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RESEARCH ARTICLE

Behavioral and Neurochemical Studies in Stressed and Unstressed Rats Fed on Protein, Carbohydrate and Fat Rich Diet

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ABSTRACT

Stress produces behavioral and neurochemical deficits. To study the relationship between adaptation to stress and macronutrient intake, the present study was designed to monitor the effects of different diets on feed intake, growth rate and serotonin (5-Hydroxytryptamine, 5-HT) metabolism following exposure to restraint stress in rats. Rats were divided into four groups (n=12) as control, sugar, protein and fat rich diet fed rats. After 5 weeks of treatment animals of each group were divided into unrestrained and restrained animals (n=6). Rats of restrained group were given immobilization stress for 2 hours/day for 5 days. Food intake and growth rates of unrestrained and restrained rats were monitored daily. Rats were decapitated on 6th day to collect brain samples for neurochemical estimation. Results show that sugar diet fed rats produced adaptation to stress early as compared to normal diet fed rats. Food intake and growth rates of unrestrained and restrained rats were comparable on 3rd day in sugar diet fed rats and on 4th day in normal diet fed rats. Stress decreased food intake and growth rates of protein and fat treated rats. Repeated stress did not alter brain 5-HT and 5-HIAA levels of normal diet fed rats and sugar diet fed rats. Protein diet fed restrained rats showed elevated brain 5-HT levels. Fat diet fed restrained rats significantly decreased brain TRP and 5-HIAA levels. Finding suggested that carbohydrate diet might protect against stressful conditions. Study also showed that nutritional status could alter different behaviors in response to a stressful environment.

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INTRODUCTION

Stress describes a general reaction of mammalian central nervous system which plays a vital role in the way an organism monitors internal conditions in order to attempt to survive (Kaneyuki *et al.,* 1991). The stress response is known to lead to behavior and metabolic changes. Exposure to chronic stress can promote the development of physiological and behavioral dysfunction, including alteration in feeding behavior possibly via serotonergic mechanisms (Samad *et al.,* 2007). Monoaminergic system in the brain is closely involved in behavioral and physiological response to stress (Buwalda *et al.,* 2001).

A number of stress stimuli increased brain tryptophan and 5-HT metabolism (Lowry *et al.,* 2008). If a particular stress is repeated it might result in adaptation (Haleem, 1999; Haleem *et al.,* 2007). Increased brain 5-HT might improve the ability to cope with stress, whereas a decline in brain serotonergic activity was involved in depression (Markus *et al.,* 2000). The metabolic changes to serotonin in response to stress have been considered to develop because of changes in the availability of TRP to brain (Joseph and Kennet, 1983). The uptake of serotonin precursor TRP into the brain has been reported dependent on the nutrients that influence cerebral availability of TRP (Markus *et al.,* 2000). Activity of tryptophan hydroxylase, the initial and rate limiting enzyme for the synthesis of 5-HT also increased in many brain regions of rats exposed acutely to restraint stress (Haleem and Parveen, 1994).

The present study was designed to assess the consequences of a change in dietary macronutrient composition on responses to stress and to investigate whether ingestion of sugar, fat and protein rich diets could help to cope stress demands producing adaptation to stress.

MATERIALS AND METHODS

Animals: Forty eight locally bred albino Wistar male rats (8-10 months) weighing 180-200 grams used for the experiment were purchased from the Aga Khan University Animal House. All animals were housed individually under a 12 hour light-dark cycle (light on at 6:00h) and controlled room temperature (22±2°C) with free access to cubes of standard rodent diet and tap water for 3 days before initiation of experiments so that rats could adapt themselves to the new environment. All experiments were conducted according to a protocol approved by Local (Department of Biochemistry, University of Karachi) Animal Care Ethical Committee.

Preparation of diets: Basal diet (standard rodent diet) available in the form of cubes was crushed. For the preparation of sugar rich diet, crushed basal diet was mixed with table sugar in the ratio of 2:1 (basal diet: sugar w/w) to prepare pallets of sugar rich diet. For the preparation of protein rich diet, crushed basal diet was mixed with minced beef in the ratio of 2:1 (basal diet: minced beef w/w). For the preparation of fat rich diet, animal fat was taken and heated until fat was obtained in liquid form and then crushed basal diet was mixed with it in the ratio of 2:1 (basal diet: animal fat w/w) to prepare pallets of fat rich diet. Pallets for basal diet were also prepared from the same crushed rodent diet without mixing sugar, fat and meat in it.

