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ORIGINAL CONTRIBUTION

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Investigations on possible serotonergic involvement in effects of OB-200G (polyherbal preparation) on food intake in female mice

Summary *Background* OB-200G is a polyherbal preparation containing aqueous extracts of *Garcinia cambogia*, *Gymnema sylvestre*, *Zingiber officinale*, *Piper longum* and resin from *Commiphora mukul*, all possessing thermogenic properties. Our previous studies reveal OB-200G to exert antiobesity effects in dietary animal models of obesity. *Aim of the study* The present study investigated the possible involvement of serotonergic system in the effect of OB-200G on food intake. We examined the effects of systemic pretreatment with 5-HT depletor, p-chlorophenylalanine (PCPA, 300 mg/kg, i. p. for 6 days), 5-HT_{1A} agonist, (8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT, 0.1 mg/kg, i. p.), nonselective 5-HT antagonist, cyproheptadine (1 mg/kg, i. p.), 5-HT^2 receptor antagonist, seganserin (1 and 2 mg/kg, i. p.) and 2-deoxy-D-glucose (2-DG, glucose antimetabolite, 500 mg/kg, i. p.) on satiety induced by OB-200G (500 mg/kg, p. o.) in non-deprived female mice. The results were compared with fluoxetine (10 mg/kg, i. p.), a selective serotonin reuptake inhibitor. *Methods* Fifteen minutes after the last drug administration, groups of mice were presented with sweetened chow and the amount of food consumed was recorded at 0.5, 1, 2, 3 and 4 h time intervals. *Results* The hyperphagic effect of PCPA, 8-OH-DPAT, cyproheptadine and 2-DG was significantly ($p < 0.05$) antagonized by both OB-200G and fluoxetine. However, the anorectic effect of fluoxetine was not reversed by centrally acting 5-HT^2 antagonist, seganserin but the latter markedly attenuated the satiety action of OB-200G. *Conclusion* The present observations suggest the role of serotonin in mediation of satiety by OB-200G and hence its antiobesity effect.

Key words Serotonin – Food intake – OB-200G – Fluoxetine – female mice

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Introduction

The energy balance of the body is regulated by a complex interplay of the various gut and hypothalamic neurotransmitters and neuropeptides [1]. Of these, serotonin (5-hydroxytryptamine, 5-HT) has been extensively implicated as one of the principal neurotransmitters in the control of food intake, food selection and body weight. There is also considerable evidence that stimulants of this monoamine reduce food intake and weight gain and increase energy expenditure [2]. Two drugs that are reported to enhance 5-HT neurotransmission, (+)-fenfluramine and fluoxetine, have been shown to be effective anorectic drugs, both in animals and in humans [3, 4]. In contrast, 5-HT antagonists have been reported to increase feeding [5, 6]. Furthermore, small doses of the selective 5-HT_{1A} agonist 8-OH-DPAT have been reported to increase food consumption in rats by inhibiting the firing rate of dorsal raphe serotonergic neurons and thereby decreasing central serotonergic activity [7]. The glucostatic and lipostatic feedback systems have also been reported to play an important role in regulation of appetite. The inhibition of oxidation of metabolic fuels such as glucose and fatty acids has been reported to stimulate feeding behavior

and vice-versa. 2-deoxy-D-glucose is a glucose analog which prevents the intracellular utilization of glucose and is known to elicit eating in rats and is an animal model of hyperphagia [8].

