Repeated Acute Stress Alters Activity of Serum Aminotransferases and Lactate Dehydrogenase in Rat

*Devaki , M. *, Nirupama, R. & Yajurvedi, H. N.*

Abstract

Serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) and lactate dehydrogenase (LDH) activities increased significantly in rats restrained (RS) for 1 hour and exposed to force swimming exercise (FS) for 15min after an interval of 4 h thereby indicating tissue damage due to stress. The results further reveal that after initial stress exposure rats do respond to a second stressor either with increased intensity (SGPT activity) or at the level similar to first exposure (LDH & SGOT activity).(**JPBS 2010;Volume 23 No.2:1-4**)

Key Words: *SGOT, SGPT, LDH, Stress*

Different types of acute stressors cause an increase in activities of serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) in humans and laboratory animals which is an indication of tissue damage. For instance, 16 h exercise in rats caused a marked rise in activity levels of serum LDH, GOT and GPT[1, 2]. A significant increase in SGOT and SGPT activities in rats is reported following exposure to flickering of light of 80 lux intensity for 30 minutes duration[3]. Similarly foot shock for 12 h.[4], ether exposure for 1 minute [5], water-immersion for 6 h[6, 7, 8, 9] or 4 h[10] in rats and restraint for 12 minutes in pigs [11] and tape-immobilization for 1 minute in mice [12] lead to a significant increase in activity of these enzymes. Stress due to surgery [13] and severe exercise [14] caused a significant elevation in the activity of SGOT, SGPT and LDH in humans. These studies reveal the effect of a single stressor on the activity of these enzymes. However, whether exposure to a second stressor after an initial stressful experience augments stress response or animals get habituated and fail to respond is not known. Hence the present study aims at investigating effect of restraint followed by forced swimming exercise applied after an interval of 4h on SGPT, SGOT and LDH activity to understand whether or not exposure to two stressors within a day augment stress response in rat.

Materials and methods

Adult male Wistar rats (25) weighing 180-220 g were obtained from inbred colony of central animal facility and five rats were killed prior to commencement of the experiment (initial control) and remaining rats were randomly divided into two groups (10 rats each), controls and stress group. The rats were provided with standard rat chow and tap water *ad libitum* and were housed on a 12h: 12h light and dark cycle. The experimental protocols were approved by the Institutional Animal Ethics Committee and guide lines of the committee for care and maintenance of animals were followed. Controls were maintained in their cages without any disturbance whereas rats in stress group were exposed to two stressors. The rats were restrained (RS) for 1h by placing each rat in an open cylindrical restrainer measuring 6.7cm in diameter and 22.3 cm in length and kept in a clean cage with bedding material. After a gap of 4 h each rat in stress group was forced to swim in a glass chromatography jar (18'' height X 8.75'' outer diameter) filled 2/3rd full of water (forced swimming exercise, FS) for 15 minutes at room temperature. Two hours after RS, 5 rats in control and stress group were killed under ether anesthesia and remaining rats were similarly killed 4 h after FS.

At each autopsy, the adrenal gland and blood samples were collected for assay of different enzymes. The blood was collected by heart puncture and serum was separated and used for assay of glutamate oxaloacetate transaminase (GOT) [15], glutamate pyruvate transaminase (GPT) [16] and lactate dehydrogenase (LDH) [17]activity .The adrenal gland homogenate was used for assay of 3ß- hydroxyl steroid dehydrogenase (3ß- HSDH) [18] activity. All data were expressed as the mean ± SE and one way analysis of variance (ANOVA) followed by Duncan's multiple range test were used to test the significant difference between mean values of different groups.

Results

There was a significant increase in the adrenal 3β-HSDH activity following RS which showed further significant elevation after exposure to FS compared to controls (Table 1).The activity levels of SGOT and LDH, determined after an interval of 2 h after RS showed a significant increase over controls and

Department of Zoology, University of Mysore, Manasagangotri, Mysore- 570 006, India (DM., NR., YHN.)

Corresponding Authors
Devaki, M.
Department of Zoology
University of Mysore
Manasagangotri, Mysore-570 006, India
E-mail : devaki.chm@gmail.com

remained elevated at this level after FS. The SGPT activity, recorded 2 h after RS was significantly increased compared to controls and showed further significant increase after FS compared to that after RS (Table 1).

Discussion

Increased aderenocortical activity is considered as an index of stress response in vertebrates as there is unspecific activation of hypothalamo-pituitary-adrenal

Table 1.Effect of restraint and forced swimming exercise on the activity levels of serum GOT, GPT and LDH and adrenal 3β-hydroxy steroid dehydrogenase in rat.

