Effect of sugar, protein and fat rich diet on cumulative food intake and brain serotonin metabolism in rats

Samia Moin1, Saida Haider1, M. Abdul Haleem1, Sadia Saleem1, Saima Khaliq2 and Darakhshan J. Haleem1*
1Department of Biochemistry, Neurochemistry and Biochemical Neuropharmacology Research Unit, University of Karachi, Karachi, Pakistan
2Department of Biochemistry, Federal Urdu University, Karachi, Pakistan

Abstract: Central 5-hydroxytryptamine (5-HT; serotonin) system plays a key role in the regulation of eating behaviour; cerebral level of serotonin in animal models is inversely related to food intake and body weight. The present study concerns the effects of long-term consumption of sugar, protein and fat rich diet on food intake, growth rate and brain 5-HT metabolism in rats. The study was conducted on 24 male albino Wistar rats. Rats were divided into three groups, and each group was given a different macronutrient-specific diets including carbohydrate-, protein- and fat-rich diets. Sugar, protein and fat rich diet were prepared by mixing standard rodent diet with table sugar, minced beef and beef fat in the ratio of 2:1 (w/w) respectively. Control rats were fed freely on standard rodent diet whereas the three test group rats were fed freely on their respective diet for 5 weeks. Cumulative food intakes and growth rates were monitored weekly. After 5 weeks of treatment, animals were decapitated to collect the brain samples for the analysis of tryptophan (TRP), 5-HT and 5-Hydroxyindoleacetic acid (5-HIAA) by HPLC-EC. After 5 weeks of treatment, growth rates were significantly decreased (p<0.01) in sugar rich diet treated rats and not altered in protein and fat rich diet treated animals. Brain 5-HT levels were significantly decreased in sugar (p<0.05) and protein (p<0.01) rich diet treated rats and significantly increased (p<0.05) in fat rich diet treated rats. Brain 5-HIAA and TRP levels were significantly decreased following the intake of sugar (p<0.05), protein (p<0.01) or fat (p<0.01) rich diet. Results showed that ingestion of sugar rich diet induced hyperphagia while ingestion of protein and fat rich diet induced hypophagia. The findings are discussed in the context of a role of serotonin in the regulation of appetite.

Keywords: Food intake, eating behaviour, serotonin metabolism.

INTRODUCTION

Large number of data on animals links brain serotonergic system to the regulation of appetite and feeding behavior. Neurochemical research shows that serotonin has a role in the normal termination of feeding1, 2 and in disorders of appetite3. 5-HT is believed to have an inhibitory influence over feeding behavior because drugs that tend to increase 5-HT functions in synaptic cleft decrease food intake in experimental animals1,4,5. Meal consumption depending on the proportion of carbohydrate, protein and fat can enhance or inhibit brain serotonin metabolism in animals thereby affecting appetite regulation6.

Neurochemical research for a relationship between central serotonergic system and feeding were stimulated as certain dietary manipulations were found to alter brain serotonin metabolism7, 8. The synthesis of serotonin depends on the availability of its precursor tryptophan9. It was found that a protein rich diet increased plasma levels of aromatic amino acids and decreased brain serotonin metabolism10, 11. Previous studies reported that fat rich diet increased food intake and elicits weight gain12, 13. Carbohydrate rich diet decreased food intake and had more satiating capacity by increasing 5-HT metabolism13, 14. It has also been reported that sugar rich diet produced hyperphagia and decreased serotonin metabolism in rats15. Decrease in brain serotonin metabolism could increase food intake while an increase in brain serotonin metabolism could suppress appetite to decrease the normal consumption of food16.

The present study was therefore designed to investigate the effects of long-term intake of dietary carbohydrate, protein and fat rich diet on total food consumption, body weight changes and brain 5-HT metabolism in rats. The aim was to investigate the possible neurochemical mechanism behind the macronutrient rich meal, food consumption and serotonin metabolism.