OB-200G is a polyherbal preparation containing aqueous extracts of *Garcinia cambogia, Gymnema sylvestre, Zingiber officinale, Piper longum and* resin from *Commiphora mukul*. All the ingredients of the preparation have been reported to possess thermogenic properties [9–11]. Furthermore, *Commiphora mukul* has been traditionally reported to exert a hypolipidemic [12] and antiobesity effect [11]. *Garcinia cambogia* also possesses antiobesity action [13]. *Zingiber officinale* has been reported to exert hypolipidemic effect and lower cholesterol levels. Gymnemic acid isolated from the leaves of *Gymnema sylvestre* has been reported to inhibit the intestinal absorption of glucose and oleic acid [14] and reported to exert beneficial effect in diabetes and obesity. *Piper longum* (pippali) posseses heat generating property and has been referred to by Charak as the best digestive stimulant [11]. We have also reported OB-200G to decrease body weight, fat pad weights and total cholesterol levels in female rats fed on cafeteria and atherogenic diets [15]. With the above background in mind, the present study was designed to elucidate the mechanism(s) of antiobesity effects of OB-200G by studying its effect on modulation of food intake by serotonin modulators like PCPA, 8-OH-DPAT, cyproheptadine, seganserin and 2-deoxy-D-glucose. The results were compared with fluoxetine, a selective serotonin reuptake inhibitor.

Materials and methods

Animals

Female albino mice of Laca strain (Central Animal House, Panjab University, Chandigarh), weighing 20–25 g, were housed six per cage with free access to food and water at laboratory conditions. The animals were used following at least a 2-day period of adaptation to the laboratory conditions. The experiments were carried out between 9.00 and 16.00 h at room temperature (22 ± 2 °C). All the experimental protocols have been approved by the Institutional Animals Ethics Committee and conducted according to the Indian National Science Academy Guidelines on the Use and Care of Experimental Animals.

Drugs

OB-200G, a polyherbal preparation, was provided as a dry powder mixture by the Himalaya Drug Company, Bangalore, India. The constituents include *Commiphora mukul* (gum resin, purified resin – 20 %), *Garcinia cambogia* (fruit rind, aqueous extract–50 %), *Gymnema sylvestre* (leaves, aqueous extract – 15 %), *Piper longum* (fruit, aqueous extract – 10 %) and *Zingiber officinale* (rhizome, aqueous extract – 5 %). The ingredients as well as their compositions were identified and confirmed with the in-house authentic specimens of the Himalaya Drug Company and the voucher deposition specifications of herbarium specimens lie with the company. OB-200G was suspended in distilled water and administered at a dose of 0.5 g/kg, p. o. Fluoxetine HCl (Divis Pharma, India), ± 8-OH-DPAT (Sigma Chemical Co., USA), cyproheptadine (Merck Sharp and Dohme Research Lab, USA) and seganserin HCl (Jannsen Research Foundation, Belgium) were dissolved in distilled water and 2-DG (Sigma Chemical Co., USA) in 0.9 % saline. PCPA (Sigma Chemical Co., USA) was dispersed in 0.1 % Tween 80 and the volume made up with distilled water. All the drugs were administered intraperitoneally at constant volume of 1 ml/100 g.

Experimental protocols

Effect of OB-200G and fluoxetine on modulation of food intake by PCPA, 8-OH-DPAT, cyproheptadine, seganserin and 2-DG

Mice were kept in groups of six in test cages and included a vehicle-treated control group and the various drug treatment groups. The food and water were withheld 1 h before the experimentation. PCPA (300 mg/kg, i. p. for 6 days), 8-OH-DPAT (0.1 mg/kg, i. p.), cyproheptadine (1 mg/kg, i. p.), seganserin (1 and 2 mg/kg, i. p.) and 2-DG (500 mg/kg, i. p.) were administered to the respective treatment groups 30 min prior to OB-200G or fluoxetine administration. Then 15 min later, preweighed sweetened chow [16] was presented to the different groups of mice in petri dishes in the test cages. The amount remaining was weighed to the nearest 0.1 g with correction for spillage after 0.5, 1, 2, 3 and 4 h intervals and the cumulated food intake/mouse (g/20 g body weight) was calculated.

Statistical analysis

All the results are expressed as mean ± SEM. The data was subjected to analysis of variance (ANOVA) separately at each time interval using the STAT program that was developed at our institute. Post hoc comparison was made using Duncan's Multiple Range test. In all tests, $p < 0.05$ was used as the criterion for statistical significance.

Results

Effect of OB-200G and fluoxetine on food intake *per se*

As depicted in Fig. 1A, fluoxetine *per se* produced hypophagia at all time intervals but OB-200G did not significantly alter food intake as compared to the control.

