

ORIGINAL ARTICLE

REPEATED ACUTE STRESS INDUCED ALTERATIONS IN CARBOHYDRATE METABOLISM IN RAT

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

Department of Zoology, University of Mysore, Mysore-570 006, India

*Telephone no. : 0821 2419778 (O)
09740970928 (Mob)

*Email- hnyajurvedi@rediffmail.com

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Acute stress induced alterations in the activity levels of rate limiting enzymes and concentration of intermediates of different pathways of carbohydrate metabolism have been studied. Adult male Wistar rats were restrained (RS) for 1 h and after an interval of 4 h they were subjected to forced swimming (FS) exercise and appropriate controls were maintained. Five rats were killed before the commencement of the experiment (initial controls), 5 control and equal number of stressed rats were killed 2 h after RS and remaining 5 rats in each group were killed 4 h after FS. There was a significant increase in the adrenal 3 β - hydroxy steroid dehydrogenase activity following RS, which showed further increase after FS compared to controls and thereby indicated stress response of rats. There was a significant increase in the blood glucose levels following RS which showed further increase and reached hyperglycemic condition after FS. The hyperglycemic condition due to stress was accompanied by significant increases in the activities of glutamate- pyruvate transaminase, glutamate- oxaloacetate transaminase, glucose -6- phosphatase and lactate dehydrogenase and significant decrease in the glucose -6- phosphate dehydrogenase and pyruvate dehydrogenase activities, whereas pyruvate kinase activity did not show any alteration compared to controls. Further, the glycogen and total protein contents of the liver were decreased whereas those of pyruvate and lactate showed significant increase compared to controls after RS as well as FS.

The results put together indicate that acute stress induced hyperglycemia results due to increased gluconeogenesis and glycogenolysis without alteration in glycolysis. The study first time reveals that after first acute stress exposure, the subsequent stressful experience augments metabolic stress response leading to hyperglycemia. The results have relevance to human health as human beings are exposed to several stressors in a day and such an experience might lead to insulin resistance because prolonged hyperglycemic condition is known to cause insulin resistance.

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The results put together indicate that acute stress induced hyperglycemia results due to increased gluconeogenesis and glycogenolysis without alteration in glycolysis. The study first time reveals that after first acute stress exposure, the subsequent stressful experience augments metabolic stress response leading to hyperglycemia. The results have relevance to human health as human beings are exposed to several stressors in a day and such an experience might lead to insulin resistance because prolonged hyperglycemic condition is known to cause insulin resistance.

Key words: carbohydrate metabolism / Gluconeogenesis / hyperglycemia / stress

Acute stress affects carbohydrate metabolism as shown by altered blood glucose levels and activities of carbohydrate metabolizing enzymes. For instance, restraint for 1h (Torres *et al.*, 2001; Zardooz *et al.*, 2006) or 2.5h (Macho *et al.*, 1999); immobilization

for 1h (Odio and Maickel, 1985) or 2h (Kulkarni and Juvekar, 2008); low environmental temperature (Odio and Maickel, 1985); random foot shock (Odio and Maickel, 1985); hypokinesia (Macho *et al.*, 1999); water immersion (Radahmadi *et al.*, 2006) in rats,

snare restraint for 5 minutes in pigs (Neubert *et al.*, 1996) and water spray bath for 15 minutes in cats (Rand *et al.*, 2002) resulted in increased serum glucose levels. Likewise, increase in activities of creatine phosphokinase, lactate dehydrogenase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase following water immersion for 4h (Arakawa *et al.*, 1997) and oxidation of proteins due to immobilization for 8h (Liu *et al.*, 1996) in rats have also been reported.

