INTRODUCTION

The effect of sweetness on appetite control is important for the reason of unwanted overconsumption associated with the tendency to gain weight.1-3 Many industry prepared food items derive a relatively high percentage of their energy from sucrose or other sugars.4 Hyperphagia, excessive food consumption, is also a prominent feature of untreated diabetes in man.5,6 Serotonin (5-hydroxytryptamine, 5-HT) has a key role in the normal termination of feeding7-9 and perhaps also in the disorders of appetite.10,11 Neurochemical research on a relationship between serotonin and feeding shows that brain 5-HT metabolism increases following ingestion of particularly carbohydrate-rich diet.12 Increased metabolism may generate a satiety signal for the termination of meal is suggested by pharmacological research.7,13,14 Conversely, a decrease in brain 5-HT metabolism following long-term ingestion of carbohydrate has been seen to be associated with hyperphagia in rats.15

Synthesis and release of 5-HT is under the control of an effective feedback mechanism involving stimulation of receptors located on cell body and dendrites of serotonergic neurons. The somatodendritic receptors are of 5-HT-1A type.16 Stimulation of these receptors decreases the availability of 5-HT at postsynaptic hypophagic receptors to elicit hyperphagia.17-20 Administration of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a 5-HT-1A selective agonist, increased food intake of freely feeding rats.21 The present study was conducted to determine the effects of long-term consumption of sugar as part of meal in rats.

METHODOLOGY

This experimental study was conducted in the Department of Biochemistry, University of Karachi, from June to August 2005. Twenty-four locally bred male albino Wistar rats, weighing 200-230 gm, 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.

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

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

Twenty-four animals were randomly divided into two equal groups: normal diet and sugar-rich diet treated groups of same mean weights and accordingly weighed amount of respective diet were placed in the hopper of rat's cages. Animals of both the groups were divided into saline and 8-OH-DPAT injected subgroups of same mean weights, which had been previously shown to produce acute hyperphagia. Control animals were injected with saline in volume of 1 ml/kg bodyweight.

For the HPLC-EC determination of tryptophan, 5-HT and 5-HIAA, 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 0.8 volts and tryptophan at 1.0 volts.

The 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.

**RESULTS**

Figure 1 shows the effects of 8-OH-DPAT (0.5 mg/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 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 (Table I).

Figure 2 and Table II show the effects of 8-OH-DPAT (0.5 mg/kg) on the levels of (a) tryptophan, (b) 5-HT, (c) 5-HIAA in normal and sugar-rich diet treated rats. Two-way ANOVA (df=1,20) showed that effects of 8-OH-DPAT were 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 were 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 post-hoc 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 normal and sugar diet treated rats.

**Table I:** Effects of 8-OH-DPAT (0.5 mg/kg) on 4h food intakes (g) in sugar diet and normal diet treated rats.

<table>
<thead>
<tr>
<th></th>
<th>Normal diet</th>
<th>Sugar-rich diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>4h intakes of food (g)</td>
<td>0.48±0.11</td>
<td>0.76±0.16*</td>
</tr>
</tbody>
</table>

Values are means ± S.D (n=6). Significant difference by Newman-Keuls test, from respective control diet treated rats, *p<0.01; from respective control diet treated rats, +p<0.05 following two-way ANOVA: drug (F=2.35, p>0.05); sugar diet (F=0.45, p>0.05); interaction (F=14, p<0.01).

**Table II:** Effects of 8-OH-DPAT on tryptophan, 5-HT and 5-HIAA levels in the hypothalamus of sugar rich diet and normal diet treated rats.

<table>
<thead>
<tr>
<th></th>
<th>Normal diet</th>
<th>Sugar-rich diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan(µg/g)</td>
<td>2.7±0.52</td>
<td>2.5±0.45</td>
</tr>
<tr>
<td>5-HT(ng/g)</td>
<td>440.19±1.2</td>
<td>364.4±21.5*</td>
</tr>
<tr>
<td>5-HIAA(ng/g)</td>
<td>488.9±43.5</td>
<td>345.2±23.8*</td>
</tr>
</tbody>
</table>

Values are means ± S.D (n=6). Significant difference by Newman-Keuls test, from respective saline-injected rats: *p<0.05; from respective control diet treated rats, +p<0.05 following two-way ANOVA: tryptophan: drug (F=0.01, p>0.05); sugar diet (F=4.25, p>0.05); interaction (F=0.84, p>0.05); 5-HT: drug (F=6.6, p<0.01); sugar diet (F=71.2, p<0.01); interaction (F=0.58, p>0.05); 5-HIAA: drug (F=2.7, p<0.05); sugar diet (F=20.1, p<0.01); interaction (F=16, p<0.05).

**Table III:** Effects of 8-OH-DPAT on tryptophan, 5-HT and 5-HIAA levels in the brain of sugar-rich diet and normal diet treated rats.