Effect of OB-200G and fluoxetine on PCPA-induced hyperphagia

As shown in Fig. 1B, administration of PCPA (300 mg/ kg – i. p. for 6 days) produced significant increase in food intake as compared to the control on day 7. The hyperphagic effect of PCPA was significantly antagonized by OB-200G (0.5 g/kg, p. o.) as well as by fluoxetine (10 mg/kg, i. p.).

Effect of OB-200G and fluoxetine on 8-0H-DPAT-induced hyperphagia

As illustrated in Fig. 2A, 8-OH-DPAT (0.1 mg/kg, i. p.) produced a significant increase in food intake as compared to the control. Both OB-200G (0.5 g/kg) and fluoxetine (10 mg/kg) significantly ($p < 0.05$) antagonized the hyperphagic effect of 8-OH-DPAT.

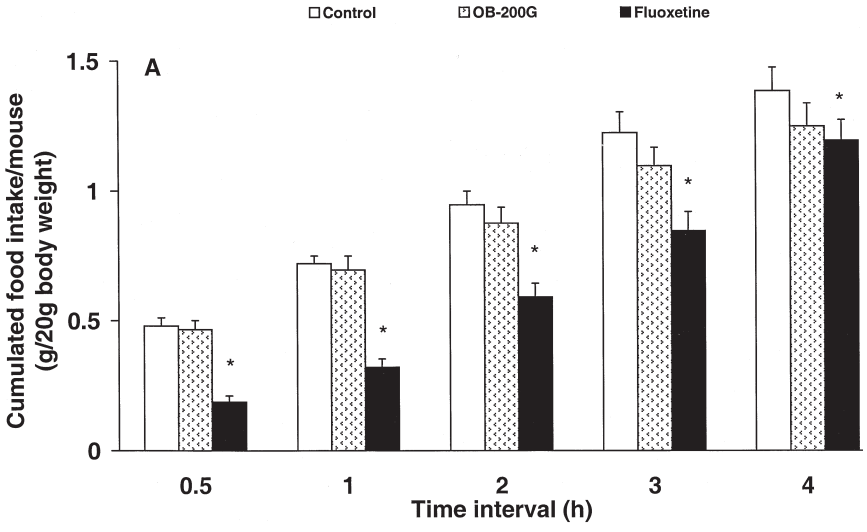

Fig. 1A Effect of OB-200G (0.5 g/kg, p. o.) and fluoxetine (10 mg/kg, i. p.) *per se* on food intake in female mice at 0.5, 1, 2, 3 and 4 h time intervals. Each bar represents the mean ± SEM (n=24 animals per group). *P < 0.05 as compared to vehicle treated group (ANOVA followed by Duncan's new multiple range test).