Experimental protocol: In the beginning of experiment animals were randomly divided into four groups (n=12) as control, sugar, fat and protein rich diet rats. The rats were given their respective diets for 5 weeks and food intake and growth rates were monitored. After 5 weeks of dietary treatment animals of each group was further divided into restrained and unrestrained rats (n=6). The rats of restrained group were given immobilization stress for 2 hours/day for 5 days whereas rats of unrestrained groups were kept as such in their home cages. Feed intake and growth rates of rats were monitored daily. On day 6 immediately after the termination of restraining all rats were decapitated between 10:00 and 11:00h. After decapitation blood was collected in heparinized tubes and centrifuged to get plasma. These plasma samples were then stored below -70°C for estimation of tryptophan (TRP) by high performance liquid chromatography with electrochemical detector (HPLC-EC). Brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation. Fresh brains were dipped in chilled saline (0.9% w/v) and stored at low temperature (-70°C) until the analysis of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and TRP by HPLC-EC. HPLC-EC determination was carried out according to standard procedure (Haleem and Haider, 1996). A 5-II Shim-Pack ODS separation column of 4.0mm internal diameter and150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer at pH 2.9 at an operating pressure of 2000–3000 psi on Schimadzu LEC 6A detector at an operating potential of 0.8V for 5-HT and 5-HIAA and 1.0 V for TRP (Haider *et al*., 2006).

Statistical Analysis: Data were analyzed by two-way ANOVA. Post hoc analysis was done by Newman-Keuls test by the help of software prepared in our laboratory. P values <0.05 were considered significant.

RESULTS

Table 1 and 2 show the effect of repeated restraint stress 2 h/day for 5 days on daily food intake and growth rates in rats treated with normal, sugar, protein and fat rich diet.

In normal rats, stress significantly decreased (P<0.01) the feed intake (Table 1) but the deficits was attenuated after 3rd day than unrestrained controls. Feed intake was increased during 2nd to 5th day compared to 1st day control. Stress significantly decreased the growth rates (Table 2) of normal rats during 1st and 2nd day than unrestrained controls but deficits were attenuated after 2nd day. Growth rate of rats was increased during 3rd to 5th day than respective day one control.

In sugar fed rats, stress significantly decreased (P<0.01) feed intake during 1st and 2nd day than unrestraint controls but the deficits was attenuated after 2nd day. Feed intake was significantly (P<0.01) increased during 2nd to 5th day in unrestrained compared to respective 1st day control rats. Stress significantly decreased (P<0.01) the growth rates of sugar treated rats during 1st day than unrestrained controls but deficits were attenuated after 2nd day. Growth rate of rats increased (P<0.01) during 2nd to 5th day than respective day one control.

In protein fed rats, stress significantly decreased (P<0.01) feed intake (Table 1) during 1st to 5th day than unrestrained controls. Feed intake was significantly increased (P<0.01) during 2nd to 5th day in unrestrained rats compared to 1st day control. Stress significantly decreased (P<0.01) the growth rates (Table 2) during 1st to 5th day compared to unrestrained control.

In fat fed rats, stress significantly decreased (P<0.01) feed intake (Table 1) during 1st to 5th day than unrestrained controls. Stress significantly decreased (P<0.01) the growth rates (Table 2) during 1st to 5th day compared to unrestrained controls.

Figures 1a and b show the effect of repeated restraint stress 2h / day for 5 days on 5-HT and 5-HIAA levels in rats fed normal, sugar, protein and fat rich diet. Stress significantly increased (P<0.01) 5-HT levels (Fig. 1a) in protein rich diet fed rats. Stress induced alteration of brain 5-HT in normal, sugar and fat fed rats were not significant. 5-HT levels were lower (P<0.01) in sugar and protein fed rich and were higher (P<0.01) in fat fed unrestrained than normal diet unrestrained rats.

Stress significantly decreased (P<0.01) 5-HIAA levels (Fig. 1b) in protein and fat rich diet treated rats. Repeated stress did not alter brain 5-HIAA levels in sugar fed diet and normal diet fed rats. 5-HIAA levels were lower (P<0.01) in sugar, protein and fat fed treated unrestrained than normal diet fed unrestrained control rats.