Though a few studies showed altered enzyme activity due to acute stress, these have not focused on rate limiting enzymes of different pathways of carbohydrate metabolism. Since, altered blood glucose levels due to stress might be combined effect of alterations in different pathways of carbohydrate metabolism; there is a need to investigate the stress-induced alterations in these pathways. Although chronic stress response is known to alter different pathways of carbohydrate metabolism (Khasina and Kirillov, 1986; Nagaraja *et al.*, 2006; Saggu and Kumar, 2007), these studies reveal changes after several days of exposure. Whether or not a short duration exposure induces changes in activity levels of different enzymes of carbohydrate metabolism within a day is not known. Further, following initial stress experience, whether a subsequent stress exposure after an interval of a few hours augments stress response or the system gets habituated and fails to respond is also not known. Hence the present study aims at investigating the effect of restraint followed by forced swimming exercise after an interval of 4h on the activities of rate limiting enzymes of different pathways of carbohydrate metabolism in adult male rats.

MATERIALS AND METHODS

Adult male Wistar rats weighing 200-230g were obtained from inbred population of the Central animal facility and were maintained (3 rats/ cage)

under 12h: 12h light-dark cycle (lights on 7am to 7pm). The rats had free access to standard rat chow and water.

Animals were divided into 3 groups viz., initial controls (n=5), treatment controls (n=10) and stress group (n=10). At the commencement of the experiment, rats in the initial controls were killed by anesthesia. The treatment controls were kept in home cage without any disturbance. The rats in the stress group were exposed to two different types of acute stressors, i.e. restraint (RS) for 1 hour (each rat placed in a cylindrical glass restrainer measuring 22 cm in length and 6.7cm diameter) and 4 hours later to forced swimming exercise (forced to swim in a chromatographic jar of 18" height and 8" diameter with 2/3rd full of the jar filled with water) for 15 minutes. Five rats in stress group and equal number in treatment controls were killed 2 hours after RS and remaining rats in both the groups were killed 4 hours after FS by deep anesthesia.

At each autopsy, blood sample was drawn by heart puncture and the liver, skeletal muscle and the adrenal glands were collected quickly. The tissue and blood samples were stored at -80° C until they were used. One ml blood was used to estimate pyruvate and lactate concentrations (Marbach and Well, 1967). Remaining blood was centrifuged to get serum for determination of glucose levels by GOD-POD method (Tenscher and Richterich, 1971; Barham and Trinder, 1972). The activities of the liver glutamate oxaloacetate transaminase (GOT) (Kit manufactured by Agappe diagnostics, Kerala, India), glutamate pyruvate transaminase (GPT) (Segal, 1962), lactate dehydrogenase (LDH) (Kit manufactured by Agappe diagnostics, Kerala, India), glucose-6-phosphatase (G6Pase) (Wood, 1982), pyruvate kinase (PK) (Immamura and Tanaka, 1982), glucose-6-phosphate dehydrogenase (G6PDH) (Lee, 1982) and pyruvate dehydrogenase (PDH) (Nachlas *et al.*, 1960) were

determined. The total protein content of the liver (Lowry *et al.*, 1951) and glycogen content in the liver and the skeletal muscles (Vies, 1954) were estimated. The activity of the adrenal 3 β -hydroxy steroid dehydrogenase (3 β -HSDH) was determined by the method described by Shivanandappa and Venkatesh (1997).

RESULTS

There was a significant increase in the adrenal 3 β -HSDH activity following RS and further elevation after exposure to FS compared to controls (Fig. 1).

Blood levels of glucose, pyruvate and lactate showed a significant increase following RS as well as FS compared to initial controls and respective treatment control groups. Glucose and lactate levels showed further increase following FS in RS exposed rats whereas pyruvate concentration was significantly lower after FS compared to RS group (Table. 1).

Liver glycogen content was significantly lower after exposure to RS compared to initial controls and treatment controls and there was a further significant reduction after FS compared to controls (Table. 1).

There was a significant increase in skeletal muscle glycogen content following RS compared to controls. However, it did not significantly differ from controls following FS (Table. 1).

