INTRODUCTION

Serotonin has long been implicated in the control of food and water intake. Generally, it has been observed that injection of serotonin into different areas of the brain decreases food intake (1-5). It has also been observed that injection of various serotonin antagonists increases food intake (6-10). However, reports are also conflicting on the action of serotonin on ingestive behaviors. It is reported that 5-HT<sub>1A</sub> receptor agonist increases food intake (11-16). It is also reported that 5-HT<sub>3</sub> antagonist, ondansetron injected intraperitoneally decreases food intake (16, 17). Thus, the

EFFECTS OF INJECTION OF SEROTONIN INTO NUCLEUS CAUDATUS ON FOOD AND WATER INTAKE AND BODY WEIGHT IN ALBINO RATS

G. K. PAL*, KANNAN N. AND PRAVATI PAL

Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry - 605 006

(Received on September 6, 2003)

Abstract : Serotonin is known to inhibit food and water intake. However, the effect of its injection into nucleus caudatus on food and water intake is not known. In the present study, serotonin hydrochloride, buspirone (the serotonin 5-HT<sub>1A</sub> agonist) and ondansetron (the 5HT<sub>3</sub> antagonist) were injected into nucleus caudatus through stereotaxically implanted cannulae in three different dosages (1, 2 and 5 µg) and their effects on 24 h food and water intake, and body weight were recorded. The injection of serotonin hydrochloride resulted in a dose-dependent decrease in food intake attaining maximum of 27.3% at 5 µg dose, whereas water intake and body weight were decreased 12% and 4.3% respectively only at the highest does. Buspirone elicited a dose dependent inhibition of food and water intake and body weight (22.3%, 19.8% and 5.1% respectively), whereas ondansetron elicited an increase in food and water intake (37.8% and 36.3% respectively) without significantly altering bodyweight. It was concluded that serotonin hydrochloride injected into nucleus caudatus inhibits food and water intake significantly. These effects are mediated via 5-HT<sub>1A</sub> and 5HT<sub>3</sub> receptors. The effect of injections of 5-HT<sub>1A</sub> receptor agonist is more pronounced on water intake. The effect of injections of 5HT<sub>3</sub> receptor antagonist is also more pronounced on water intake.

Key words : serotonin buspirone ondansetron nucleus caudatus food intake water intake body weight

INTRODUCTION

Serotonin is known to inhibit food and water intake. However, reports are also conflicting on the action of serotonin on ingestive behaviors. It is reported that 5-HT<sub>1A</sub> receptor agonist increases food intake (11-16). It is also reported that 5-HT<sub>3</sub> antagonist, ondansetron injected intraperitoneally decreases food intake (16, 17). Thus, the
action of agonists and antagonists of serotonin on ingestive behaviors is contradictory.

Injection of 8-OH-DPAT, serotonin 1A receptor agonist produces reduction in polydipsia and anorexia (18, 19). Injection of serotonin into paraventricular nucleus (PVN) of hypothalamus inhibits feeding by unknown mechanisms. It was proposed that serotonin induced anorexia (20). The suppressive effect of serotonin on carbohydrate intake was attenuated by injection of mianserin, 5-HT$_{2A}$ antagonist. Intraperitoneal injection of fenfluramine results in depression of food intake. Chronic treatment with citalopram, a selective serotonin reuptake inhibitor, decreases both food intake and bodyweight gain in well nourished rats (21). It is suggested that effect of serotonin on food intake is mediated primarily via 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors (4, 19). Both these receptor-subtypes are expressed in hypothalamic regions that are involved in regulation of feeding behaviors. Agents that activate 5-HT$_{2C}$ and 5-HT$_{1B}$ receptors produce hypophagia which indicate that serotonin through these receptors decreases food intake (22). Though injection of 8-OH-DPAT, 5-HT$_{2A}$ agonist 15 minutes before refeeding of starved animals decreases food intake, injection of the same chemical after one hour of the start of refeeding, increases food intake (11). This indicates that serotonin has strong influence on food intake. However, the effect of 8-OH-DPAT on water intake was negligible in water-deprived animals.

