Neurochemical Profile of 8-Hydroxy-2-(Di-N-propylamino) tetralin (8-OH-DPAT) Induced Hyperphagia in Sugar Rich Diet Treated Rats

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Summary: To assess the role of hyperphagic serotonin-1A (5-hydroxytryptamine, 5-HT) receptors, if any, in the precipitation of hyperphagia associated with long term consumption of sugar rich diet, the present study is designed to investigate hyperphagic and brain serotonin metabolism attenuating effects of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a 5-HT-1A selective agonist, following long term consumption of sugar as part of meal in rats. Sugar-rich diet was prepared by mixing standard rodent diet and table sugar in the 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. Five-week food intakes were greater in sugar-rich-diet-treated than normal diet treated rats. After five weeks of treatment control and sugar-diet-treated animals were injected with 8-OH-DPAT at a dose of 0.5 mg/ ml/ kg. 8-OH-DPAT induced hyperphagia and decreases in serotonin metabolism were smaller in sugar-diet than normal-diet-treated rats. The results suggest that decrease in the responsiveness of somato-dendritic 5-HT-1A receptors is not involved in sugar-rich-diet-induced hyperphagia.

Introduction

Sucrose, a dietary sugar, is widely consumed as part of meal. Evidence suggests that excessive consumption of sugar produces overeating and weight gain [1-3]. Hyperphagia, excessive food consumption, is also a prominent feature of untreated diabetes in man [4,5]. Clinical studies provide evidence that serotonin (5-hydroxytryptamine, 5-HT) is involved in the dysregulation of appetite and eating disorders [6]. Neurochemical research on a relationship between 5-HT and feeding shows that brain 5-HT metabolism is increased following the ingestion of particularly carbohydrate rich diet [7-9]. Increased 5-HT metabolism may generate a satiety signal for the termination of meal is suggested by pharmacological research [10-11]. Hypothalamic serotonergic receptor mechanism has been playing an important role in the regulation of appetite and in transducing satiety signals of 5-HT [10,12,13]. Administration of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a 5-HT-1A selective agonist [14], or other compounds with selectivity for 5-HT-1A sites, increased foodintake of freely feeding rats [15-16]. These studies explained 8-OH-DPAT-induced hyperphagia in terms of stimulation of 5-HT-1A receptors, located on the soma and dendrites of serotonergic neurons that control the synthesis and release of 5-HT by feedback mechanism [17-19]. Stimulation of these receptors decreases the availability of 5-HT at postsynaptic hypophagic receptors [20-22] to elicit hyperphagia. The present study is designed to investigate the effects of long term consumption of sugar-rich diet on 8-OH-DPAT induced hyperphagia and decreases serotonin metabolism in the hypothalamus and whole brain to assess the sensitivity of somatodendritic 5-HT-1A receptors and their role, if any, in the precipitation of hyperphagia associated with the long term consumption of sugar-rich diet.

Results and Discussion

Fig. 1 shows the effects of sugar rich diet on five-week food intake. Statistical analysis by t-test showed that food intakes were significantly greater ($p<0.01$) in sugar diet than normal diet treated 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

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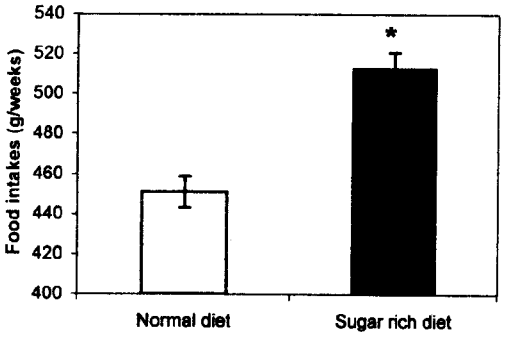

Fig. 1: Effects of sugar-rich-diet ingestion on five-week food intakes (g) in freely feeding rats. Values are means ± S.D (n=12). Significant difference by t-test *p<0.01.

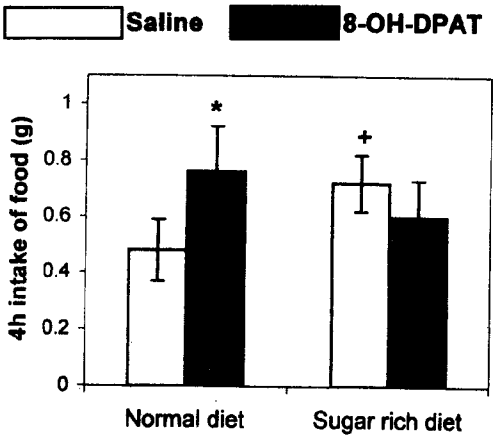

Fig. 2: The effects of 8-OH-DPAT on 4h food intake (g) in normal and sugar-rich-diet treated rats. Values are means ± S.D (n=6). Significant difference by Newmans Keuls test *p<0.01 from respective saline injected rats, +p<0.05 from respective control-diet-treated rats following Two-way ANOVA.

