Ondansetron, a selective 5-HT₃ antagonist, antagonizes methamphetamine-induced anorexia in mice


Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

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Abstract

Effects of some selective serotonergic (5-HT) antagonists on methamphetamine-induced anorexia were investigated in male mice. The least possible dose of methamphetamine alone that caused significant anorectic activity was 11 μmol kg⁻¹, i.p. (2mg kg⁻¹). Various doses of some selective serotonergic receptor antagonists were administered half an hour before the above mentioned dose of methamphetamine. Methiothepin potentiated, whereas NAN-190, methysergide, mianserin and ondansetron antagonized methamphetamine-induced anorectic activity. The least possible doses of these antagonists which modified methamphetamine-induced anorexia were as follows: methiothepin (1.1 μmol kg⁻¹, i.p.), NAN-190 (4.2 μmol kg⁻¹, i.p.), methysergide (2.1 μmol kg⁻¹, i.p.), mianserin (3.3 μmol kg⁻¹, i.p.) and ondansetron (0.003 μmol kg⁻¹, i.p.). The serotonergic antagonists at the above mentioned doses did not modify the food intake of animals not treated with methamphetamine, except for methiothepin, which produced a significant reduction, and mianserin, which produced a significant increase in food intake. The results of the present study indicated that the anorectic activity induced by methamphetamine is related to the interactions of methamphetamine with 5-HT receptor. Since a very small dose (0.003 μmol kg⁻¹) of ondansetron (the 5-HT₃ antagonist), as compared with the other antagonists used in this study, antagonized the anorexia induced by methamphetamine, the 5-HT₃ receptor is likely to be the site for this interaction.

Keywords: Methamphetamine; NAN-190; Methiothepin; Methysergide; Mianserin; Ondansetron; Anorexia; Food intake; 5-HT antagonists

1. Introduction

There is considerable evidence supporting the role of serotonin (5-HT) in the control of food intake [1,2]. For example, the serotonin releaser/reuptake inhibitor fenfluramine produces a reduction in food intake in both animals [3] and man [4]. In addition, drugs that are considered to act primarily by a direct action at serotonin receptors, such as quipazine or m-chlorophenyl piperazine (m-CPP), are capable of reducing food intake [5,6].

Recent pharmacological studies have more precisely characterized the nature of the inhibitory effect of brain serotonin on feeding behavior. The medial hypothalamus is believed to be a critical location in the mediation of serotonin’s action. Specifically, the paraventricular and ventromedial nuclei are known to be involved in controlling energy balance, while the suprachiasmatic nucleus determines circadian patterns of eating. Serotonergic stimulation of these three nuclei with exogenous serotonin or drugs that release endogenous serotonin, preferentially reduces carbohydrate intake in naturally feeding animals through satiety mechanisms involved in the termination of feeding. This phenomenon is mediated by serotonin and serotonin receptors, in contrast to serotonin autoreceptors which potentiate feeding possibly by inhibiting serotonin release [7—9].

Interest in the mechanisms controlling food intake and involving the serotonergic system is
continually increasing. Indeed there is a flow of information concerning the heterogeneity of serotonin receptors [10] and a parallel surge in the development of new compounds acting selectively as agonists or antagonists on these receptor subtypes [11]. New tools are thus becoming available to explore the exact role of serotonin-acting drugs in the control of appetite and possibly also in the treatment of obesity.

Methamphetamine, a known drug of abuse, causes anorexia in rodents and in humans. It is the most of the amphetamines that are linked with serotonin. The decrease in the food intake induced by methamphetamine has been related to the central serotonergic component, since this effect of methamphetamine is partially attenuated by lesions to fibres of the ventral tegmental area [12—14] or due to inability of the animals to eat due to the locomotor consequences of the serotonin syndrome induced by methamphetamine [15].

Appetite suppression has been reported to depend on serotonin receptors rather than on serotonin availability [16]. Therefore, the present study was designed to explore whether the anorectic activity of methamphetamine is selectively, or at least partially, induced through stimulation of a specific serotonin receptor subtype.

2. Materials and methods

2.1. Drugs

Drugs used in this study were: methamphetamine hydrochloride (E. Merck, Germany), NAN-190 hydrobromide (Sigma, USA), methiothepin (Winlab, UK), methysergide (Research Biochemical International, USA), mianserin hydrochloride (Sigma, USA) and ondansetron hydrochloride di-hydrate (Glaxo Laboratories, UK). All drugs were dissolved in 0.9% NaCl solution. Doses are expressed as mg kg⁻¹ of the salt. The dose volume administered was 10 ml kg⁻¹, i.p.

