ORIGINAL ARTICLE

EFFECTS OF LONG TERM CONSUMPTION OF SUGAR AS PART OF MEAL ON SEROTONIN 1-A RECEPTOR DEPENDENT RESPONSES

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ABSTRACT
In view of an effect of high intake of sugar on brain serotonin (5-hydroxytryptamine, 5-HT) and a role of serotonin in the regulation of appetite, the present study concerns pre and postsynaptic responses to a selective 5-HT-1A receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) following long term consumption of sugar as part of meal in rats. Sugar diet was prepared by mixing standard rodent diet and table sugar in ratio of 3:1 (w/w) and rats were fed freely on this diet for five weeks. Control rats were fed freely on standard rodent diet. After five weeks 8-OH-DPAT at a dose of 0.5mg/kg/ml was injected to both the groups to compare effectiveness of the drug to elicit hyperphagia (presynaptic response) and elicited hyperactivity syndrome (postsynaptic response). Results showed that 8-OH-DPAT-induced forepaw treading and flatbody posture were smaller in sugar than normal diet treated rats. Conversely 8-OH-DPAT-induced hyperlocomotion was greater in sugar than normal diet treated rats. 4h Food consumption was greater in sugar than normal diet treated rats while 8-OH-DPAT-induced hyperphagia significant in normal diet treated rats was not observed in sugar diet treated rats. The results show a decrease in the effectiveness of pre as well as postsynaptic 5-HT-1A receptor dependent responses following long term consumption of sugar diet. Role of serotonin receptor responsiveness on mood and impaired adaptation to stress is discussed.

Keywords: Hyperphagia, serotonin, sugar-diet, somatodendritic 5-HT 1A receptors.

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INTRODUCTION

The effect of sweetness on appetite control is important for the reason of unwanted over consumption associated with the tendency to gain weight. Many food items, for example reduced fat and non-fat items prepared by food industry also derive a relatively high percentage of their energy from sucrose or other sugars. Serotonin (5-hydroxytryptamine, 5-HT) have a role in the normal termination of feeding (Curzon, 1990; Leibowitz and Alexander, 1998; Reis *et al*., 2005) and perhaps also in the disorders of appetite (Kaye *et al*., 2005). Previous studies have shown that high carbohydrate diet elevates brain tryptophan concentration (Fernstrom and Wurtman, 1972) because feeding-induced insulin secretion decreases the circulating levels of large neutral amino acids (LNAAs) which compete with tryptophan for a carrier for transport to the brain (Fernstrom, 1983; Wurtman *et al*., 2003). Increases of brain tryptophan, increase brain serotonin metabolism because the rate limiting enzyme of 5-HT biosynthesis exists unsaturated with its substrate (Fernstrom and Wurtman, 1972; Fernstrom, 1983; Fernstrom and Fernstrom, 1995). Pharmacological research shows that drugs that tend to increase serotonin functions via postsynaptic 5-HT-2C receptors decrease appetite (Haleem, 1993; Schuhler *et al*., 2005). Conversely stimulation of somatodendritic 5-HT-1A receptors elicits hyperphagia in experimental animals (Haleem, 1992; De Vry and Schreiber, 2000) because the availability of 5-HT at postsynaptic receptors is decreased (Liu *et al*., 2005). Previously we have shown that ingestion of sugar diet increased food intake in rats that was associated with a decrease in brain 5-HT metabolism