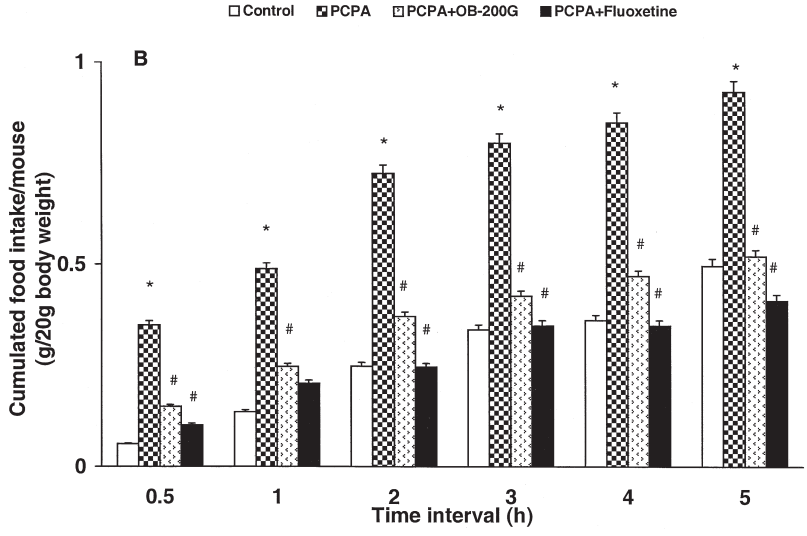

Fig. 1B Effect of OB-200G (0.5 g/kg, p. o.) and fluoxetine (10 mg/kg, i. p.) administration on p-chlorophenylalanine (PCPA, 300 mg/kg, i. p. for 6 days)-induced hyperphagia in female mice at 0.5, 1, 2, 3, 4 and 5 h time intervals. Each bar represents the mean ± SEM (n=6 animals per group). *P < 0.05, #P < 0.05 as compared to vehicle and PCPA-treated groups respectively (ANOVA followed by Duncan's new multiple range test).

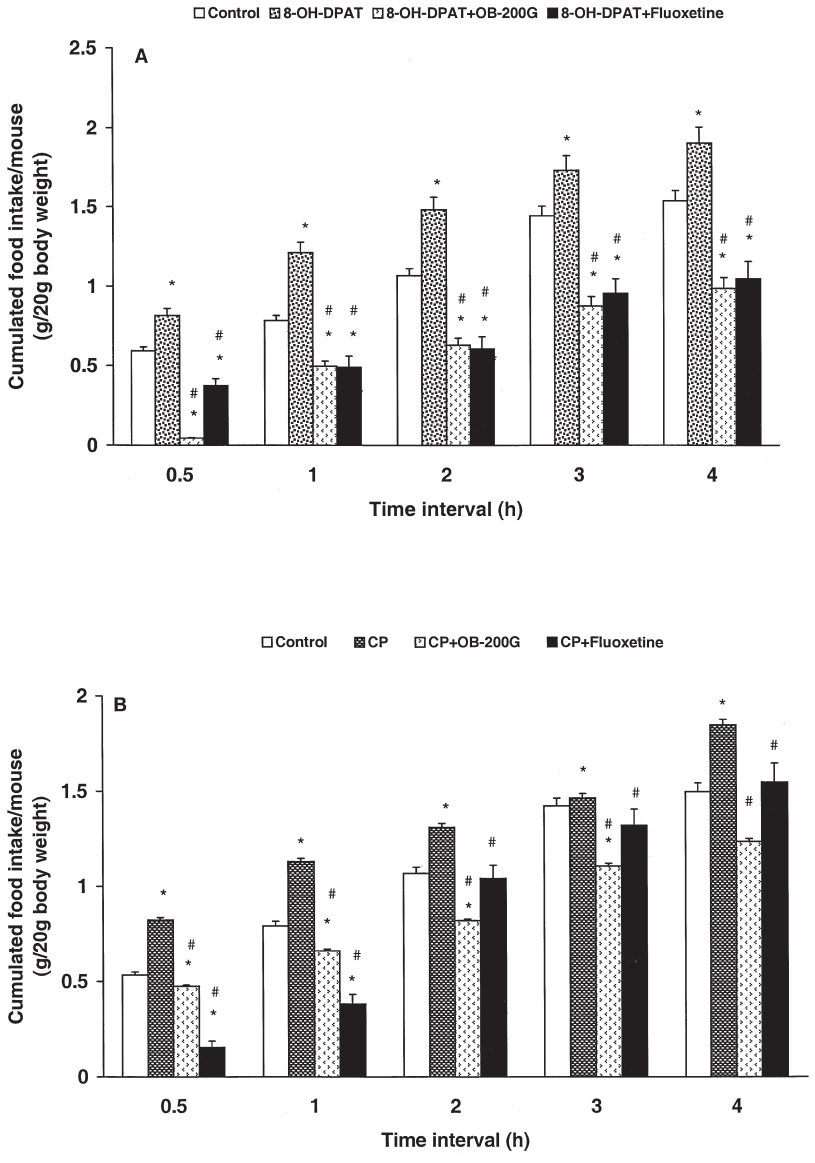

Fig. 2 Effect of OB-200G (0.5 g/kg, p. o.) and fluoxetine (10 mg/kg, i. p.) administration on the hyperphagia induced by 8-OH-DPAT (0.1 mg/kg, i. p.; **A**) and cyproheptadine (CP, 1 mg/kg, i. p.; **B**) in female mice at 0.5, 1, 2, 3 and 4 h time intervals. Each bar represents the mean ± SEM (n=6 animals per group). *P < 0.05, #P < 0.05 as compared to vehicle and 8-OH-DPAT or cyproheptadine-treated groups, respectively (ANOVA followed by Duncan's new multiple range test).