	Enzyme activity			
Group	**3β-HSDH (nmol/mg/min)**	**SGOT (U/L)**	**SGPT (U/L)**	**LDH (U/L)**
Initial controls (Zero hour)	0.10 ± 0.03^{a}	233.8 ± 14.5^{a}	8.33 ± 0.45^{a}	304.5700 ± 24.83^{a}
2h after restraint (RS)	0.22 ± 0.02^{b}	478.08 ± 31.06^{b}	11.70 ± 0.41^{b}	484 ± 29.64^{b}
Controls for RS group	0.11 ± 0.02^{a}	246.86 ± 13.58^{a}	8.66 ± 0.78^{a}	313.834 ± 15.57^{a}
4h after forced swimming (RS+FS)	0.28 ± 0.03^{c}	530.18 ± 26.67^{b}	13.53 ± 0.86^{c}	510.66 ± 15.93^{b}
Controls for (RS+FS)	0.11 ± 0.01^{a}	251.4 ± 16.99^{a}	9.41 ± 0.30^{a}	306.32 ± 20.9^{a}
ANOVA (F-Value) df=4,20	15.66 P<0.001	43.92 P<0.001	18.36 P<0.001	22.29 P< 0.001

Note: All values are Mean ±SE; ANOVA, Analysis of variance
Mean values with same superscript letters in a given column are not significantly different, whereas those with different superscript letters are significantly (P<0.05) different as judged by Duncan's multiple test.

steroidogenesis, following RS as well as FS in the present study indicates that the animals were undergoing stressful experience.

An increase in the activity levels of serum GOT, GPT and LDH under different pathological conditions, viz. liver necrosis [20], hepatitis [21], excess alcohol ingestion [22, 23], muscle injury [24] and heart attack [25] has been reported. Stress also causes an increase in the activity of these enzymes[6, 7, 8, 9, 10, 26, 27], there by indicating stress induced tissue injury. Since, SGOT is more specific marker for disruption of cell integrity of the cardiac musculature [28], the stress induced increase in SGOT activity following RS or FS in the present study might be due to leakage of the enzyme from damaged cardiac muscles. Similarly, emotional stress caused an increase in SGOT activity in mice [12]. SGPT is primarily found in the liver, making it a more specific test for detecting liver abnormalities. Increase in serum LDH activity is mainly due to the release from damaged liver, heart and skeletal muscles into bloodstream. In the

present study, elevated activities of serum GOT, GPT and LDH demonstrated tissue injury in rats exposed to RS for 1h followed by FS for 15minutes after an interval of 4 hours. Similarly, rotating drum exercise in rats for 16h resulted in 2-6 fold increase in activities of serum LDH, GOT, GPT [1, 2] and elevation in LDH and GOT activities in the rats exposed to ether for 1 minute[5]**.** Further, foot shock for 12 h[4] and water immersion restraint [6, 7, 8, 9, 10] in rats**,** restraint in pigs[11] and immobilization in mice[12] resulted in the elevated levels of LDH, GOT and GPT activities. It is evident from early reports that alterations in enzymes activity levels indicating tissue injury were observed following one time exposure of animals to a acute stressor. However, present study reveals either increased response (SGPT) or persistence of altered activity (SGOT & LDH) following exposure to a second stressor. Hence, the study indicates that after initial stress exposure, animals do respond to second stressor. Further total duration of altered enzyme activity due to two exposures was approximately more than 6 h, thereby indicating that repeated stress episodes cause tissue injury for a prolonged period. The observation has relevance to human situations, wherein a person is exposed to different types of stressors within a day which might result in marked tissue damage.