(F=14) was significant (p<0.01). Post-hoc analysis showed that administration of 8-OH-DPAT increased food intake in rats treated with normal diet but not 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.

Fig. 3 shows the effects of 8-OH-DPAT (0.5mg/ kg) on the levels of (a) tryptophan (b) 5-HT and (c) 5-HIAA in the hypothalamus of normal and sugar rich diet treated rats. Two way Anova

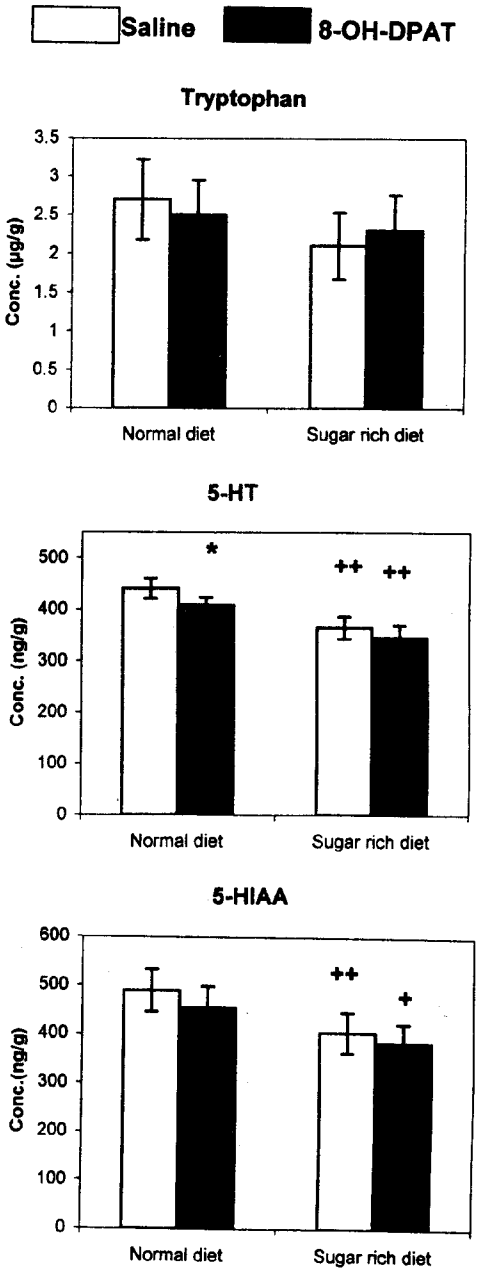

Fig. 3: Effects of 8-OH-DPAT on the levels of tryptophan, 5-HT and 5-HIAA in the hypothalamus of normal and sugar-diet-treated rats. Values are means ± S.D (n=6). Significant difference by Newmans Keuls test *p<0.05 from respective saline injected rats, $^{+}$p<0.05, $^{++}$ p<0.01 from respective control-diet-treated rats following Two-way ANOVA.

(df=1,20) showed that effects of 8-OH-DPAT not significant for tryptophan (F=0.01, p>0.05), and 5-HIAA (F=2.7, p>0.05), were significant for 5-HT (F=9.6, p<0.01). Effects of sugar rich diet not significant for tryptophan (F=4.25, p>0.05), were significant (p<0.01) for 5-HT (F=71.2) and 5-HIAA (F=20.1). Interaction between 8-OH-DPAT and sugar diet was not significant (p>0.05) for tryptophan (F=0.84), 5-HT (F=0.58) and 5-HIAA (F=0.16). The posthoc test showed that administration of 8-OH-DPAT decreased 5-HT levels in normal diet but not sugar-diet-treated rats. The levels of 5-HIAA were not altered by 8-OH-DPAT in sugar or normal diet treated rats. 5-HT and 5-HIAA levels were smaller in saline-injected, sugar-diet than saline-injected, normal-diet-treated rats. These were also smaller in 8-OH-DPAT-injected sugar diet than 8-OH-DPAT-injected normal diet treated rats.