Effect of OB-200G and fluoxetine on cyproheptadine-induced hyperphagia

Cyproheptadine (1 mg/kg, i. p.) administration produced a significant increase in food intake as compared to the control. This hyperphagic effect was again significantly ($p < 0.05$) antagonized by both OB-200G and fluoxetine (Fig. 2B).

Modulation of food intake by seganserin preadministration

As shown in Fig. 3A, seganserin administration at 1 and 2 mg/kg dose *per se* did not significantly alter food intake in mice. Prior administration of seganserin (1 and 2 mg/kg) failed to reverse fluoxetine-induced hypophagia and a further decrease in food intake was observed with a 2 mg/kg seganserin dose (Fig. 3B). However, as depicted in Fig. 3C, prior administration of seganserin (1 and 2 mg/kg) in OB-200G-treated mice elicited a marked reversal of the satiety action of OB-200G as compared to the control as well as the seganserin-treated group.

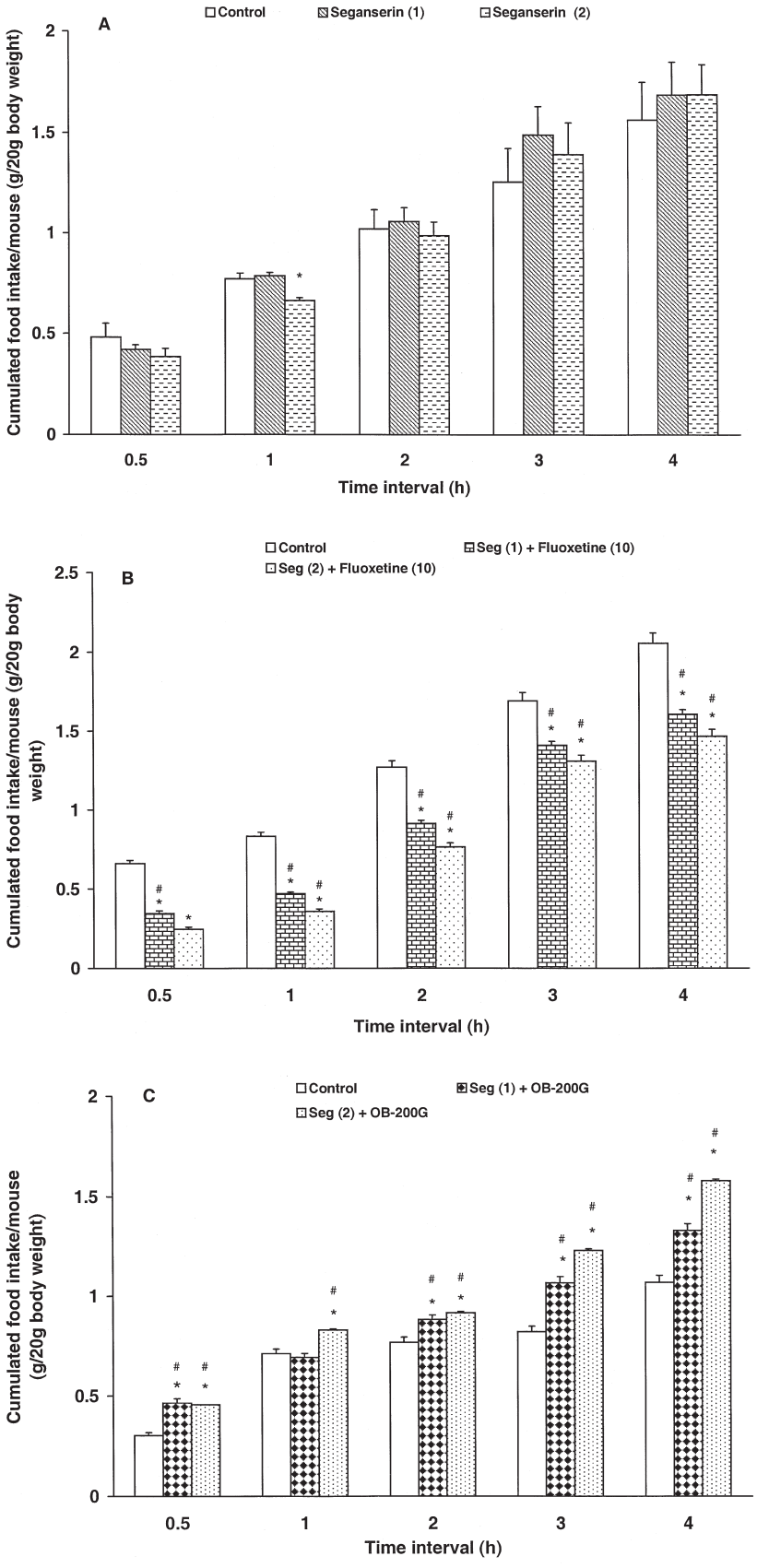