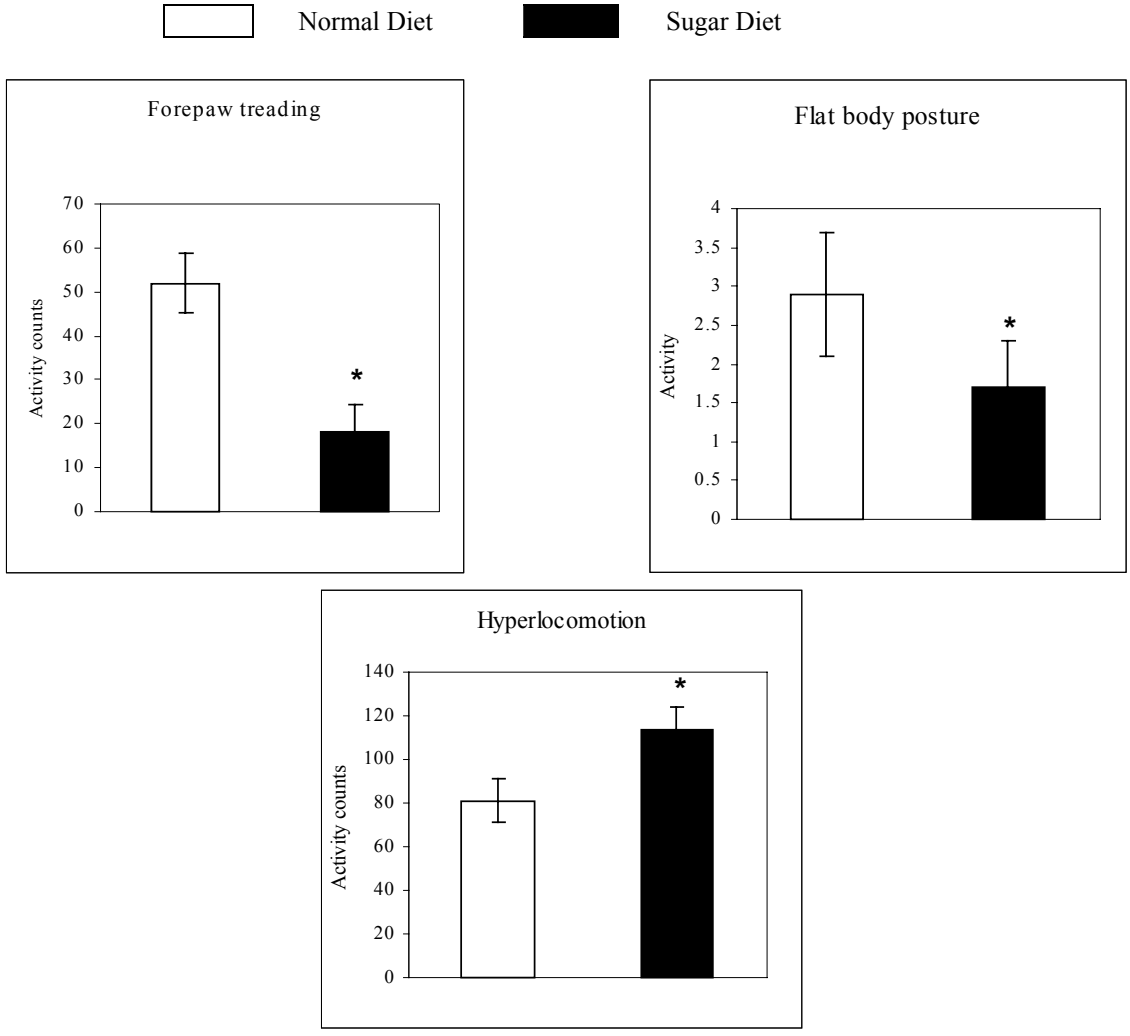

Fig. 1: Components of serotonin syndrome elicited by 8-OH-DPAT (0.5 mg/kg) in normal and sugar-diet treated rats. Values are means ±S.D (four scoring periods each of 1 minute duration). Significant difference by t-test *$p<0.01$ from normal diet + 8-OH-DPAT injected rats.

(Haleem *et al*., 2000). Quantification of 8-OH-DPAT–induced 5-HT syndrome is often taken as a measure of postsynaptic receptor responsiveness (Haleem *et al*., 2002b). The purpose of the present study was to monitor pre and postsynaptic serotonin-1A receptor dependent responses in rats treated with sugar diet. The intensity of 5-HT syndrome and hyperphagia induced by a selective 5-HT-1A agonist 8-OH-DPAT is therefore compared in rats treated with normal or sugar diet.