Fig. 4 shows the effects of 8-OH-DPAT (0.5mg/ kg) on levels of (a) tryptophan (b) 5-HT (c) 5-HIAA in whole brain of normal and sugar-rich-diet-treated rats. Two way Anova (df=1,20) showed that effects of 8-OH-DPAT not significant for tryptophan (F=1.2, p>0.05), were significant (p<0.01) for 5-HT (F=28.5) and 5-HIAA (F=17.5). Sugar-rich diet effects were significant for tryptophan (F=4.6, p<0.05), 5-HT (F=55, p<0.01) and 5-HIAA (F=46.5, p<0.01). Interaction between 8-OH-DPAT and sugar diet was not significant (p>0.05) for tryptophan (F=0.3), 5-HT (F=0.002) and 5-HIAA (F=0.13). The posthoc test showed that administration of 8-OH-DPAT produced no effect on tryptophan levels in rats treated with normal or sugar diet but decreased 5-HT and 5-HIAA levels in rats treated with normal or sugar diet. The concentration of tryptophan was comparable in normal diet and sugar-diet-treated animals but 5-HT and 5-HIAA levels were smaller in saline injected sugar diet than saline injected normal-diet-treated rats. 8-OH-DPAT-injected sugar-diet and normal diet treated rats exhibited comparable values of tryptophan but 5-HT and 5-HIAA levels were smaller in 8-OH-DPAT-injected sugar-diet than 8-OH-DPAT-injected, normal-diet-treated rats.

Previously it has been shown that rats treated with sugar rich diet for three weeks exhibited an increase in food intake which was associated with the decrease in 5-HT metabolism in the whole brain and in the hypothalamus [23]. The present results are also consistent with the previous data that prolonged consumption of sugar-rich-diet produces hyperphagia

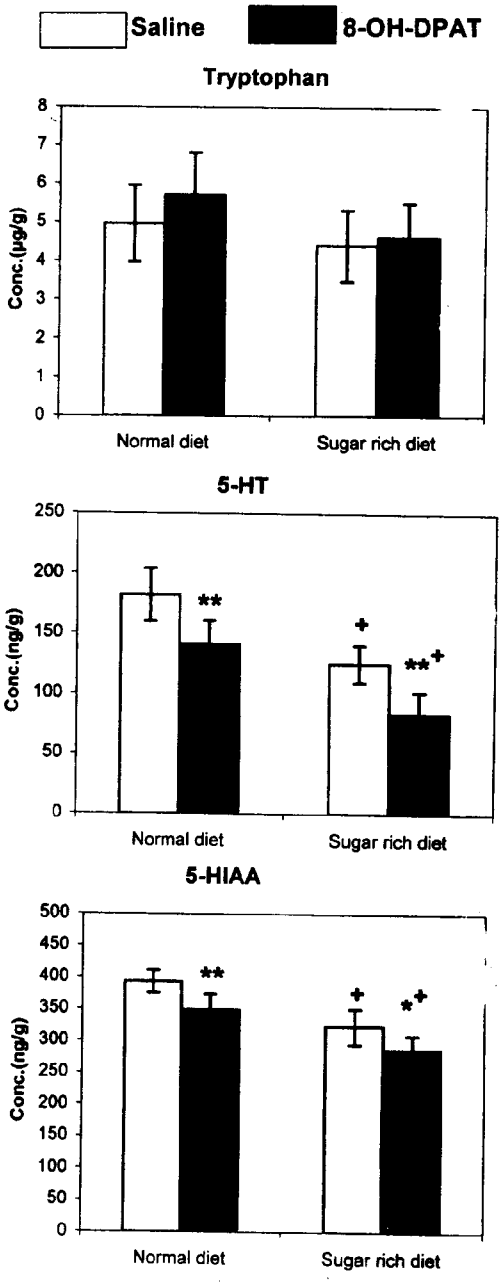

Fig. 4: Effects of 8-OH-DPAT on the levels of tryptophan, 5-HT and 5-HIAA in the brain of normal and sugar-diet-treated rats. Values are means ± S.D (n=6) significant difference by Newmans Keuls test *p<0.05, **p<0.01 from respective saline-injected rats, $^{+}$p<0.01 from respective control-diet-treated rats following Two-way ANOVA.