Fig. 2a and 2b show the effect of repeated restraint stress 2h / day for 5 days on plasma TRP and brain TRP in rats fed normal, sugar, protein and fat rich diets. Stress significantly increased (P<0.01) plasma TRP levels (Fig. 2a) in normal diet fed rats. Stress induced alteration of

Table 1: Effect of repeated stress on daily food intake (Means±SD)

Diet	Unrestrained Rats					Restrained Rats				
	1st Day	2nd Day	3rd Day	4th Day	5th Day	1st Day	2nd Day	3rd Day	4th Day	5th Day
Normal diet	9.7±1.35	10.58±0.6	11.55±0.78	11.31±0.8	12.9±0.92	3.5±0.6*	4.4±0.3*+	6.31±0.4*+	11.03±0.5+	12.1±0.8+
Sugar diet	9.98±0.61	12.8±0.7	12.05±0.42	11.03±0.56	12.1±0.5	3.8±0.46*	5.78±0.38*+	11.8±0.9+	10.9±0.7+	12.2±1.2+
Fat diet	10.3±0.4	10.35±0.6	10.05±0.67	10.36±0.5	10.15±0.65	5.1±0.2*	5.36±0.37*	6.01±0.37*	6.13±0.3*	6.08±0.3*
Protein diet	9.76±0.66	11.8+±0.3	12.23+±0.35	11.05±0.6+	14.35±0.73+	5.18±0.3*	5.36±0.37*	6.0±0.3*	7.05±0.4*	9.6±0.56*

Effect of repeated stress 2h/day for 5 days on daily food intake in rats treated with normal, sugar, protein and fat rich diet. Significant difference by Neuman-Keuls test following two-way ANOVA. *P<0.01 from respective day unrestrained rats; +P<0.01 from respective day one control.

Table 2: Effect of repeated stress on growth rates

Diet	Unrestrained Rats					Restrained Rats				
	1st Day	2nd Day	3rd Day	4th Day	5th Day	1st Day	2nd Day	3rd Day	4th Day	5th Day
Normal diet	100.4±1.04	100±1.2	99.9±0.6	101±0.9	100.3±0.9	97±2.3*	98.1±2.47*	98.8±1.49+	99.4±2.35+	99.6±1.23+
Sugar diet	99.6±1.6	100.5±1.2	99.28±1.76	101.7±1.76	100±2.1	95.05±0.7*	98.8±0.97+	99.5±0.8+	99.4±2.5+	98.4±2.01+
Fat diet	100.1±1.38	101±1.6	101.7±1.19	101±0.8	101.6±1.03	96.3±2.5*	97.8±1.31*	98.7±1.58*	98.3±0.4*	98.8±0.56*
Protein diet	102±0.99	102±1.2	101±1.29	101.4±0.94	102+±0.3	96.6±0.8*	97.3±0.77*	97.9±1.13*	98±1.5*	98.0±0.76*

Effect of repeated stress 2h/day for 5 days on growth rates in rats treated with normal, sugar, protein and fat rich diet. Values are means ± S.D. Significant difference by Neuman-Keuls test following two-way ANOVA. *P<0.01 from respective day unrestrained rats; +P<0.01 from respective day one control.