Fig. 3 Effect of seganserin (1 and 2 mg/kg, i. p.; **A**) administration *per se*, and combination of seganserin (1 and 2 mg/kg, i. p.) with fluoxetine (10 mg/kg, i. p.; **B**) and OB-200G (0.5 g/kg, p. o.; **C**) administration on modulation of food intake in female mice at 0.5, 1, 2, 3 and 4 h time intervals. Each bar represents the mean ± SEM (n=10 animals per group). *P < 0.05, #P < 0.05 as compared to vehicle and seganserin-treated groups, respectively (ANOVA followed by Duncan's new multiple range test).

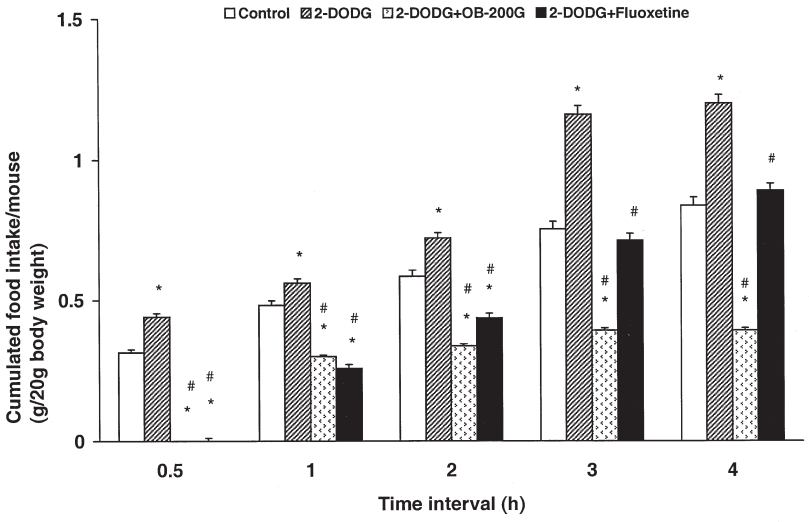

Fig. 4 Effect of OB-200G (0.5 g/kg, p. o.) and fluoxetine (10 mg/kg, i. p.) administration on 2-deoxy-D-glucose (2-DODG, 500 mg/kg, i. p.)-induced hyperphagia in female mice at 0.5, 1, 2, 3 and 4 h time intervals. Each bar represents the mean ± SEM (n=6 animals per group). *P < 0.05, #P < 0.05 as compared to vehicle and 2-DG-treated groups, respectively (ANOVA followed by Duncan's new multiple range test).