MATERIALS AND METHODS

Animals and treatment

Twenty-four locally bred male albino Wistar rats weighing 200-230gm purchased from The Agha Khan University, Pakistan, were housed individually under 12h light dark cycle (lights on at 6:00 h) with free access to cubes of standard rodent diet and tap water 3 days before experimentation. Experiments were performed according to a protocol approved by the local animal care committee.

Preparation of sugar diet

The diet was prepared essentially in the same way as described before (Haleem *et al*., 2000).

Standard rodent diet available in the form of cubes was crushed finely. The crush mixed with sugar in the ratio of 3:1 (rat diet: sugar; w/w) was used to prepare pellets of sugar diet. Pellets for normal diet were also prepared from the same crushed rodent diet without mixing sugar in it.

Drug

8-OH-DPAT purchased from Research Biochemical (RBI, USA) was dissolved in saline injected intra peritoneally (i.p) at a dose of 0.5mg/ml/kg bodyweight. Control animals were injected with saline in volume of 1ml/kg bodyweight.

Experimental protocol

Animals were randomly assigned into normal diet and sugar diet treated groups and accordingly weighed amount of respective diet were placed in the hopper of rat's cages. After 5 weeks of treatment animals of the two groups were further divided into saline and 8-OH-DPAT injected subgroups while control animals were injected with saline (0.9% NaCl) between 10:00-11:00 h using a balanced design. 8-OH-DPAT-elicited behavioral syndrome was monitored for 20 minutes, starting 5 minutes postinjection. A weighed amount of food was placed in the hopper of the cages immediately after monitoring the activity. Intake during 4h was monitored.

8-OH-DPAT- Elicited 5-HT syndrome

Normal diet or sugar diet treated rats were transferred in perspex activity cages (26x26x26cm) with sawdust covered floor 15 minutes before injecting 8-OH-DPAT between 10:00 to 11:00 h. the drug was injected i.p at a dose of 0.5mg/kg/ml. Forepaw treading, flat body posture and hyperlocomotion elicited by 8-OH-DPAT were scored as described earlier (Haleem, 1992). The experiment was conducted on a group of four rats at a time. Normal and sugar diet treated rats placed in separate observation cages and injected with 8-OH-DPAT were used in each group in a balanced design. The scoring period (5-25 minutes postinjection) was divided into four sessions of 5 minutes each. During a scoring session each animal was continuously observed for 1 minute and similarly observed for 1 minute at 5 minutes intervals in the next session for a total of 4 scoring sessions. In each session, the number of cage crossings and forepaw treadings were counted. Flat body posture was scored on 0-4 scale of absent to maximum intensity. A total of four scoring periods was later determined.

STATISTICAL ANALYSIS

The data on 5-HT syndrome were analyzed by t-test. Data on food intake were analyzed by two-way ANOVA. Post hoc comparisons were done by Newman-Keuls test, p values < 0.05 were taken to be significant.

RESULTS

Fig. 1 shows the components (forepaw treading, flat body posture and hyperlocomotion) of 5-HT syndrome elicited by 0.5 mg/kg/ml 8-OH-DPAT in normal diet and sugar diet treated rats. Mean values of forepaw treading and flat body posture were significantly smaller ($p<0.01$) while cage crossings (hyperlocomotion) were significantly greater ($p<0.01$) in sugar diet than normal diet treated rats.

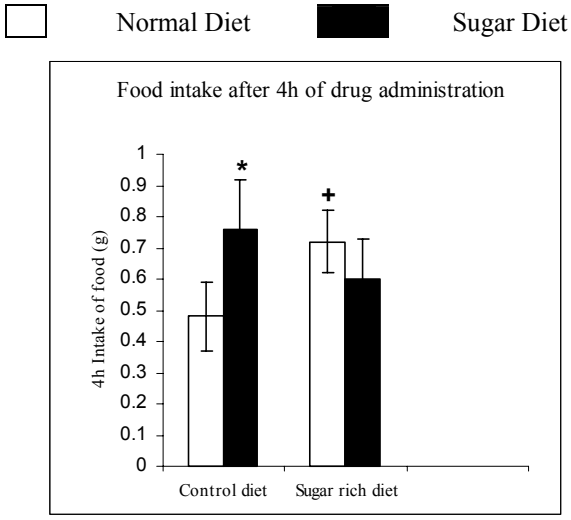

Fig. 2: The effects of 8-OH-DPAT on 4h food intake (g) in normal and sugar-diet treated rats. Values are means ±S.D (n=6). Significant difference by Newman Keuls test *$p<0.01$ from respective saline injected rats, $^{+}p<0.01$ from respective control diet rats.

