ± 0.45**	57.33 ± 0.88**	91.00 ± 1.73	85.66 ± 0.88

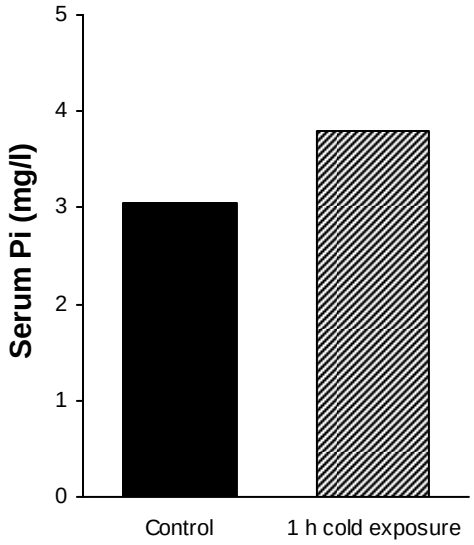
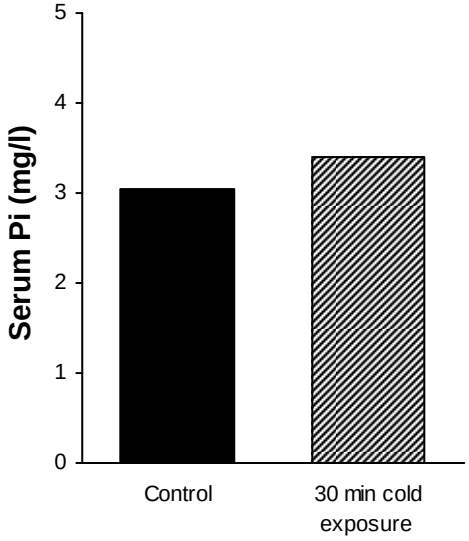


Fig. 1(a)



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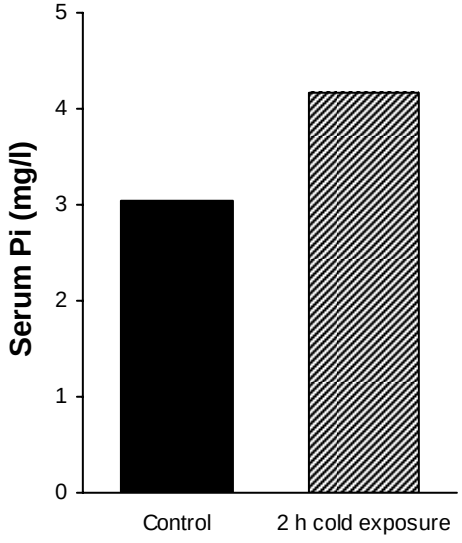

Fig. 1(c)

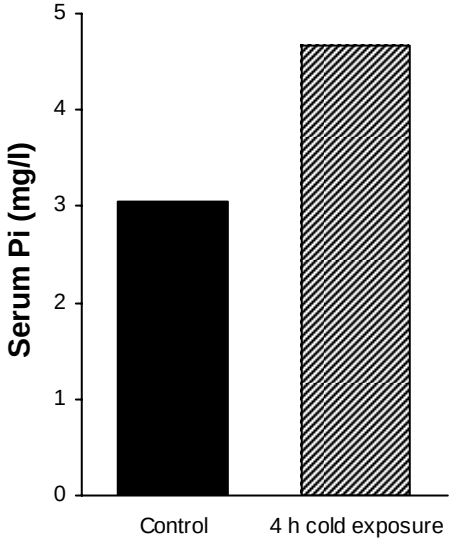

Fig. 1(d)

Fig. 1. Effects of cold exposure on serum inorganic phosphate (Pi) content of rats. The rats exposed to cold for 30 min (a), 1 h (b), 2 h (c) and 4 h (d) were divided into four groups accordingly and were kept in the cold chamber. After cold exposure, the rats were immediately anesthetized with di-ethyl ether and blood was taken from the jugular vein. The serum was prepared and was analyzed for inorganic phosphate (Pi) content. Control rats were similarly used for inorganic phosphate (Pi) estimation except giving cold exposure. The data are ± SE for 3 rats in each group.