2.2. Animals

Male Swiss albino mice (obtained from the Animal Care Center, College of Pharmacy, King Saud University) weighing 25—30g were used. The animals were housed, 10 mice per cage (35 cm x 25 cm x 15 cm) with woodchip bedding, under conditions of constant room temperature (23 ± 1 °C), humidity and light cycle (7 a.m.—7 p.m.). They were given access to food (standard lab chow purchased from Grain silos and flour mills organization, Riyadh) and water ad libitum. The experiments described in this study were approved by the local Ethical Committee for the Conduction of Animal Experiments.

2.3. Treatment protocols

Four groups of animals were used in each of the serotonin (5-HT) antagonist studies, as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control group)</td>
<td>First treatment was saline; second treatment was saline.</td>
</tr>
<tr>
<td>Group 2</td>
<td>First treatment was antagonist; second treatment was saline.</td>
</tr>
<tr>
<td>Group 3</td>
<td>First treatment was saline; second treatment was methamphetamine.</td>
</tr>
<tr>
<td>Group 4</td>
<td>First treatment was antagonist; second treatment was methamphetamine.</td>
</tr>
</tbody>
</table>

The first treatment was given 30 min before the second treatment.

2.4. Procedure

Animals were deprived of food for 24 h before the experiment. The animals, however, were allowed free access to water. Animals were divided randomly into groups of 10. Each group was weighed and placed in a separate large cage. Animals were injected by the test drug (or saline as appropriate). A weighed amount of the normal mouse food (~20 g) was placed at the top of the animal’s cage. After a certain period of time (i.e., 1, 2, 3, 4, and 5 h), reweighing of the animal’s food was done to determine the amount of food taken by the animals. Four groups of 10 mice were used per each dose of the test drugs or saline.
2.5. Statistics

All data are expressed as mean ± S.E.M. Statistical analysis of the results was performed by using one-way ANOVA. For significant results, a post hoc comparison between any two means was done by Tukey—Kramer test. The level of significance adopted was at \( P < 0.05 \).

3. Results

All the figures presented in this section show the time course of drug’s action on food consumption at 1-h time intervals, following injection of the test drug. Appropriate dosages for the various 5-HT antagonists were determined from pilot experiments in accordance to doses which have previously been used by various investigators cited in the literature.

Methamphetamine (2 mg kg\(^{-1}\)) significantly reduced the food consumption of food-deprived as compared to food non-deprived male mice at 1 and 2 h after its administration. Food-deprived animals, which were treated with methamphetamine, started eating before or at 3 h following methamphetamine administration, indicating loss of the anorectic effect of the drug. For that reason it was decided to concentrate on the effect of the antagonist on methamphetamine-induced anorexia only during the first 2 h following methamphetamine administration. The 0.5 and 1 mg kg\(^{-1}\) doses of methamphetamine, on the other hand, did not reduce the food consumption of food-deprived as compared to food non-deprived male mice at any time interval after its administration (Fig. 1). The former dose of methamphetamine (i.e., 2 mg kg\(^{-1}\)) was selected for challenge with some serotonin antagonists in subsequent experiments.

From pilot experiments, the least possible dose of NAN-190 that modified the anorectic activity induced by methamphetamine was 2 mg kg\(^{-1}\) (4.21 p~mol kg\(^{-1}\)). Fig. 2 shows that NAN-190 (2 mg kg\(^{-1}\)) significantly reversed \((P < 0.001)\) the anorectic activity of methamphetamine (2 mg kg\(^{-1}\)) in food-deprived mice throughout the first 2-h observation period (the period for maximal anorectic action of methamphetamine). It also reversed the effect of methamphetamine at the third and fourth hour after its administration. That dose of NAN-190, however, did not modify the food consumption of food-deprived mice when used alone.

The effect of various doses of methiothepin on methamphetamine (2 mg kg\(^{-1}\)) induced anorectic activity in food-deprived male mice was studied. From pilot experiments, the least possible dose of methiothepin that modified the anorectic activity induced by methamphetamine was 0.5 mg kg\(^{-1}\) (1.11 p.mol kg\(^{-1}\)). Fig. 3 shows that methiothepin (0.5 mg kg\(^{-1}\)) significantly enhanced \((P < 0.05)\) the anorectic activity of methamphetamine (2 mg kg\(^{-1}\)) in food-deprived mice throughout the first 2-h observation period. That dose of methiothepin, however, significantly reduced \((P < 0.001)\) the food consumption of food-deprived mice when used alone, throughout the first 2-h observation period.