MATERIALS AND METHODS

Animals
Twenty four locally bred albino Wistar rats (180-200g) purchased from Aga Khan University Hospital were used in the study. All animals were housed individually under a 12 h 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 at least 3-4 days before experimentation. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

Experimental procedure
Animals were randomly divided into control and 3 test groups (carbohydrate, protein and fat rich diet treated groups). Sugar, protein and fat rich diet were
prepared by mixing standard rodent diet with table sugar, minced beef and beef fat in the ratio of 2:1 (w/w) respectively. Control rats were fed freely on standard rodent diet whereas the three test group rats were fed freely on their respective diet for 5 weeks. Cumulative food intakes and growth rates were monitored weekly. Rats were decapitated after 5 weeks to collect brain samples. The experiments were performed in a balanced design in such a way that the control and test rats were killed alternatively to avoid the order effect. Brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation. Fresh brains were dipped in chilled saline and stored at low temperature (-70°C) until analysis of 5HT and 5HIAA by HPLC-EC.

**Neurochemical estimations**

HPLC-EC determination was carried out as standard. A 5-II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 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 Shimadzu LEC 6A detector at an operating potential of 0.8 volts for biogenic amines.

**Statistical analysis**

Neurochemical and behavioral data were analyzed by one-way ANOVA. Post hoc comparisons were made using the Newman-Keuls test; p values <0.05 were considered significant.

**RESULTS**

Figure 1 shows weekly food intake of rats consuming normal, sugar rich, and protein rich and fat rich diet. One way ANOVA revealed a significant effect of diet on food intake (F=380, p<0.01). Post hoc comparison by Neuman Keuls test showed that food intake was significantly greater in sugar rich diet (p<0.01; 23%) treated rats and significantly smaller in protein (p<0.01; 17%) and fat rich diet (p<0.01; 30%) treated rats.

Figure 2 shows growth rate of rats consuming normal, sugar rich, protein rich and fat rich diet. One way ANOVA revealed a significant effect of diet on growth rate (F=78, p<0.01). Post hoc comparison by Neuman Keuls test showed that intake of sugar rich diet significantly decreased (p<0.01; 16%) the body weight of rats. Effect of protein (p>0.05) and fat (p>0.05) rich diet on growth rates was not significant.

Figure 3 shows the effect of normal, sugar rich, protein rich and fat rich diet on brain TRP levels in rats. One way ANOVA revealed a significant effect of diet on brain TRP levels (F=78.8, p<0.01). Post hoc comparison by Neuman Keuls test showed that intake of sugar (p<0.01; 30%), protein (p<0.01; 32%), and fat (p<0.01; 19%) rich diet significantly decreased the TRP levels of rats. Protein rich diet decreased brain TRP levels to a greater extent.
Effect of diet on food intake and serotonin metabolism in rats

Figure 3: Effect of sugar rich, protein rich and fat rich diet on brain tryptophan levels in rats. Values are mean±SD (n=6). Significant difference by Newman Keuls test; *P<0.01 vs control group following one way ANOVA.

Figure 4 shows the effect of normal, sugar rich, protein rich and fat rich diet on brain 5-HT levels in rats. One way ANOVA revealed a significant effect of diet on brain 5-HT levels (F=16.8, p<0.01). Post hoc comparison by Newman Keuls test showed that intake of sugar (p<0.05; 24%) and protein (p<0.01; 29%) rich diet significantly decreased the brain 5-HT levels of rats. However fat rich diet significantly increased the brain 5-HT levels in rats (p<0.05; 23%).

Figure 5 shows the effect of normal, sugar rich, protein rich and fat rich diet on brain 5-HIAA levels in rats. One way ANOVA revealed a significant effect of diet on brain 5-HIAA levels (F=12.0, p<0.01). Post hoc comparison by Newman Keuls test showed that intake of sugar (p<0.05; 19%), protein (p<0.01; 30%), and fat (p<0.01; 34%) rich diet significantly decreased the 5-HIAA levels of rats.