Fig. 2 shows the effects of 8-OH-DPAT (0.5mg/kg) on 4h food intake of normal and sugar diet treated rats. Two-way ANOVA (df=1,20) revealed that the effects of 8-OH-DPAT (F=2.3) and sugar diet (F=0.45) were not significant ($p>0.05$). Interaction between 8-OH-DPAT and sugar diet (F=14) was significant ($p<0.01$). Post-hoc analysis showed that 8-OH-DPAT increased food intake in rats treated with normal diet but no effect was observed in rats treated with sugar diet. Food intake was greater in saline injected sugar diet than normal diet treated rats. 8-OH-DPAT injected sugar diet and normal diet treated animals exhibited comparable values.

DISCUSSION

Presynaptic response to 8-OH-DPAT following long term ingestion of sugar diet

Previously it has been shown that long term ingestion of sugar diet produces hyperphagia in rats and weekly food intakes are greater in sugar diet than normal diet treated rats (Haleem *et al*., 2000).The present study shows that 4h intakes are also greater in sugar than normal diet treated rats

A negative feed back loop exist between consumption of carbohydrate and turnover of 5-HT i-e carbohydrate ingestion enhances the synthesis and release of 5-HT which in turn causes a selective decrease in carbohydrate ingestion (Leibowitz and Alexander, 1998). It was suggested that eating a meal of palatable food causes a short term increase in extra cellular serotonin which in turn suppress the food intake but long term ingestion of sugar diet decreases 5-HT concentrations in the brain and produces hyperphagia (Haleem *et al*., 2000).

Important findings of the present study is that hyperphagic effects of 8-OH-DPAT were smaller in sugar than normal diet treated rats (fig. 2). These smaller hyperphagic effects of 8-OH-DPAT in sugar diet treated animals are explainable in terms of a decrease in the effectiveness of somatodendritic 5-HT-1A receptors. Thus stimulation of somatodendritic 5-HT-1A receptors decreases the availability of 5-HT (Liu *et al*., 2005) at postsynaptic hypophagic 5-HT-2C receptors (Haleem *et al*., 2004, Schuhler *et al*., 2005) to elicit hyperphagia (De Vry and Schreiber, 2000) and a decrease in the sensitivity of these receptors results in the absence of hyperphagic effects of 8-OH-DPAT in sugar diet treated rats.

Postsynaptic response to 8-OH-DPAT following long term ingestion of sugar diet

Stimulation of postsynaptic 5-HT-1A receptors by 8-OH-DPAT elicits hyperactivity syndrome (O' Connell and Curzon 1996). A decrease in the intensity of forepaw treading and flat body posture as observed in the present study is explainable in terms of a decrease in the responsiveness of the postsynaptic 5-HT-1A receptors in sugar diet treated rats. On the other hand an increase in locomotor activity may occur due to an increase in dopaminergic activity because compounds that enhance serotonergic functions tend to diminish dopamine-mediated behaviors, whereas manipulation that diminish serotonin activity augment dopamine-mediated behavior (Ramusson *et al*., 1994, Tanda *et al*., 1994)

CONCLUSION

The present study shows that long term intake of sugar diet decrease the responsiveness of presynaptic as well as postsynaptic 5-HT-1A receptors. An upregulation of postsynaptic 5-HT-1A receptors and a down regulation of presynaptic 5-HT-1A receptors is involved in adaptation to stree (Haleem *et al*., 2002a). The present results suggest that a decrease in the responsiveness of postsynaptic 5-HT-1A receptors dependent responses may impair adaptation to stress (Haleem *et al*., 2002b) and may have a causal role in the prevalence of depression in people taking sugar rich meal.

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