The activities of G6Pase and LDH were significantly elevated after RS as well as FS compared to controls. However, the activity of LDH although showed a significant increase over controls after FS, it was significantly lower compared to RS group (Table. 2).

The activity of G6PDH was significantly reduced following RS as well as FS compared to respective controls (Table. 2). The activity of PDH was significantly reduced following RS. However

following FS though its activity was reduced, it was not significant when compared to controls (Table. 2).

The activity of PK was not changed after RS as well as following FS compared to controls (Table. 2).

The activities of GOT and GPT in the liver were significantly higher following RS as well after FS compared to initial controls and respective control groups. However, GPT activity was significantly lower following FS compared to RS group (Table. 3). The total protein content of the liver was significantly reduced following exposure to RS as well to FS compared to respective controls (Table. 3).

DISCUSSION

A familiar stress response in vertebrates is the activation of adrenocortical activity. Acute (Arakawa *et al.*, 1997) or chronic (Zhou *et al.*, 1999) stress causes an elevation in corticosterone levels in mammals. Hence increased adrenocortical activity is considered as an index of stress response in vertebrates. In the present study an increase in the activity of 3 β -HSDH, a key enzyme of steroid genesis, following RS as well as FS indicates stress-induced activation of adrenocortical activity.

Restraint induced increase in the serum glucose level in the present study is comparable to a significant elevation in blood glucose level due to one time exposure to acute stressors viz., restraint (Odio and Maickel, 1985; Kulkarni and Juvekar, 2008); immobilization (Macho *et al.*, 1999); low environmental temperature (Odio and Maickel, 1985); random foot shock (Odio and Maickel, 1985); hypokinesia (Macho *et al.*, 1999); immobilization (Macho *et al.*, 1999); water immersion (Arakawa *et al.*, 1997; Radahmadi *et al.*, 2006); water immersion restraint (Ohta *et al.*, 2009) in rats; snare restraint in pigs (Neubert *et al.*, 1996) and water spray bath in

Table 1: Effects of restraint and forced swimming exercise on substrates of carbohydrate metabolism in rats.

Groups	Concentration of				
	Serum Glucose (mg/dL)	Lactate (mM)	Pyruvate (mM)	Glycogen mg/g	
				Liver	Skeletal muscle
Initial Control	68.14±2.1 ^a	7.28±1.0 ^a	0.24±0.04 ^a	5.78±0.4 ^a	6.35±0.3 ^{a,b}
Stress group , 2h after restraint (RS group)	106.14±1.2 ^b	19.07±2.2 ^b	0.61±0.07 ^b	3.03±0.4 ^b	7.52±0.8 ^b
Controls for RS group	85.88±4.4 ^a	12.05±1.8 ^c	0.17±0.01 ^a	5.74±0.2 ^a	5.23±0.4 ^a
Stress group 4h after forced swimming (RS+FS) group	185.00±4.2 ^c	24.16±1.3 ^d	0.46±0.04 ^c	2.61±0.3 ^b	6.33±0.3 ^a
Controls for RS+FS group	107.12±1.6 ^b	12.98±0.7 ^c	0.12±0.02 ^a	4.5±0.12 ^c	5.39±0.1 ^a
ANOVA	220.79	18.94	25.42	26.23	4.09
F value	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01

All values are mean ± SE

Values with same superscript letters are not significantly different whereas those with different superscript letters are significantly ($P<0.05$) different as judged by Duncan multiple test.

Table 2: Effects of restraint and forced swimming exercise on activities of different enzymes of carbohydrate metabolism.