Effect of OB–200G and fluoxetine on 2-deoxy-D-glucose-induced hyperphagia

As shown in Fig. 4, administration of 2-DG (500 mg/ kg, i. p.) elicited a marked hyperphagia over the 4 h period as compared to the control. Treatment with both fluoxetine (10 mg/kg) and OB-200G (0.5 g/kg) significantly antagonized the hyperphagia produced by 2-DG.

Discussion

The result of the study showed that PCPA induces hyperphagia, which is consistent with the previous reports that central and peripheral administration of PCPA depletes 5-HT and increases feeding [17]. Furthermore, the hyperphagia is dependent on the dose, the degree and duration of 5-HT depletion. Interestingly, both OB-200G and fluoxetine significantly reduced food intake in PCPA-treated mice. The persistence of the hypophagic effect of fluoxetine following PCPA administration as previously reported [18] gives strong evidence against increased synaptic availability as the mechanism responsible for its hypophagic effect. But according to a report by Tohyama *et al.*, a small percentage of 5-HT-like immunoreactive fibers in the anterior or lateral hypothalamus are resistant to PCPA administration [19]. Thus, an increase in synaptic availability of 5-HT by fluoxetine in this region can account for its hypophagic effect. Furthermore, since PCPA prevents the operation of presynaptic mechanisms, OB-200G may mediate this effect through postsynaptic receptors.

The results of our study are in accordance with the previous finding that 8-OH-DPAT, a 5-HT_{1A} agonist, causes inhibition of the endogenous satiety system and increases food intake at low doses [7]. Furthermore, studies have demonstrated that the hyperphagic effects of 8-OH-DPAT were a consequence of the reduced 5-HT synthesis and release caused by the agonistic action of the drug at somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei [20]. The antagonism of 8-OH-DPAT-induced hyperphagia by fluoxetine is also consistent with the earlier findings [21]. Thus, it is likely that OB–200G may also mediate its effect on food intake through 5-HT_{1A} receptors as well since it significantly antagonized 8-OH-DPAT-induced hyperphagia.

Increase in food intake after cyproheptadine administration is in good agreement with the previous reports suggesting appetite stimulant, calorie intake and body weight increasing properties of cyproheptadine in animals [6] and human subjects [22]. Besides, cyproheptadine exerts potent serotonin receptor blocking action and has been reported to antagonize the anorectic effect of 5-hydroxytryptophan and fenfluramine [23]. Antagonism of cyproheptadine-induced hyperphagia by fluoxetine and OB-200G further instigates some involvement of serotonergic system in their effect on food intake.

At a dose of 1 and 2 mg/kg, seganserin did not significantly alter food intake as compared to the control during the 4 h test period. A similar observation has also been reported with the other 5-HT^2 antagonist ritanserin in satiated rats [24]. The failure of the specific 5-HT^2 antagonist, seganserin to reverse the fluoxetine hypophagia is also consistent with previous report indicating the lack of reversal of anorectic effect of fluoxetine by ketanserin and ritanserin [25]. In contrast, seganserin markedly reversed the satiety action of OB-200G, thus, again corroborating the serotonergic involvement in effects of OB-200G on food intake.

In accordance with reported evidence 2-deoxy-D-glucose produced glucoprivic-feeding response in female mice [8]. It does so by inhibiting glucose oxidation

in the brain and periphery by competitive inhibition of phosphohexoisomerase enzyme activity. Reduction in cellular glucose availability evokes sympatho-adrenal activation, hyperglycemia and hyperphagia [27, 28]. Antagonism of 2-deoxy-D-glucose-induced hyperphagia by fluoxetine implicates serotonergic receptor involvement in the central processing of metabolic regulatory signals. This finding is in good agreement with the previous reports indicating such antagonism by other serotonergic agents like (±)-fenfluramine, p-chloramphetamine, quipazine and even fluoxetine [29, 30]. Antagonism of 2-DODG hyperphagia by OB-200G also implicates perhaps the involvement of a common central pathway(s) regulating glucose substrate homeostasis.

In conclusion, the results of our study suggest the possible involvement of serotonergic mechanisms in the effect of OB-200G on food intake. OB-200G may prove useful to supplement the current armamentarium for the treatment of obesity.

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