<table>
<thead>
<tr>
<th></th>
<th>Normal diet</th>
<th>Sugar-rich diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan (µg/g)</td>
<td>4.96±0.99</td>
<td>5.7±1.1</td>
</tr>
<tr>
<td>5-HT (pg/g)</td>
<td>181721.97</td>
<td>124.9±15.5*</td>
</tr>
<tr>
<td>5-HIAA (ng/g)</td>
<td>3927±17.9</td>
<td>286.7±22.7*</td>
</tr>
</tbody>
</table>

Values are means ± S.D (n=6). Significant difference by Newman-Keuls test, from respective saline-injected rats: *p<0.05; **p<0.01; from respective control diet treated rats: +p<0.01 following two-way ANOVA: tryptophan: drug (F=1.2, p<0.05); sugar diet (F=4.6, p<0.05); interaction (F=0.3, p<0.05); 5-HT: drug (F=28.5, p<0.01); sugar diet (F=55, p<0.01); interaction (F=0.002, p<0.05); 5-HIAA: drug (F=17.5, p<0.01); sugar diet (F=65, p<0.01); interaction (F=0.002, p<0.05).
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.

Figure 3 and Table III show the effects of 8-OH-DPAT (0.5 mg/kg) on levels of (a) tryptophan, (b) 5-HT, (c) 5-HIAA in the brain of normal and sugar diet treated rats. Two-way ANOVA (df=1,20) showed that effects of 8-OH-DPAT were 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 post-hoc 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.

**DISCUSSION**

Rats treated with sugar-rich diet for three weeks exhibit an increase in food intake, which was associated with the decrease in 5-HT metabolism in the whole brain and in the hypothalamus. The present study shows that 4h intake was also greater in sugar diet than normal diet treated rats. The results of the present and previous studies have shown that prolong consumption of sugar rich diet produces hyperphagia.

An important finding of the present study is that hyperphagic effects of 8-OH-DPAT were smaller in sugar than normal diet treated rats (Figure 1). These smaller hyperphagic effects of 8-OH-DPAT in sugar diet treated animals can be explained in terms of a decrease in the effectiveness of somatodendritic 5-HT-1A receptors. Stimulation of somatodendritic 5-HT-1A receptors decreases the availability of 5-HT at postsynaptic hypophagic 5-HT-2C receptors to elicit hyperphagia. A decrease in the responsiveness of these receptors would be expected to decrease 8-OH-DPAT induced hyperphagia in sugar diet treated rats.

The synthesis and release of 5-HT is under the control of an effective feedback mechanism involving stimulation of somatodendritic 5-HT-1A receptors which inhibit neuronal firing, consequently, the synthesis of 5-HT is decreased. A decrease of 5-HT metabolism by 8-OH-DPAT in the hypothalamus of control diet treated animals is consistent with the notion that the drug elicits hyperphagia by decreasing the availability of 5-HT in the hypothalamus. Smaller 5-HT metabolism in the hypothalamus of sugar diet treated animals strengthens our previous report suggesting that a decrease in the availability of 5-HT in the hypothalamus is involved in the induction of hyperphagia following long-term consumption of sugar-rich diet. In the present study, 8-OH-DPAT induced decrease in 5-HT was smaller in the hypothalamus (Figure 2) but not in the whole brain of sugar diet treated rats.

In the present study, the levels of both 5-HT and 5-HIAA were smaller in the hypothalamus of rats treated with sugar-rich diet suggesting that the decreases are not due to an increase in the effectiveness of negative feedback mechanism. The present results, therefore, suggest that a decrease in the 5-HT metabolism but not an increase in the responsiveness of somatodendritic 5-HT-1A receptors is involved in sugar diet induced hyperphagia. In the present study, 5-HT and 5-HIAA but not tryptophan levels were smaller in the hypothalamus of rats treated with sugar-rich diet. Apart from brain levels of tryptophan affecting the synthesis of 5-HT due to suppression of tryptophan hydroxylase, evidence suggests that kinetic properties of tryptophan hydroxylase are sometimes modulated in vivo and may be responsible for changing the rate of 5-HT synthesis in the absence of any change in precursor level. The present results, therefore, tend to suggest that long-term consumption of sugar-rich diet decreases the activity of synthesizing enzyme without increasing the effectiveness of feedback regulatory mechanism. Conversely, the effectiveness of somatodendritic 5-HT-1A receptors is decreased. These findings would help in the development of effective pharmacological compounds and therapeutic strategies to combat the hyperphagia and excessive weight gain in people taking sugar-rich meal.

**CONCLUSION**

A decrease in brain 5-HT metabolism and not an increase in the responsiveness of somatodendritic 5-HT-1A receptors was involved in sugar diet induced hyperphagia, conversely somatodendritic 5-HT-1A receptors seemed to have been desensitized in rats treated subchronically with sugar-rich diet. Decrease in serotonin metabolism is not due to an increase in the effectiveness of negative feedback mechanism or smaller availability of serotonin precursor tryptophan. Changes in the kinetics of tryptophan hydroxylase may well be involved in the observed decrease of 5-HT metabolism.

**REFERENCES**