Buspirone, 5-HT$_{1A}$ agonist injected subcutaneously fifteen minutes prior to presentation of food produced a dose related inhibition of food intake in food deprived rats (3). It was suggested that inhibition of food intake in food-deprived rats was not due to non-specific disruption of behavior, rather due to the specific action of chemical on specific neural centers. However, it was also observed that regardless of the route of administration, buspirone increases food intake in a dose dependent manner for almost two hours from the start of presentation of food. Therefore, the role of buspirone on food intake is also found to be contradictory (in one experimental setting it decreases and in other setting it increases). It is also observed that buspirone, selectively increases food intake when the diet is rich in carbohydrate (13). Injection of low dose of 8-OH-DPAT is also observed to increase food intake (23). Thus, the effect of serotonin on same receptors studied by different investigators is found to be conflicting in nature.

Mesolimbic structures are strongly involved with regulation of behavioral functions (24). Nucleus caudatus, being a mesolimbic structure is also involved in the regulation of food and water intake in rats as reported from our laboratory (25–28). However, effect of injection of serotonin in nucleus caudatus on ingestive behaviors has not been studied yet. Moreover, 5-HT$_{1A}$ and 5-HT$_{3}$ receptors mainly mediate the effects of serotonin on ingestive behaviours. Therefore, in the present study we have assessed the role of serotonin, buspirone (5-HT$_{1A}$ agonist) and ondansetron (5-HT$_{3}$ antagonist) injected into nucleus caudatus on food and water intake and body weight in rats.
METHODS

A total number of 24 male albino rats of Wistar strain were taken for this study. They were about six months old with body weight ranging between 200–250 g. All animals were kept in separate cages. Food and water were provided ad lib.

Animals were allowed one week to recover from isolation stress, after which experiments were carried out. The temperature of the room in which animals were caged was between 28 to 32°C. Animals were exposed to 24 h natural light – dark cycle.

Measurement of basal food and water intake and body weight

Food and water were provided at 1400 h everyday, following which 24 h food and water intake were measured for each animal. For water intake, tap water at room temperature (30°C) was provided in calibrated glass cylinder having a metal spout. The minimum amount of change in water intake that could be recorded accurately was 0.5 ml. The food was provided in the form of standard rodent chow. Measurement of food was done by electronic weighing machine in which minimum change of 0.01 g could be recorded. The basal daily food and water intake were recorded on seven consecutive days to determine the 24 h basal mean food and water intake of each animal. Body weight of rats was recorded using electronic weighing machine. Basal body weight was obtained as the average of seven days recording.

Cannulation of nucleus caudatus

Cannulae were made by appropriately cutting the stainless steel injection needles of 20 G size. During cannulation of nucleus caudatus, animals were anaesthetized by intraperitoneal administration of Ketamine Hydrochloride (100 mg per kg body weight). Cannulations were performed by stereotaxic method using the co-ordinates of Konig and Klippel (29), following the procedures as described earlier (27).

Cannulations were performed unilaterally on the right side in all animals of all groups. Cannulae were allowed to remain in the brain for injecting chemicals till the animals were sacrificed. After cannulation, five days were given for the animal to recover completely and during the period animal was closely monitored for any infection. In our study there was no postoperative infection at all.

Preparation of dosages of chemicals

Serotonin hydrochloride (Lancaster, U. K.), was diluted in ethanol to prepare concentrations of 1 µg, 2 µg and 5 µg per µL respectively. Similarly, Buspirone (Rankem, New Delhi), a 5HT$_{1A}$ receptor agonist, was diluted in ethanol to prepare concentrations of 1 µg, 2 µg and 5 µg per µL respectively and Ondensetron (Rankem, New Delhi), a 5HT$_{3}$ receptor antagonist was diluted with ethanol to make 2 µg and 5 µg per µL solutions.

Experimental protocol

All the animals (24 totally) were divided equally into four groups with six animals
in each group. Group I was the serotonin group, Group II was the agonist group, Group III was the antagonist group and Group IV was the control group. Their 24 h basal food intake, water intake and body weight were measured before injection of chemicals.

