Original Article

Anatomy

Anti-stress Role of Cyanocobalamin in Heat- induced Weight Loss in Albino Rats

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ABSTRACT

Objective: To determine the deleterious effects of heat-stress on the body weight and to evaluate the anti – stress role of the Cyanacobalamin.

Study Design: An Experimental study

Place and Duration of the Study: Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from November 2010 to February 2011.

Materials and Methods: Forty five Albino rats (180-200 grams) were selected and divided into group A (Control), group B (Heat - induced) and group C (Protective). Each group was further subdivided into three subgroups, based on the period of the study. The animals of the subgroups B and C received heat and the temperature was set at 42 C for six hours daily. Group C (C1, C2 and C3) animals were protected with Cyanocobalamin at the dosage of 0.8 mg/ kg of body weight intraperitonealy, two hours before heat induction. All the animals were weighed before the commencement of the study. Blood samples were collected for the hormonal assay of plasma ACTH level.

Results: Heat –stress and its consequences significantly decrease the body weight in group B animals. It could be due to hyper activation of the HPA and is and resultant ACTH corticosterone secretions causecatabolism of muscle and other bodily proteins. The prophylactic use of Cyanocobalamin group C animals by its cytoprotective of and anti-apoptotic role significantly increase the body weight and restore ACTH secretions near to normal.

Conclusion: Findings of the current study conclude that Cyanocobalamin is beneficial to alleviate the detrimental effects of the heat-stress.

Key Words: Albino rats, Heat- stress, Body weight, ACTH, Cyanocobalamin

INTRODUCTION

Numerous environmental factors evoke in an animal organism a set of functional and morphological changes, called stress[1]. Stress affects our daily lives[2].A major neuroendocrine mechanism in a stress reaction in both animals and humans is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in a rapid increase in circulating corticotropin (ACTH) and subsequent rise in glucocorticoids (Corticosterone in rats, cortisone in humans) which are critical for successful adaptation [3,4]. Adaptive processes not only restore and protect but also damage the body[5]. It is predicted that global warming will cause an increase in the frequency and severity of heat waves with an associated rise in mortality, unless proactive measures are taken [6]. The concentration of antioxidant vitamins decrease with heat stress [7,8]. A large number of micronutrients (vitamins, essential minerals and other compounds) are required for normal metabolism in the human diet [9]. Cyanocobalamin (vitamin B12) is an important water soluble vitamin belonging to the vitamin B-complex [10]. and isunique among the nutrients in that it straddles the divide between vitamin and minerals [11]. Cyanocobalamin is a nutrient necessary for normal DNA synthesis, red cell production and maintenance of the nervous system[12,13]. Cyanocobalamin is an indispensable vitamin for sustaining life [14]. Antioxidant vitamins counteract the free radicals and decrease the ACTH and cortisol levels, protecting the metabolism from the effects of stress [15].

MATERIALS AND METHODS

This experimental study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. Inthisexperimental study 45 male albino rats of sprauge-dawely variety, 90-120 days of age, weighing180-200 grams were used. They were obtained from the Animal house of the Jinnah PostgraduateMedical Center, Karachi. These animals were housed in the experimental room of the animal house for a week prior to the commencement of the study and maintained on the balanced diet and water wasprovided ad libitum.

Study design: The animals weresubdivided into three groups A, B and C. Each group was further subdivided into three subgroups, A1, A2, A3, B1, B2, B3, C1, C2 and C3 based on the period of the treatment, that was two four and six weeks respectively, whereas each subgroup comprised of five animals.