The effect of various doses of methysergide on methamphetamine (2 mg kg\(^{-1}\)) induced anorectic activity in food-deprived male mice was studied. From pilot experiments, the least effective dose of methysergide that modified the anorectic activity induced by methamphetamine was 1 mg kg\(^{-1}\) (2.13 jmol kg\(^{-1}\)). Fig. 4 shows that methysergide (1 mg kg\(^{-1}\)) significantly reversed \((P < 0.001)\) the anorectic activity of methamphetamine (2 mg kg\(^{-1}\)) in food-deprived mice during the second hour and up to the end of the 5-h observation period. That dose of methysergide, however, did not affect the food consumption of food-deprived mice when used alone, throughout the first 2-h observation period.

food-deprived male mice which were also administered methamphetamine (2 mg kg\(^{-1}\)) at time intervals 2 and 4 h \((P < 0.001 \text{ and } 0.05, \text{ respectively})\). That dose of ondansetron (0.001 mg kg\(^{-1}\)) did not modify the food consumption of food-deprived male
mice when used alone.

4. Discussion

In this study, methamphetamine caused a significant reduction in the food intake of mice. With the exception of methiothepin and mianserin, all other serotonin antagonists tested in this study (NAN-190, methysergide or ondansetron) did not modify the food intake of mice when they were used alone.

Methiothepin, which is known to block 5-HT1B/1D receptors, when used alone caused a significant decrease in food intake. Thus, it could be suggested that 5-HT1B/1D receptors are possible sites for stimulation of food intake. The reduction in the food intake induced by methiothepin may be related to its ability to increase serotonergic transmission by acting on the cell body autoreceptors [17,18].

On the contrary to methiothepin, mianserin when used alone caused a significant increase in food intake. Thus 5-HTA receptor, which is blocked by mianserin, is a possible site for suppression of food intake. The increase in the food intake induced by mianserin may be related to the ability of mianserin to induce down regulation of receptors [19]. Similar findings have previously been reported by Dekeyne et al. [20], and Poeschla et al. [21]. Mianserin-induced hyperphagia may also be related to the ability of mianserin to induce hypoglycemia as a result of either inhibition of glycogenolysis in the liver, or through increased insulin secretion [22].

Pre-treatment with any of the serotonin antagonists used in this study, with the exception of methiothepin, blocked methamphetamine-induced anorexia. These findings indicate the possible involvement of 5-HT1,3 receptors in the regulation of methamphetamine-induced anorectic activity. However, 5-H1,4,7 receptors, which were not investigated in this study, should not be excluded as possible sites for
methamphetamine-induced anorectic activity. These findings could be explained by the ability of these antagonists to stimulate the serotonergic somatodendritic autoreceptors in the raphe nuclei that, in turn, decrease the release of serotonin [23], or may be related to the reduction in the serotonin turnover [24], or as the result of the involvement of multisystems in the regulation of behavioural eating. Blockade of dopaminergic receptors [11,25] has also been reported to increase food intake as an indirect activity of these serotonin receptor antagonists. The anxiolytic properties of these antagonists or the ele-

Fig. 6. Effect of ondansetron alone (0.001 mg kg⁻¹, i.p.) and in combination with methamphetamine (2 mg kg⁻¹, i.p.) on the food intake of food-deprived male mice. (El) Saline + saline, (U) ondansetron 0.001 + saline, (~) saline + met 2, (~ ondansetron 0.001 + met 2; #p < 0.05, as compared to the methamphetamine-induced anorexia. Similar findings have previously been reported by Montgomery and Fineberg [30].

The effect of various doses of mianserin on methamphetamine (2 mg kg⁻¹) induced anorectic activity in food-deprived male mice was also studied. A dose of 1 mg kg⁻¹ mianserin (3.33 nmoles kg⁻¹), when used alone, significantly increased the food consumption throughout the first 2-h observation period (P < 0.001), as shown in Fig. 5. That dose of mianserin caused a significant reversal in food consumption of food-deprived male mice, which were treated with methamphetamine, throughout the first 2-h observation period. However, it did not alter the effect of methamphetamine during the rest of the 5-h observation period.

The effect of ondansetron on methamphetamine (2 mg kg⁻¹) induced anorectic activity in food-deprived male mice is shown in Fig. 6. From pilot experiments, a dose of 0.001 mg kg⁻¹ ondansetron (0.003 p.mol kg⁻¹) caused a significant increase in the food consumption of

The results of the present study indicated that the anorectic activity induced by methamphetamine is related to the interactions of methamphetamine with 5-HT₃ receptor. Since a very small dose (0.003 p.mol kg⁻¹) of ondansetron (the 5-HT₃ antagonist), as compared with the other antagonists used in this study, antagonized the anorexia induced by methamphetamine, the 5-HT₃ receptor is likely to be the site for this interaction.

References

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