**Group I**

In this group, different doses of serotonin hydrochloride (1 µg, 2 µg and 5 µg) were injected separately into nucleus caudatus of all six animals of Group I, on every alternate day and their 24 h food intake, water intake and body weight were measured following each injection. The schedule of injection was such that 1 µg was injected on Day 1, no injection was given on Day 2, 2 µg was injected on Day 3, no injection was given on Day 4, 5 µg was injected on Day 5, and no injection was given on Day 6, and so on and so forth till each dose of serotonin was injected on six occasions. Mean of these six recordings of each parameter was obtained as experimental post-injection values. These values were compared with their basal values and with the values of control animals.

**Group II**

In this group, different doses of buspirone (1 µg, 2 µg and 5 µg), 5-HT<sub>1A</sub>-receptor agonist, were injected separately into nucleus caudatus of all six animals of Group II. The protocol was otherwise identical to that employed in Group I.

**Group III**

In experiment 2, the maximally effective doses of buspirone were found to be 2 µg and 5 µg per µL. Therefore, in this experiment 2 µg and 5 µg per µL doses of ondansetron, a 5-HT<sub>3</sub> receptor antagonist, was injected into nucleus caudatus in animals of Group III.

In Group IV, the control group, equal amount (1 µL) of vehicle (ethanol) without drug was injected every alternate days into nucleus caudatus like that of experimental animals and their 24 h food intake, water intake and body weight were recorded and compared with their basal values.

**Confirmation of cannulation**

After completion of all the experiments, the animals were sacrificed and their brains were fixed by perfusing with 10% formalin solution. After perfusion, brains were removed for sectioning, staining and confirmation under microscope, which were performed by the procedures as described earlier (27).

The observations were recorded in all three groups and the obtained results were analyzed by one-way ANOVA, following which the level of significance was tested by paired ‘t’ test.

**RESULTS**

**Group I**

The mean basal food intake, water intake and body weight in this group of animals were 11.37 ± 0.62 g, 26.1 ± 1.03 mL and 210.25 ± 2.58 g respectively. After injection of different doses of serotonin hydrochloride, there was significant and dose-dependent decrease in food intake of
animals (Table I). However, significant decrease in body weight and water intake was observed only at 5 µg dose of serotonin.

**Group II**

The mean basal food intake, water intake and body weight in this group of animals were 22.46 ± 0.81 g, 32.58 ± 0.94 mL and 225.50 ± 1.86 g respectively. After injection of 1 µg of buspirone, serotonin 5-HT$_{1A}$ receptor agonist, there was significant decrease in water intake without significant change in food intake and body weight (Table II). However, with injection of higher doses of buspirone, there was significant and dose-dependent decrease in food intake, water intake and body weight of animals.

**Group III**

The mean basal food intake, water intake and body weight in this group of animals were 18.64 ± 0.78 g, 31.16 ± 0.55 mL and 221.58 ± 1.98 g respectively. There was a significant increase in food and water intake following injection of 2 µg dose of ondansetron. The increase in water intake was proportionately greater than the increase in food intake. At 5 µg dose of ondansetron, there was highly significant increase in both the food and water intake (Table III). However, at both the doses, no change in body weight was observed.

<table>
<thead>
<tr>
<th>SH-Dose</th>
<th>FI (g)</th>
<th>WI (mL)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg (Basal*)</td>
<td>11.37±0.62</td>
<td>26.1±1.03</td>
<td>210.25±2.58</td>
</tr>
<tr>
<td>1 µg</td>
<td>9.85±0.68* (-13.4%)</td>
<td>25.83±0.99 (-1.0%)</td>
<td>209.78±1.94 (-0.2%)</td>
</tr>
<tr>
<td>2 µg</td>
<td>9.66±0.62* (-15.0%)</td>
<td>24.41±0.67 (-6.5%)</td>
<td>206.3±1.23 (-1.9%)</td>
</tr>
<tr>
<td>5 µg</td>
<td>8.26±0.4*** (-27.4%)</td>
<td>22.96±0.49* (-12%)</td>
<td>201.18±1.05** (-4.3%)</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001.

*Baseline indicates values recorded before injection of serotonin hydrochloride.

Values in brackets indicate % decrease from the basal value.