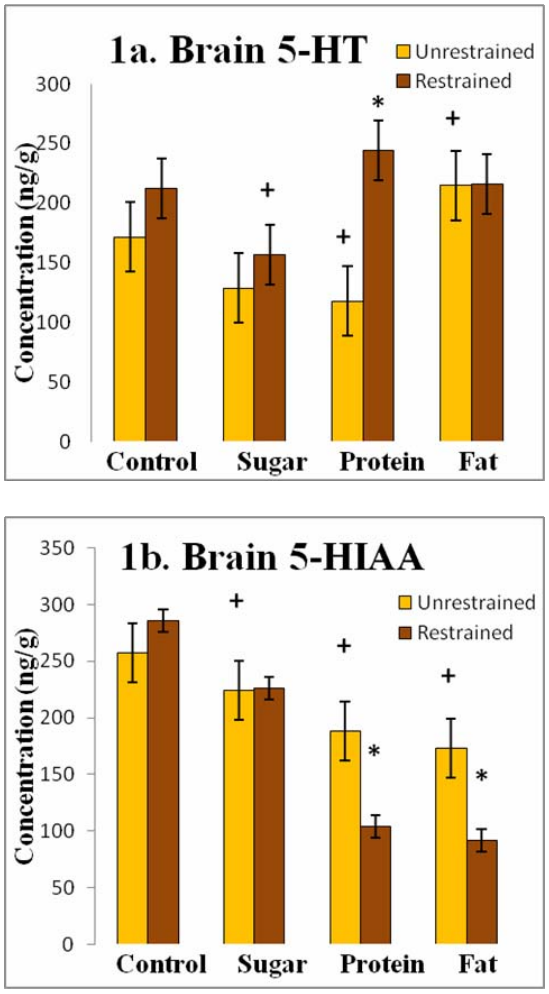

Fig. 1: Effect of repeated restraint stress 2h/day for 5 days on brain 5-HT (Fig. 1a) and brain 5-HIAA (Fig. 1b.) in rats treated with normal, sugar, protein and fat rich diet. Values are means ± S.D. Significant difference by Neuman-*Keuls test* following two- way ANOVA. *P<0.01 from respective diet treated unrestrained rats; + P<0.01 from normal diet treated unrestrained control rats.

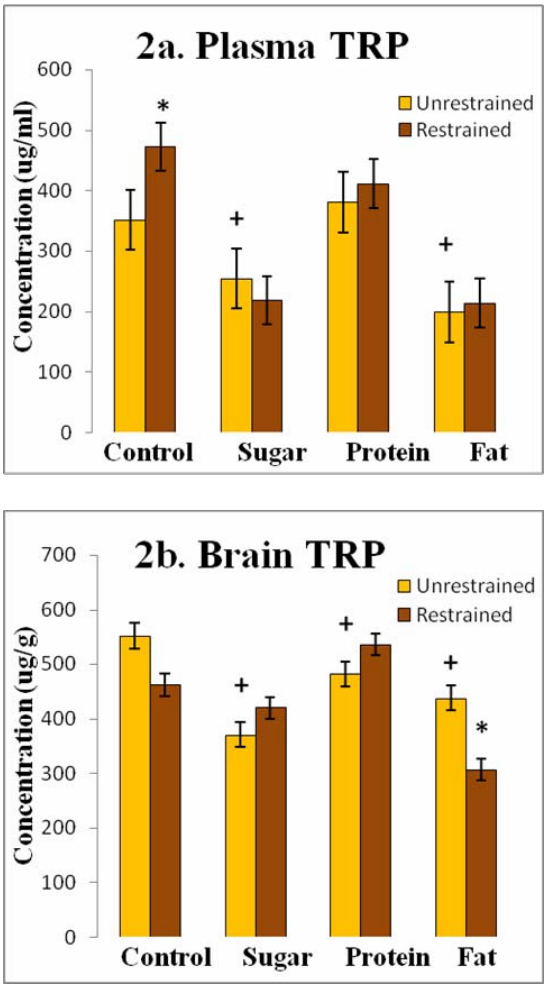

Fig. 2: Effect of repeated restraint stress 2h/day for 5 days on plasma TRP (Fig. 2a) and brain TRP (Fig. 2b.) in rats treated with normal, sugar, protein and fat rich diet. Values are means ± S.D. Significant difference by Neuman-*Keuls test* following two- way ANOVA. *P<0.01 from respective diet treated unrestrained rats; +P<0.01 from normal diet treated unrestrained control rats.

plasma TRP levels in sugar, protein and fat rich diet treated rats were not significantly different. Plasma TRP levels significantly decreased (P<0.01) in sugar and fat rich diet fed unrestrained than normal diet fed unrestrained animals. Stress significantly decreased (P<0.01) brain TRP levels (Fig. 2b) in fat rich diet fed rats. Stress induced alteration of brain TRP levels in sugar, protein and normal diet fed rats were not significant. Brain

TRP levels significantly decreased ($P<0.01$) in sugar, protein and fat rich diet fed unrestrained than normal diet fed unrestrained control rats.

DISCUSSION

In the present study, repeated restrained stress of 2h/ day for five days decreased food intake and growth rate but these animals exhibited normal food intake and growth rate after 3rd day of the treatment. These results suggested that adaptation to a stress schedule occurred when similar type of stress was administered repeatedly. These results are in agreement with those reported previously (Haleem and Parveen, 1994; Samad and Haleem, 2007).