References

1. Atland DP, Highman B. Effect of exercise on serum enzyme values and tissues of rats. Am J Physiol, 1961; 201: 393-395.
2. Garbus J, Benjamin, Highman, Altland DP. Serum enzymes and lactic dehydrogenase isoenzymes after exercise and training in rats. Am J Physiol. 1964; 207: 467-472.
3. Lalitha R, Suthanthirarajan N , Namasivayam A. Effect of flickering light stress on certain biochemical parameters in rats. Ind J Physiol Pharmacol.1988; 32(3):182-186.
4. Miller GD, Mallov S. Quantitative determination of stress induced myocardial damage in rats. Pharma Biochm & Behavior. 1977; 7: 139-145.
5. Gartner K, Buttoner D, Dohler K, Friedel R, Lindena J , Trautschold I. Stress response of rats to handling and experimental procedures. Lab. Ani. 1980; 14**:** 267.
6. Arakawa H, Kodama H, Matsuoka N , Yamaguchi I. Stress increases plasma enzyme activities in rats differential effects of adrenergic and cholinergic blockades. J Pharmacol Exp Ther.1997; 280:1296-1303.
7. Iwai K, Onodera A, Matsue H. Antioxidant activity and inhibitory effect of Gamazumi (Viburnum dilantaum THUNB) on oxidative damage induced water immersion restraint stress in rats. Int J Food Sci Nutr. 2001; 52: 443-451.
8. Ohta Y, Chiba S, Tada M, Imai Y , Kitagawa A. Development of oxidative stress and cell damage in the liver of rats with water-immersion restraint stress. Redox Rep. 2007; 12(3): 139-147.
9. Ohta Y, Kaida. S, Chiba S, Tada M, Teruya A, Imai Y, Kawanishi M. Involvement of oxidative stress in increases in the serum levels of various enzymes and components in rats with water immersion restraint stress. J Clin Biochem Nutr. 2009; 45: 347-354.
10. Starec M, Fierova A, Rosina J, Malek J, Kriak M. Effect of Agroclavine on NK activity *in Vivo* under normal and stress conditions in rats. Physiol Res. 2001; 50*:* 513-519.
11. Thoren T K, Jonsson L. Creatine kinase isoenzymes in serum of pigs having myocardial and skeletal muscle necrosis. Can J Comp Med. 1983; 47: 207-216.
12. Sanchez O, Arnau A, Pareja M, Poch E, Ramırez I, Soley M. Acute stress-induced tissue injury in mice: differences between emotional and social stress. Cell Stress & Chaperones. 2002; 7 (1): 36-46.
13. Ota K, Namiki A, Takahashi I, Iwasaki H, Ujike Y. Effects of ulinastatin on operative stress in major surgery. Masui. 1989; 38(4): 540-545.
14. Kayashima S, Ohno H, Fujioka T, Taniguchi N , Nagata N . Leucocytosis as a marker of organ damage induced by chronic strenuous physical exercise. Eur J Appl Physiol. 1995; 20(5): 413-420.
15. Jenkins TW, David A, Yphantis, Sizer WI. Glutamic aspartic transaminase. J biol chem. 1958; 51-57.
16. Segal LH, fDiana S, Beattie, Hopper. S. Purification and Properties of Liver Glutamic-alanine transaminase from normal and corticoid-treated rats. J biol chem. 1962; 237.
17. Clark BJ, Nicklas WJ. The metabolism of rat brain mitochondria. J biol chem. 1970; 245: 4724-4731.
18. Shivanandappa T , Venkatesh S. A colorimetric assay method for 3β-hydroxy-δ^{5-} steroid dehydrogenase. Anal. Biochem. 1997; 254: 57.
19. Tsigos C , Chrousos GP. Hypothalamic- pituitary-adrenal axis, neuroendrocrine factors and stress. J Psychosom Res. 2002; 53(4): 865-971.
20. Kim JH, Oh SW, Kim JD , Choi YE. Abundance of immunologically active alanine aminotransferase in sera of liver cirrhosis and hepatocellular carcinoma patients. Clin chem. 2009; 55(5): 1022-1025.
21. Kaito, Sato S, Nakiamura T, Ishii T , Onodera H. Clinical evaluation of LDH isoenzyme in hepatic disease. J Gastroenterology. 1970; 5(1): 66.
22. Alatalo PI, Koivisto MH, Hietala PJ, Puukka SK, Bloigu R , Niemelia JC. Effect of moderate alcohol consumption on liver enzymes increases with increasing body mass index. Alcohol & Alcoholism. 2008; 88(4) :1097-1103.
23. Alatalo PI, Koivisto MH, Puukka SK, Hietala PJ, Anttila P, Bloigu R , Niemelia JC. Biomarkers of liver status in heavy drinkers, moderate drinkers and abstainers. Alcohol & Alcoholism. 2009; 44(2):199-203.

24. Singh SR, Agrawal PS, Dikshit KS. Serum enzymes in nutritional muscular wasting. I J Pediatrics. 1972; 39(12): 383-388.
25. Honda. Serum lactic acid dehydrogenase isoenzyme determination in myocardial infraction postgrand. med. J. 1967; 43 : 141-145.
26. Raab W, Chaplin JP & Bajusz E, Myocardial necroses produced in domesticated rats and in wild rats by sensory and emotional stresses, Proc Soc Exp Biol. 1964; 116: 665.
27. Matsuoka N, Arakawa H, Kodama H & Yamaguchi I, Characterization of stress-induced sudden death in cardiomyopathic hamsters, J Pharmacol Exp Ther.1998; 284:125.
28. Fontes J P, Goncalves M, & Ribeiro V G, Serum markers for ischemic myocardial damage, Rev Port Cardiol.1999;18: 1129.