Groups	Activity of				
	(μmol/ mg/ min)				LDH
	G6Pase	G6PDH	PDH	PK	(U/L)
Initial Control	0.10±0.02 ^{a,b}	0.02±0.01 ^a	0.17±0.03 ^{a,c}	1.0±0.24 ^a	354.3±28.6 ^a
Stress group, 2h after restraint (RS group)	0.16±0.03 ^{b,c}	0.01±0.01 ^b	0.07±0.01 ^b	0.79±0.2 ^a	525.9±19.9 ^b
Controls for RS group	0.09±0.01 ^{a,b}	0.02±0.02 ^a	0.21±0.04 ^a	1.0±0.18 ^a	381.5±31.4 ^a
Stress group ,4h after forced swimming (RS+FS) group	0.21±0.04 ^c	0.01±0.01 ^b	0.05±0.01 ^b	1.06±0.63 ^a	484.1±21.7 ^c
Controls for RS+FS group	0.04±0.01 ^a	0.02±0.01 ^a	0.10±0.01 ^{b,c}	1.21±0.18 ^a	394.3±25.6 ^a
ANOVA	7.05	10.29	8.77	0.20	8.02
F value	P< 0.001	P<0.001	P<0.001	P<0.94	P<0.001

All values are mean ± SE

Values with same superscript letters are not significantly different whereas those with different superscript letters are significantly ($P<0.05$) different as judged by Duncan multiple test.

Table 3: Effects of restraint and forced swimming exercise on total protein content and activity of aminotransferases in the liver.

Groups	Activity of		Total protein content (mg/ml)
	GPT (IU/L)	GOT(U/L)	
Initial control	21.39±3.01 ^a	9.32±0.89 ^{a, b}	1.32±0.08 ^a
Stress group 2h after restraint (RS group)	38.50±5.07 ^b	13.72±0.26 ^c	0.65±0.12 ^b
Controls for RS group	23.38±2.79 ^a	7.73±0.67 ^{a, d}	1.6±0.13 ^a
Stress group 4h after forced swimming (RS+FS) group	38.12±3.11 ^b	11.33±0.54 ^b	0.69±0.16 ^b
Controls for RS+FS group	19.2±3.97 ^a	6.62±1.04 ^d	1.4±0.08 ^a
ANOVA	6.52	14.88	13.46
F value	P<0.002	P<0.001	P<0.001

All values are mean ± SE

Values with same superscript letters are not significantly different whereas those with different superscript letters are significantly ($P < 0.05$) different as judged by Duncan multiple test.

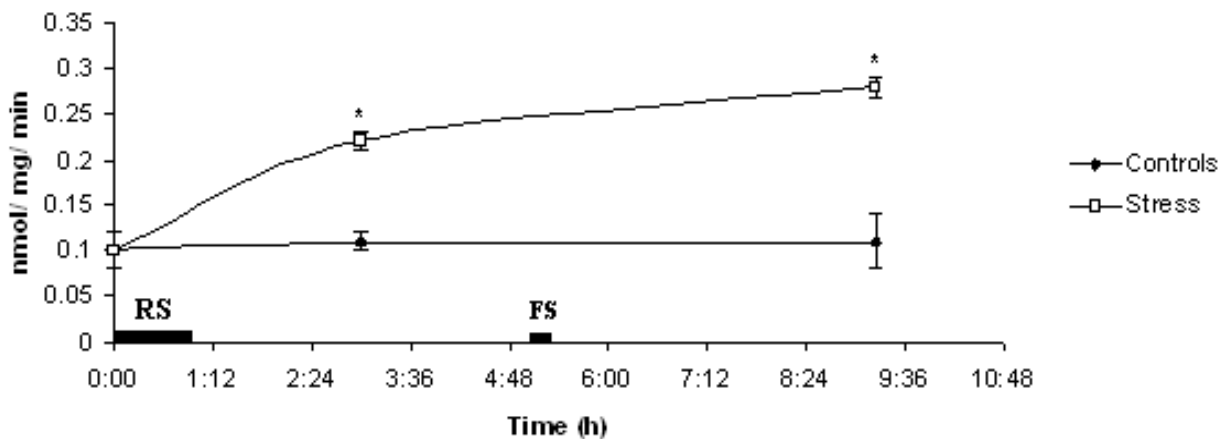


Fig 1: Changes in the adrenal 3β-HSDH activity following exposure to restraint and later to forced swimming in rats. RS, restraint; FS, forced swimming;