It was reported previously that carbohydrate rich feed diminished depressive mood and cortisol response to stress (Markus *et al.*, 1998) and enhanced memory functions in rats (Jabeen *et al.*, 2007). Milk chocolate often chosen during stress might increase the availability of TRP to the brain and enhanced 5-HT concentration to elevate mood (Parsad, 1998). It was previously reported that male and female subjects to restrained stress and chocolate consumption are differently affected (Fachin *et al.*, 2008). In the present study we have reported that sugar rich diet treated restrained rats exhibited lesser feed intake but it became normal after 2nd day of treatment.

Previous study reported that protein rich diet produced stress induced rise in depression, increased in vigor and cortisol elevation (Markus *et al.*, 1998). It has also been reported that protein rich diet has negative effect on mood and a high proportion of protein has been predicted to greater depression in rats under laboratory induced stress conditions (de Castro, 1987). Previously effect on mood and behavior following dietary composition has not been reported (Deijen *et al.*, 1989). Fat rich diet has been previously reported to decrease feed intake and growth rate by affecting insulin levels (Clegg *et al.*, 2011). In non-stressed animal consuming fat rich diet increased the body weight and adiposity (Paternain *et al.*, 2011). In the present study restrained stress for five days significantly decreased food intake and growth rate in protein and fat rich diet fed rats.

An important role of brain monoaminergic system in behavioral and physiological stress responses has been established (Flügge, 2000). Central nervous system has been shown involved in macronutrient selection (Leibowitz *et al.*, 1989). Therefore, it has been suggested that variations in the macronutrient composition of a diet may affect mood and stress responsiveness (Tannenbaum *et al.*, 1997). The present study reported that repeated immobilized stress for five days did not alter 5-HT and 5-HIAA level in the brain of normal diet treated rats. It has been reported earlier that whole brain and regional 5-HT synthesis increased following 2-3 hours restraint but these increases were not observed in repeatedly restrained rats (Haleem and Perveen, 1994; Haleem, 1999).

Serotonin-releasing brain neurons are unique in that the amount of neurotransmitter released by them is normally controlled by food intake; Carbohydrate consumption- acting via insulin secretion and the "plasma tryptophan ratio" increased serotonin release whereas protein intake lacked this effect (Wurtman and Wurtman, 1995). The present study showed that restrained stress in sugar rich diet fed rats did not alter TRP, 5-HT and 5-HIAA levels. Previously it was reported that restrained induced increase of serotonin metabolism were smaller in sugar rice bran treated rats (Jabeen and Haleem, 2008; Inam *et al.*, 2009). It is, however, possible that long term increase of sugar rich diet might decreased the efficacy of negative feedback control over the synthesis and release of 5-HT (Haleem, 1999; Inam *et al.*, 2008). Long term consumption of sugar diet decreased the effectiveness of presynaptic as well as postsynaptic 5-HT_{1A} receptor dependent responses (Inam *et al.*, 2006). The restrained induced increase in the availability of 5-HT at terminal regions might help coping the stress demand to attenuate stress induced behavioral deficits. Markus *et al.* (1998) reported that carbohydrates prevented a functional shortage of serotonin during acute stress due to their potentiating effect on brain serotonin.

In the present study protein rich diet fed restrained rats showed higher brain 5-HT levels whereas brain 5-HIAA levels were significantly decreased. Present study also reported that fat rich diet fed restrained rats had significantly lower brain TRP and 5-HIAA levels and did not alter brain 5-HT levels. Stress induced activation of sympathetic system has been shown to stimulate lipolysis resulting in activation of non-esterified fatty acids, competing with TRP for binding to albumin thus elevating the plasma pool of TRP (Chaouloff, 1993) and also increased brain TRP uptake by affecting the permeability of blood brain barrier and thereby increased 5-HT metabolism.

It has been concluded that meal consumption depending on the proportion of carbohydrate, fat and protein rich diets can affect brain serotonin release in animals thereby regulating stressful events. Stressful experience could induce neurobiological changes which might impede adequate handling of subsequent stressor. The finding of present study suggested that a carbohydrate rich feed prevented functional shortage of serotonin during stress due to its potentiating effect on brain serotonin to help coping the stress demand and produce adaptation to stress. Thus restrained induced increase in the availability of 5-HT at terminal regions might have attenuated the stress effects.

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