Group A- (A1, A2 and A3) served as Control

Group B - (B1, B2 and B3) received heatonly(Heat-induced)

Group C- (C1, C2 and C3) received heat and Cyanocobalamin (Protected)

Group C (C1, C2 and C3) were protected with Cyanocobalamin (BETOLVEX) manufactured by Alpharma Aps, Denmark at the dosage of0.8 mg/ kg of body weight intraperitonealy, two hours before heat induction. Then animals of group B (B1, B2, and B3) and C (C1, C2, and C3) were shiftedin another experimental room for heat induction provided by double rod electric room heater of 2000 WATT. The temperature was set at 42 C for six hours daily [16] according to their time duration. All the animals were weighed by using a digital balance.before the commencement of the study and at the time of the end of the study. Blood samples about 2 ml were collected from each animal by cardiac puncture in the plastic vacutainers containing EDTA-K2 as an anticoagulant(BD- Franklin NJ ,USA) for the hormonal assay of plasma ACTH level by using Mouse /Rat adrenocorticotropic hormone (ACTH) ELISA antibody test Kit (Catalog#40 - 109 – 325002;Genway Biotech ,INC ,CA).

RESULTS

Observations on Body Weight: The mean values of the body weight (table-1) in group B animals showed a moderately significant decrease ($p<0.01$) in subgroups B-and B-2 and highly significant decrease ($p<o.oo1$) in subgroup B-3 as compared to the control subgroups A-1 and A-2, and A-3 respectively the data (table-1) of group c animals showed insignificant decrease ($p>0.05$) in the subgroups c-1, c-2 and c-3 when compared with the final body weight of control subgroups A-1, A-2 and A-3 respectively when compared with the data (table-1) of the group B animals, these was a modestly significant increase ($p<0.01$) in the final body weight in subgroup c-1 than subgroup B-1 and a highly significant increase ($p<0.001$) in subgroups C-2 and C-3 than the subgroups and B-2 and B-3 respectively.

Analysis of Plasma Acth Level: The mean values of the plasma acth levels (table-2) showa highly significant increase ($p<0.001$) in subgroups b-1, b-2 and b-3 compared to control subgroups a-1, a-2 and a-3. the data (table-2) of group c animals showed an insignificant increase ($p>0.05$) in subgroups c-1 c-2 and c-3 compared to control subgroups a-1, a-2 and a-3 the data (table) also showed a highly significant decrease ($p>0.001$) in subgroups c-1,c -2 and c-3 compared to subgroups b1, b2 and b3 respectively.

Table No.1: *Mean Body Weight (G) of Albino Rats in Different Groups at Variable Time Intervals

Group	Sub-groups	Treatment Given	Initial weights	Final Body weight		
				2nd week	4th week	6th weeks
A	A1 (n=5)	Control	186.60 ± 1.63	211.40 ± 1.77		
	A2 (n=5)		186.80 ± 2.45		217.0 ± 2.70	
(n=15)	A3 (n=5)		187.0 ± 2.16			234.60 ± 1.07
B	B1 (n=5)	Heat	192.20 ± 2.17	189.80 ± 2.05		
	B2 (n=5)		195.60 ± 1.63		184.60 ± 2.03	
(n=15)	B3 (n=5)		194.80 ± 2.08			182.40 ± 1.28
C	C1 (n=5)	Heat+Cyano-cobalamin	188.90 ± 2.13	208.40 ± 1.69		
	C2 (n=5)		188.34 ± 1.95		212.60 ± 1.28	
(n=15)	C3 (n=5)		190.20 ± 1.35			230.40 ± 0.87

Statistical Analysis of the Differences in Mean Body Weight within the Same Group and Between Different Groups of Albino Rats

Statistical comparison	P-value	Statistical comparison	P-value
Initial vs Final wt. at 2nd week in group-A1	P<0.001****	B1 vs A1	P<0.01***
Initial vs Final wt. at 4th week in group-A2	P<0.001****	C1 vs B1	P<0.01***
Initial vs Final wt. at 6th week in group-A3	P<0.001****	C1 vs A1	P>0.05*
Initial vs Final wt. at 2nd week in group-B1	P<0.01***	B2 vs A2	P<0.01***
Initial vs Final wt. at 4th week in group-B2	P<0.05**	C2 vs B2	P<0.001****
Initial vs Final wt. at 6th week in group-B3	P<0.01***	C2 vs A2	P>0.05*
Initial vs Final wt. at 2nd week in group-C1	P<0.01***	B3 vs A3	P<0.001****
Initial vs Final wt. at 4th week in group-C2	P<0.01***	C3 vs B3	P<0.001****
Initial vs Final wt. at 6th week in group-C3	P<0.001****	C3 vs A3	P>0.05*