<table>
<thead>
<tr>
<th>BS-Dose</th>
<th>FI (g)</th>
<th>WI (mL)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg (Basal*)</td>
<td>22.46±0.81</td>
<td>32.58±0.94</td>
<td>225.50±1.86</td>
</tr>
<tr>
<td>1 µg</td>
<td>21.71±0.86 (-3.3%)</td>
<td>29.14±0.78* (-10.5%)</td>
<td>220.46±1.92 (-2.2%)</td>
</tr>
<tr>
<td>2 µg</td>
<td>19.42±0.56** (-13.5%)</td>
<td>25.88±0.66** (-12.2%)</td>
<td>218.68±1.42* (-3.0%)</td>
</tr>
<tr>
<td>5 µg</td>
<td>17.46±0.84*** (-22.3%)</td>
<td>26.12±0.7*** (-19.8%)</td>
<td>213.82±1.10** (-5.1%)</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001.

*Baseline indicates values recorded before injection of buspirone.

Values in brackets indicate % decrease from the basal value.

<table>
<thead>
<tr>
<th>ON-Dose</th>
<th>FI (g)</th>
<th>WI (mL)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg (Basal*)</td>
<td>18.64±0.78</td>
<td>31.16±0.55</td>
<td>221.58±1.98</td>
</tr>
<tr>
<td>2 µg</td>
<td>21.45±0.38* (+15%)</td>
<td>38.83±0.46*** (+24.6%)</td>
<td>223.64±2.12 (+0.9%)</td>
</tr>
<tr>
<td>5 µg</td>
<td>25.68±0.52*** (+37.8%)</td>
<td>42.46±0.84*** (+36.3%)</td>
<td>225.12±2.10 (+1.6%)</td>
</tr>
</tbody>
</table>

*P<0.05; ***P<0.001

*Baseline indicates values recorded before injection of ondansetron.

Values in brackets indicate % increase from the basal value.
In control animals, no significant change in above-mentioned parameters was observed following injection of equal volume (1 µL) of vehicle (ethanol) into nucleus caudatus when compared with their basal values (Table IV).

**DISCUSSION**

In our study, injection of serotonin hydrochloride into nucleus caudatus resulted in decrease in food intake in a dose dependent manner, which indicates that serotonin in nucleus caudatus inhibits feeding. Furthermore, injection of buspirone, 5-HT_{1A} agonist decreased food intake. This indicates that serotonin acting via 5-HT_{1A} receptors inhibits feeding. This is contradictory to the observations of other workers who have demonstrated that buspirone increases feeding behavior (12-14). They have demonstrated that buspirone not only increases food intake, it increases it in a dose dependent manner irrespective of its route of administration (12). It was also observed that buspirone selectively increases food intake when food is rich in carbohydrates (13). However, our findings agree with the findings of other investigators, who have demonstrated that buspirone inhibits food intake in a dose dependent manner (3, 4). It was also further revealed that buspirone inhibits food intake partly by stimulating the release of dopamine in the brain, which is a known inhibitor of feeding behavior (30).

Our study also supports the findings of other workers in which 5-HT_{1A} agonists other than buspirone, like ipaspirone have been shown to inhibit food intake (15, 18). Therefore, it can be concluded that serotonin inhibits food intake via 5-HT_{1A} receptors. However, inhibition of food intake by buspirone in our study was observed only at higher doses. Moreover, injection of ondansetron, 5-HT_{3} antagonist stimulated food intake. This clearly indicates that serotonin normally inhibits feeding behavior, which also is mediated via 5-HT_{3} receptors.

In our study, serotonin also inhibited water intake, though only at the highest dose. However, the inhibition of water intake by buspirone was significant even at the lower dose, and buspirone inhibited water intake in a dose dependent manner. These findings, that no significant decrease in water intake inspite of significant decrease in food intake following injections of 1 and 2 µg doses of serotonin, and significant decrease in water intake inspite of no significant decrease in food intake at 1 µg dose of buspirone, indicate that the inhibition of water intake was independent of inhibition of food intake. Since the hypodipsia induced by these chemicals was independent of hypophagia, it is suggested that serotonin induced hypodipsia may a type of “primary hypodipsia.”

**TABLE IV**: Effect of injection of 1 µL of ethanol on food intake (FI), water intake (WI) and body weight (BW) in control rats (n = 6).