(Fig 1) and decreases brain serotonin metabolism (Fig. 3, 4). The present study was conducted to test the possibility that long-term consumption of sugar rich diet increases the effectiveness of feedback regulatory mechanism and the resultant decrease in the availability of 5-HT at postsynaptic hypophagic receptors to elicit hyperphagia. Important finding of the present study is that 8-OH-DPAT induce hyperphagia (Fig 2) and decreases of 5-HT were smaller and not significant in the hypothalamus (Fig 3) but not in the whole brain (Fig 4) of sugar diet than normal-diet-treated rats. These smaller hyperphagic and neurochemical effects of 8-OH-DPAT in sugar-rich-diet-treated animals are explainable in terms of a decrease in the effectiveness of somatodendritic 5-HT-1A receptors in sugar-rich-diet-treated rats. Stimulation of somatodendritic 5-HT-1A receptors by 8-OH-DPAT decreases the availability of 5-HT [17,18,19] at postsynaptic hypophagic receptors [20,22] to elicit hyperphagia [17,13]. A decrease in the responsiveness of these receptors would be expected to attenuate 8-OH-DPAT induced hyperphagia in sugar-diet-treated rats. The sensitivity of 5-HT-1A autoreceptors located on the cell soma and/ or dendrites of serotonergic neurons is also decreased following long term antidepressant administration [24] and adaptation to stress [25,26]. In the present study a decrease in the responsiveness of somatodendritic 5-HT-1A recaptors leading to an increase in the availability of 5-HT particularly in the hypothalamus cannot explain hyperphagia observed in sugar diet treated rats because pharmacologic manipulations that lead to increase 5-HT functions in the brain elicit satiety signal [10,11]. The present results therefore suggest that a decrease in the responsiveness of somatodendritic 5-HT-1A receptors is not involved in sugar-diet-induced hyperphagia. The results support the notion that a decrease in the metabolism of 5-HT in the hypothalamus and also in the whole brain is involved in the hyperphagic effects of sugar rich diet to precipitate overeating and weight gain. The results also suggest that long term intake of sugar rich diet may elicit antidepressant like effects on the activity of serotonergic neurons.

Experimental

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 [23].

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) dissolved in saline was injected intraperitoneally (i.p) at a dose of 0.5 mg/ ml/ kg bodyweight. The dose which has previously shown produced significant hyperphagia [16]. Control animals were injected with saline in volume of 1ml/ kg bodyweight.

Animals were randomly assigned to normal diet and sugar diet treated groups and accordingly weighed amount of respective diets were placed in the hopper of rats' cages. Food intakes were monitored weekly. After 5 weeks of treatment animals of both the groups divided into saline and 8-OH-DPAT-injected subgroups were injected with saline (0.9% NaCl) or 8-OH-DPAT (0.5mg/ ml/ kg) between 10:00-11:00 h using a balanced design. A weighed amount of food was placed in the hopper of the cages immediately after the drug administration and intake during 4h was monitored. Rats were decapitated 4h after the drug administration between 2:00-3:00 h to collect plasma and brain samples. Hypothalami and rest of the brain dissected out, cerebellum was removed as described before [27] were stored at -70°c until analysis of tryptophan, 5-HT and 5-HIAA by HPLC-EC was carried out.

For the HPLC-EC determination of tryptophan, 5-HT and 5-HIAA samples were extracted as described before [25]. A 5μ ODS (ECHPHERE) separation column of 4.0 mm internal diameter, 250 mm length was used. The mobile phase, comprising methanol (14%), octyl sodium

sulphate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of PH = 2.9 was passed through the column at an operating pressure of 2000-3000 psi with the help of Waters 510 HPLC pump. Electrochemical detection was achieved on Schimadzu LEc 6A detector (Kyoto Japan). 5-HT and 5-HIAA were detected at an operating potential of 0.8volt and tryptophan at 1.0 volt.

Statistical Analysis

The data on the effects of sugar-rich-diet ingestion on five-week food intakes were analyzed by t-test and data on the effects of 8-OH-DPAT on 4h food intakes and neurochemical data were analyzed by two-way ANOVA. Post hoc comparisons were done by Newman-Keuls test. p values < 0.05 were taken to be significant.

Conclusions

In conclusion, the present study shows that a decrease in brain 5-HT metabolism and not an increase in the responsiveness of somatodendritic 5-HT-1A receptors are involved in sugar-diet induced hyperphagia [23] conversely somatodendritic 5-HT-1A receptors seem to have been desensitized in rats treated subchronically with sugar rich diet. A hypoactive, serotonergic system is described in human depression [28], while adaptation to stress associated with a decrease in the responsiveness of somatodendritic 5-HT-1A receptors [26,29]. The present results therefore suggest that long term ingestion of sugar diet may help to cope the stress demand to produce adaptation to stress.

Acknowledgement

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