*, significantly ($P < 0.05$) different compared to controls.

cats (Rand *et al.*, 2002). However, the present study reveals the fact that exposure to second acute stressor (forced swimming) after a gap of 4 h results in marked hyperglycemia for a period of 4 h. Prevalence

of hyperglycemia for a prolonged period due to stress exposure was not reported earlier as in those studies blood glucose levels were determined immediately after exposure to stress. A significant elevation in

serum glucose levels following RS and further increase due to FS and hyperglycemic condition for a period of 4 h in the present study indicates altered carbohydrate metabolism.

Changes in the activities of key enzymes and substrates of carbohydrate metabolism reveal underlying mechanisms of acute stress-induced elevation in blood glucose level. Though, earlier studies have shown increased activity of LDH, SGPT and SGOT following exposure to acute stressors viz., rotating drum (Altland and Highman, 1961), restraint (Pearl *et al.*, 1966), water immersion (Arakawa *et al.*, 1997) and water immersion restraint (Ohta *et al.*, 2009), these studies do not reveal the changes in the concentration of substrates and the activities of other key enzymes of carbohydrate metabolism. Hence, it is difficult to comprehend the effects of acute stressor on carbohydrate metabolism from these studies whereas, the present study considers stress induced alterations in different pathways of carbohydrate metabolism. Reduction in the liver glycogen content following RS and further reduction due to FS accompanied by increased blood glucose levels indicate glycogenolysis which might have contributed for elevated blood glucose level. A similar depletion in the hepatic glycogen content due to hyper-G-stress has been reported by Daligcon and Oyama (1985). However, glycogenolytic pathway might not sustain hyperglycemia for a prolonged period. Hence another source of glucose is essential. Indeed the present study provides evidence for the activation of gluconeogenesis due to acute stress. Elevated level of glucose in the present study was accompanied by a significant increase in the activity of G6Pase, a key enzyme of gluconeogenesis following RS and showed further increase after FS indicating increased gluconeogenesis. Availability of substrates is also necessary to support prolonged gluconeogenesis due to stress. An increase in concentration of pyruvate in blood, a substrate for gluconeogenesis, increased

activity of aminotransferases (GPT and GOT) in the liver which split glucogenic amino acids to form substrates like pyruvate and oxaloacetate and increased activity of LDH in liver, which converts lactate to pyruvate accompanied by increased lactate concentration reveal increased supply of substrates for gluconeogenesis. In addition reduced PDH activity following RS and FS and unaltered activity of PK under these conditions indicate unaltered glycolytic pathway leading to pyruvate formation and reduced flow of pyruvate towards TCA cycle, which may be an attempt to increase the availability of substrates for gluconeogenesis. Further, reduction in G6PDH activity following RS and FS is indicative of reduced glucose utilization through pentose phosphate pathway which might contribute for increased oxidative stress as reported by Zhang *et al.*, 2000. The results put together reveal that an increased gluconeogenesis and glycogenolysis and unaltered glycolytic pathway lead to hyperglycemic condition following exposure to acute stressors. Alteration in blood glucose levels accompanied by altered activities of carbohydrate metabolizing enzymes has also been reported following chronic stress. For instance, restraint for 1, 5, 10, 20, 30, 45, 60, 70 and 90 days in rats caused an elevation in blood glucose levels accompanied by increases in activities of hexokinase, G6PDH, G6Pase and PEPCK (Khasina and Kirillov, 1986). Similarly, restraint for 3 h/ day for 3 days (Zhou *et al.*, 1999) or 1 h/ day for 7 days (Nagaraja *et al.*, 2006) and exposure to unpredictable stressors for 10 days (Nayantara *et al.*, 2009) in rats resulted in increased activity of SGOT, SGPT and LDH concomitant with elevation in blood glucose levels. On the other hand, decrease in hexokinase, G6PDH, citrate synthase due to cold-hypoxia-restraint was observed in rats (Saggu and Kumar, 2007). Overall changes in different pathways of carbohydrate metabolism due to stress cannot be ascertained from these studies because these studies