<table>
<thead>
<tr>
<th>Ethanol volume</th>
<th>FI (g)</th>
<th>WI (mL)</th>
<th>BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Basal*)</td>
<td>21.82±0.74</td>
<td>29.92±0.67</td>
<td>218.72±1.74</td>
</tr>
<tr>
<td>1 µL</td>
<td>20.90±0.81</td>
<td>30.18±0.72</td>
<td>216.40±1.82</td>
</tr>
<tr>
<td></td>
<td>(-4.2%)</td>
<td>(+0.9%)</td>
<td>(-1.1%)</td>
</tr>
</tbody>
</table>

*Basal indicates values recorded before injection of vehicle (ethanol). Values in brackets indicate % increase from the basal value.
Following injections of buspirone, there was also a dose dependent decrease in body weight. No significant change of body weight was observed at 1 µg dose. But, at 2 µg dose of buspirone, decrease in body weight was significant and was further significantly decreased at 5 µg dose of buspirone. It is proposed that separate centers in the brain exclusively control body weight independent of food intake (31). Nucleus tractus solitarius (NTS) and ventromedian hypothalamus (VMH) are the proposed ponderostats (centers controlling body weight). It has also been proposed that separate neurotransmitter systems in the brain control regulation of body weight. These neurotransmitters are called ponderostatic neurotransmitters.

Injection of ondansetron, 5-HT₃ receptor antagonist increased food intake in a dose dependent manner. This further substantiates the earlier finding that serotonin inhibits food intake as its antagonist increases food intake. It also can be well concluded that the inhibitory effect of serotonin was mediated via 5-HT₃ receptors, in addition to its action through 5-HT₁A receptors. Injection of ondansetron resulted in increased water intake significantly even at the dose of 2 µg (P<0.001), the dose at which increase in food intake was just significant (P<0.05). This again suggests that water intake induced by serotonin antagonist is independent of increased food intake.

In spite of increase in food and water intake, there was no significant increase in body weight following injections of ondansetron. This indicates that body weight is not solely dependent on ingestive behaviors. Though the injection of buspirone, 5-HT₁A agonist resulted in decrease in body weight, injection of ondansetron, 5-HT₃ antagonist, did not elicit significant increase in body weight. This shows that serotonin antagonist do not influence body weight though they increase food intake. It further indicates that neural mechanisms controlling body weight may be separate from the neural mechanisms controlling food intake. However, it could also be argued that the significant change in body weight was not observed as the study was a short-term study of injections of chemicals. Therefore, a long-term study should be taken up to assess the role of serotonin on change in body weight, which we plan in our future studies.

In our previous experiments, we have observed that lesion of nucleus caudatus results in decreased food and water intake and injections of catecholamines and angiotensin into nucleus caudatus increases food and water intake (25–28, 32). This shows that nucleus caudatus is an important element of neural circuitry involved in the regulation of ingestive behaviors. Injection of various chemicals into nucleus caudatus changes food and water intake without producing significant motor abnormalities as we have observed in our previous studies and also in the present study. This indicates that nucleus caudatus is an important structure that solely influences feeding and drinking behavior in rats. In the present study, as we observed that injections of serotonin agonist and antagonist into the same nucleus alter ingestive behaviors, we conclude that nucleus caudatus is an important centre in the brain controlling
these physiological parameters. Serotonin and serotonin 5-HT$_{1A}$ receptor agonist (buspirone) decreased food and water intake and body weight, whereas serotonin 5-HT$_3$ receptor antagonist (ondensetron) increased these parameters. It was concluded that serotonin injected into nucleus caudatus inhibits ingestive behaviors and these effects of serotonin are mediated by 5-HT$_{1A}$ and 5-HT$_3$ receptors. It needs further studies of confirm the role of serotonin in nucleus caudatus on the regulation of body weight.

ACKNOWLEDGEMENTS

The authors acknowledge Mrs. Selvam S. R. R., Technical Supervisor, Department of Physiology, for providing the technical assistance in cannulating the animals.

REFERENCES


17. Mazzola-Pomietto P, Aulakh CS, Murphy DL. Temperature, food intake, and locomotor activity effects of 5-HT\textsubscript{3} receptor agonist and two 5-HT\textsubscript{3} receptor antagonists in rats. *Psychopharmacology (Berl)* 1995; 121(4): 488-493.