Key: Insignificant*, Significant**, Moderately Significant***, Highly Significant***

Table No.2: *Mean Plasma Level of ACTH (pg/ml) in Different Groups of Albino Rats at Variable Time Intervals

Group	Sub-groups	Treatment Given	Plasma level of ACTH		
			2nd week	4th week	6th week
A	A1 (n=5)	Control	153.20 ± 2.31		
	A2 (n=5)			158.401± 1.93	
(n=15)	A3 (n=5)				162.80 ± 1.77
B	B1 (n=5)	Heat	355.60 ± 8.22		
	B2 (n=5)			359.20 ± 2.08	
(n=15)	B3 (n=5)				361.40 ± 3.01
C	C1 (n=5)	Heat+Cyano-cobalamin	165.0 ± 4.04		
	C2 (n=5)			166.60 ± 6.45	
(n=15)	C3 (n=5)				171.80 ± 3.59

Statistical Analysis of Mean Levels of ACTH in Different Groups of Albino Rats

Statistical comparison	P-value	Statistical comparison	P-value
B1 vs A1	P<0.001****	C2 vs B2	P<0.001****
C1 vs B1	P<0.001****	C2 vs A2	P>0.05*
C1 vs A1	P>0.05*	B3 vs A3	P<0.001****
B2 vs A2	P<0.001****	C3 vs B3	P<0.001****
		C3 vs A3	P>0.05*

Key: Insignificant*, Significant**, Moderately Significant***, Highly Significant****

DISCUSSION

The present study demonstrated that heat-stress in group B animals mediate destructive changes reflected as a significant decrease in the body weight and increase in the plasma ACTH level.

ACTH secretion is essential for survival when the stress is severe. Hyper activation of HPA axis causes excessive corticosteronesecretion that leads to protein catabolism and increased gluconeogenesis in the liver to withstand the effects of stress and to stabilize the existence of an organism in a steady but at the expenses of decrease body and organ weight[17].

The decrease in the body weight could be due to the decreased feed intake due to high ambient temperature [18], decreased activity of digestive enzymes [15,19], or may be due to stress factors that cause an increase in free radicals and the release of ACTH in circulation [15].Findings of our study are parallel with the study of the Virden etal, who suggested that weight loss is the most detrimental effect of prolonged heat lossoccurs due to the catabolism of the structural proteins induced by the ACTH and corticostcrone[20]. Our findings are also in accordance with kokoetal, who observed a significant rise in plasma ACTH in Wistar rats exposed to heat for 60 minutes[21].

Animals in group C treated with Cyanocobolamin and heat gained moderately significant increase in body weight and highly significant decrease in plasma ACTH level.These findings could be due to the cytoprotective effects of the Cyanocobalamin to promote the growth as described by the Guerra shinobaraand in accordance with our study[22].Gain in body weight is in accordance with another study[23], in whichCyanocobalamin directly reacts with the free radicals to prevent the cell damage at molecular level and prevent the apoptosis by inhibiting the cleavage of caspases-3.Plasma ACTH sections returns near to normal values, it might be due to direct or indirect inhibitory effect of the Cyanocobalamin on the ACTH session and this finding is similar to lovgren etal described inhibitory effects of vitamin B-12 on ACTH and costicosterone[24]

CONCLUSION

Findings of the current study reveals that overheating in any form damages the body structures and its consequences leads to decrease in body weight.Whereas the prophylactic use of the Cyanocobalamin is beneficial to alleviate the detrimental effects of the heat-stress.

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