have considered one or another group of enzymes. The present study for the first time demonstrates the overall changes in the carbohydrate metabolism due to acute stress exposure. Our study also reveals that changes in certain enzyme activities (G6PDH, G6Pase, SGOT, SGPT and LDH) that are observed due to chronic stress exposure after several days are also evident due to acute stress within a day. The fact that elevation in blood glucose levels following RS changed to hyperglycemic condition due to exposure to second stress exposure (FS) which was also accompanied by altered activity levels of different enzymes of carbohydrate metabolism, indicates that animals do not get habituated after exposure to first stressor; instead the response is augmented after the initial exposure.

Metabolic stress responses are mediated by hormones of the adrenal gland (Eisenstein, 1973; Mazurkiewicz- Kwilecki and Bielkiewicz, 1982; Sahin and Gumuslu, 2007). Elevation in glucocorticoid levels due to water immersion in humans was correlated with increased activities of blood LDH and CPK (Tigranyan, 1975). Similarly exposure to ether for 1 minute in rat was accompanied by elevation in LDH and GOT activities (Gartner *et al.*, 1980). Further, exogenous administration of corticosterone to normal animals induced alterations in enzyme activities similar to those found due to stress. For instance, administration of corticosterone reduced the activity of pyruvate kinase and increased the activity of LDH in rats (Hoyer and Lannert, 2008); elevated the activity of G6Pase in rats (Weber *et al.*, 1964) and sheep (Filsell *et al.*, 1969) and increased the activities of G6Pase and GOT in the liver and kidney of sheep (Fowder *et al.*, 1993).. Similarly infusion of epinephrine caused an inactivation of pyruvate kinase in rats (Stifel *et al.*, 1974.) In addition increased synthesis of gluconeogenic enzymes due to administration of glucocorticoids was also reported

(Weber *et al.*, 1964). Recent studies have shown increased expression of genes coding for PEPCK and G6Pase following administration of glucocorticoids (Andrews and Walker, 1999; Wang, 2005). Hence the altered carbohydrate metabolism in the present study might be due to adrenal hormones as increased steroidogenic activity was accompanied by alterations in the activities of different carbohydrate metabolizing enzymes.

Exposure to hyperglycemic condition is known to alter insulin sensitivity of cells. Infusion of 50% glucose (Miles *et al.*, 1998) or 10% dextrose (Midaoui and Champlain, 2002) resulted in insulin resistance in rats. Similarly infusion of 50% dextrose for 6 h in rats resulted in reduced insulin stimulated glucose uptake determined by hyperglycemic-euglycemic clamp technique and thereby indicated the development of insulin resistance (Haber *et al.*, 2003). These *in vivo* studies are supported by *in vitro* studies. For instance, exposure of cultured adipocytes to 15mM glucose resulted in reduction of phosphorylation of insulin stimulated receptor tyrosine kinase (Kroder *et al.*, 1996; Tang *et al.*, 2001). Similarly, exposure of rat adipocytes to 25mM glucose resulted in insulin resistance (Rossetti *et al.*, 1987; Kurowski *et al.*, 1999). Hyperglycemia induced by critical illness, sepsis and surgery is known to cause insulin resistance in humans (Robinson and Lindsay, 2004; Rezvanfar *et al.*, 2009). These reports put together clearly reveal deleterious effect of hyperglycemic condition, as insulin resistance is a major cause for type 2 diabetes. Hence, present study has relevance to life situations in human society wherein, human beings are exposed to different stressors several times in a day and the study reveals that repeated stress exposure can result in prolonged hyperglycemia which might lead to insulin resistance.

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