DISCUSSION

Evidence accumulated over more than one decade indicates the existence of several neurochemical systems that may influence food intake, appetite for specific nutrients and meal taking behavior. Serotonin, an indoleamine neurotransmitter is thought to play a key role in the suppression of appetite by reducing the rate of eating and size of meal through the intensification of satiety. The present study showed that long-term intake of sugar diet for 5 weeks produced hyperphagia which was associated with a decrease of body weight and 5-HT metabolism. It was reported previously that a decrease in 5-HT metabolism but not an increase in the responsiveness of somatodendritic 5-HT1A receptors is involved in sugar rich diet induced hyperphagia.

Previously it was reported that carbohydrate rich diet increases TRP and 5-HT levels. In the present study levels of brain TRP, 5-HT and 5-HIAA were significantly decreased in rats fed on sugar rich diet for 5 weeks. It has been reported that long-term intake of sugar rich diet produced hyperphagia in rats that was associated with a decrease in brain serotonin metabolism. Pharmacological research shows that drugs that tend to increase serotonin functions via post synaptic 5-HT2C receptors decrease appetite. Conversely stimulation of somatodendritic 5-HT1A receptors elicits hyperphagia in experimental animals because the availability of 5-HT at post synaptic 5-HT2C receptors sites is decreased. The results of the present study are explainable in terms of long-term intake of sugar rich diet decreasing the availability of TRP for the synthesis of 5-HT. It has been reported that metabolism of 5-HT could also be altered by changes of plasma and brain TRP concentration. It could be that TRP is an essential amino acids and its source is only dietary, so long-term intake of carbohydrate rich diet decrease plasma and brain TRP concentration due to less availability of precursor. It may be relevant that carbohydrate craving is the result of decreased 5-HT levels.

In this study, protein consumption for 5 weeks elicited hypophagia and significantly decreased brain TRP, 5-HT and 5-HIAA levels in rats. The hypophagia was not associated with a decrease in body weight. Previous studies reported that protein
consumption provides the plasma with sources of all amino acids\textsuperscript{20}. One of the effects of high protein meal is that less TRP is able to reach the brain. Most protein present in the diet contains amino acids other than TRP. It has been reported previously that protein rich diet increased the concentration of large neutral amino acids (LNAA)\textsuperscript{3} other than TRP\textsuperscript{10, 31}. These LNAA compete with TRP for uptake to the brain, thus decreasing the transport of TRP to the brain for 5-HT synthesis and its conversion to 5-HIAA\textsuperscript{27, 28}. Another explanation could be that the protein rich diet increases the synthesis of catecholamines by increasing the availability of its precursor tyrosine.

Hypophagic effect of fat rich diet as observed in the present study is explainable in terms of an increase in brain 5-HT levels although brain TRP and 5-HIAA levels were also significantly decreased. TRP in the blood is found in two forms, one is free and other is bound to albumin\textsuperscript{24}. The free fraction of TRP in circulation plays a major role in the regulation of available TRP for transport across blood brain barrier\textsuperscript{20}. It is well known that intake of high fat diet increases the lipolysis as a result more free fatty acids released in the plasma, that compete with TRP for binding to albumin as a result more TRP is released into the plasma\textsuperscript{30}. TRP is more likely to cross the BBB, where it is taken up by the serotonergic neurons to activate the synthesis of 5-HT. TRP hydroxylase, rate limiting enzyme of 5-HT synthesis is half saturated with its substrate\textsuperscript{31, 32}, and therefore an increase in 5-HT synthesis occurs. In the present study an increase in brain 5-HT levels although brain TRP concentration and serotonin synthesis remain responsive to energy balance. Eur. J. Clin. Nutr., 1999; 53: 148-165.


Liu RJ, Lambe EK and Aghajanian GK. Somatodendritic autoreceptors regulation of serotonergic neurons:

REFERENCES


23. Liu RJ, Lambe EK and Aghajanian GK. Somatodendritic autoreceptors regulation of serotonergic neurons:
Effect of diet on food intake and serotonin metabolism in rats