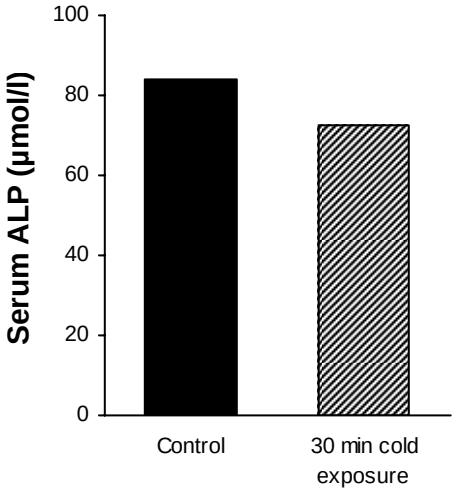

Fig. 2(a)

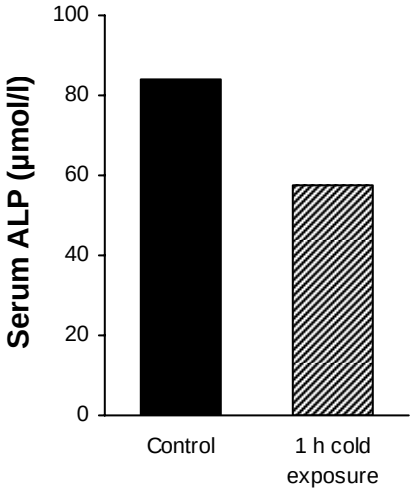

Fig. 2(b)

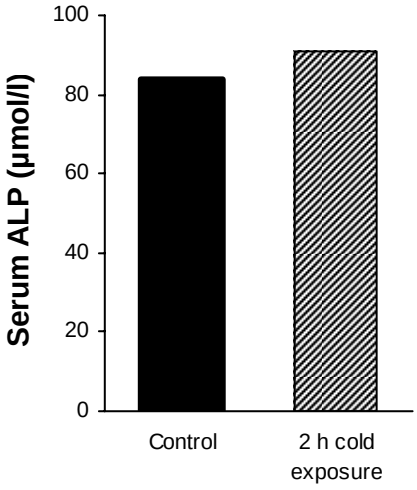

Fig. 2(c)

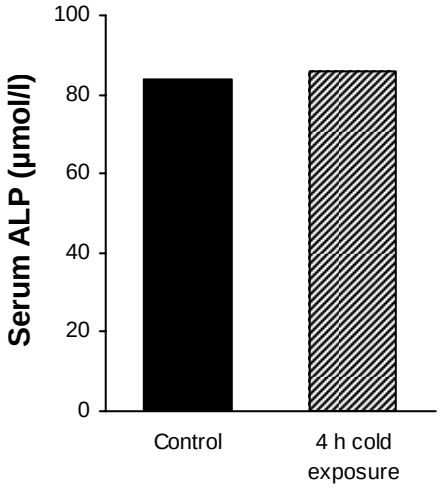

Fig. 2(d)

Fig. 2. Effects of cold exposure on serum alkaline phosphatase (ALP) levels of rats. Cold exposure (4-8^{o}C) was given to the different groups rats in the cold chamber depending on the time of exposure 30 min (a), 1 h (b), 2 h (c) and 4 h (d). After cold exposure treatment, the rats were immediately anesthetized with diethyl ether and were quickly decapitated. Blood was drawn from the jugular vein during anesthesia. Control rats were similarly used for sampling of blood except giving cold exposure. The results are ± SEM of 3 rats in each group.

Discussion

In the present study, serum inorganic phosphate (Pi) was significantly increased in cold-exposed rats. The rats which were kept in cold temperature produce heat by splitting of cellular ATP into ADP and Pi. The increased Pi might be utilized by the cell by mitochondrial oxidative phosphorylation.or appeared as higher serum Pi on hydrolysis of phosphatase enzyme. Sympathetic nervous system has the vital role on phosphatse activity. Ceramide is an essential second messenger in a variety of processes downstream of stress stimuli[8, 10]. The

finding that neurotrophins induce ceramide accumulation in certain neuronal types by binding to the p75 neurotrophin receptor and activating a sphingomyelinase[11, 12] underscores the relevance of ceramide as a second messenger in the nervous system[13]. The regulating mechanism of phosphatase by hypothalamus in response to cold exposure has been clarified. Pi is the major constituent of the blood serum. It is the essential for the formation and development of bone and teeth along eith calcium. It is required for the formation of phopholipids, nucleic acid and phosphoproteins. Alkaline phosphatase enzyme is found in a number of organs, most plentiful in bones and liver, then in small intestine, kidney and placenta. Serum ALP is a most valuable index of osteoblastic activity. Increases in serum ALP activity seen in rickets, osteomalacia, hyperparathyroidism. In primary and secondarey malignancies of bone, the level depends on the severily and degree of new bone formation. When the lesion is purely destructive as myelomatosis, the value is normal. In hypophosphatasia- where there defective calcification, low tissue and serum ALP activity is observed. Cold exposure given to rats reduces initially the activity of ALP and again it enhances to the basal level. Our results suggest that cold exposure has the dual function. Short term exposure reduces its concentration and prolonged exposure maintains the homeostasis of ALP activity. It is speculated that cold exposure might improve the pathological condition of the subject.

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All correspondence to:
Dr. Md. Shahidul Haque
Associate Professor,
Department of Biochemistry and Molecular Biology
Rajshahi University, Rajshahi-6205