NAN-190, a possible specific antagonist for methamphetamine

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Abstract

Effect of NAN-190, a selective 5-HTIA receptor antagonist, on methamphetamine-induced locomotor activity, anorexia, analgesia, and hyperthermia was investigated in male mice. Methamphetamine (1.5 mg/kg, i.p) produced a significant increase in locomotor activity, which was significantly antagonized by NAN-190 at a dose of 4 mg/kg, i.p. NAN-190 did not alter the antinociceptive activity of mice when it was administered alone. Methamphetamine (2 mg/kg, i.p) produced a significant decrease in food intake of mice, which were deprived of food during the previous 24 h. This anorectic activity of methamphetamine was significantly antagonized by NAN-190 at a dose of 2 mg/kg, i.p. NAN-190 did not alter the food intake of mice when it was administered alone. Methamphetamine (2 mg/kg, i.p) also produced a significant increase in body temperature of mice, which was significantly antagonized by NAN-190 at a dose of 0.5 mg/kg, i.p. NAN-190 did not alter the body temperature of mice when it was administered alone. In the writhing test, methamphetamine (1 mg/kg, i.p) produced a significant antinociceptive effect in mice. This was significantly antagonized by NAN-190 at a dose of 1 mg/kg, i.p. NAN-190 did not alter the antinociceptive activity of mice when it was administered alone. The results of the present study indicate a possible role for serotonergic mechanisms, in addition to the catecholaminergic systems, in the above-studied activities of methamphetamine in mice. This role is possibly mediated through direct stimulation of the 5-HTIA receptor subtype. All of the above-studied activities of methamphetamine were antagonized by NAN-190, which may indicate that NAN-190 is a possible antagonist for methamphetamine.

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I. Introduction

Many aspects of the activities induced by the amphetamines are probably a consequence of the central release of dopamine (Creese and Iverson, 1973; Kelly, 1977), norepinephrine (Taglirontec et al., 1977), and/or 5-hydroxytryptamine (5-Hi⁺) (Foote et al., 1969; Fuxe and Ungerstedt, 1970; L-Holmes and Rutledge, 1976). Methamphetamine is known to increase central 5-Hi⁺ levels more markedly than other psychomotor stimulants such as amphetamine or cocaine (Munzar and Laufert, 1999).

Other suggested mechanisms for the amphetamines’ actions include reuptake inhibition of biogenic amines and inhibition of monoamine oxidase (Brain and Hoftman, 1998). Both mechanisms may result in increased concentrations of the biogenic amines centrally and peripherally. In addition, the amphetamines may exert direct effects on central receptors for the biogenic amines.

The brain serotonergic systems, in particular, are suggested to be associated with the symptomatology of major behavioral disorders. This may explain the effectiveness of 5-Hi⁺ antagonists in some behavioral conditions. The main therapeutic potential of 5-HTIA receptors, for instance, has been in the treatment of anxiety and depression. The antianxiety actions of 5-HTIA antagonists may involve primarily presynaptic somatodendritic 5-Hi⁺ IA receptors (leading to reduced release of 5-Hi⁺ in
terminal areas), whereas the antidepressant action of 5-HTIA receptors antagonists may primarily involve post-synaptic 5-HTIA receptor. Also 5-HTIA receptor antagonists may be involved in obsessive—compulsive disorders, sexual behavior, appetite control, thermoregulation, and cardiovascular function (Westkampcr and Glennon, 2002; Westkampcr ci al., 1999, 2001). With the advent of the new 5-HT receptor classification (Martin and Humphrey, 1994), methamphetamine’s interaction with these receptors needs to be explored. Although a variety of selective 5-HTIA receptor agonists are available, few truly selective antagonists for this site have been reported (Hoyer ci al., 1992; Seiler et al., 1992).

No specific antagonist is known for the amphetamines, although haloperidol may be used for its dopaminergic antagonistic activity. I - (2-Methoxyphenyl)-4-(4-[2-phthalimido]butyl)piperazine (NAN-190) is a drug under investigation. Since the serotonergic systems are highly influenced by amphetamine’s overdose, the present study was designed to study whether the 5-HTIA receptor antagonist NAN-190 may antagonize the major activities of methamphetamine. For this purpose, the effects of NAN-190 on methamphetamine-induced locomotor, anorectic, hyperthermic, and antinociceptive activities were investigated. The latter activity of methamphetamine is not well established as the former three activities, however, the amphetamines and many other sympathomimetics have long been known to produce analgesia (Ginawi et al., 1980; Leimdorfer and Metzner, 1949; Major and Pleuvry, 1971). Moreover sympathetic stimulation at times of excitement, anger or exercise can cause physiological analgesia in human.

2. Methods

2.1. 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 x 25 x 15 cm) with wood-chip bedding, under conditions of’ constant room temperature (23 ± 1 °C), humidity, and light cycle (7a.m. to 7 p.m.). They were given access to food (standard lab chow, Grain silos and flour mills organization, Riyadh) and water ad libitum. The experiments described in this study were approved by a Local Ethical Committee for the Conduction of Animal experiments. Each mouse was used on only one occasion.

2.2. Drugs

Drugs used in this study were: I (+)-methamphetamine hydrochloride (E.Merck, Germany) and NAN-190 hydrobromide (Sigma, USA). Both drugs were dissolved in 0.9% NaCl soltion. Doses were expressed as mg/kg of’ the salt. The dose vol Lime administered was 10 ml/kg, intraperitoneally.

2.3. Experimental design

In each of the experimental procedures planned, fotir groups were used as follows:

Group I: (control group) first treatment was saline; second treatment was saline.
Group 2: first treatment was NAN-190; second treatment was saline.
Group 3: first treatment was saline; second treatment was methamphetamine.
Group 4: first treatment was NAN-190; second treatment was methamphetamine.

The first treatment was given 30mm hcf arc for the second treatment. Appropriate doses of NAN-190 were determined from pilot experiments and in accordance with doses cited in the literature (e.g., Dias Elpo Zomkowski et al., 2004; Ginawi et al., 2004; Wesolowska et al., 2002). Animals used in pilot experiments were not used in any of the subsequent experiments.

2.4. Procedures

2.4.1. Measurement of locomotor activity

Locomotor activity was recorded by an activity meter (Optovarimex, Columbus, Ohio, USA). The activity cage of this instrument is equipped with horizontal and vertical sets of infrared photocells, which send continuotls unseen light beams. The sttimer of light beam interruptions due to the animal’s
movement inside the cage was automatically recorded. Each group of five mice was placed in the activity cage and counts of motor activity were recorded automatically every 10 mm for 2 h following saline or drug administration. Experiments were run between 10:00 a.m. and 3:00 p.m. under standard conditions of temperature, lighting, and noise as was practicable.

2.4.2. Measurement of anorectic activity

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 test drug or control.

2.4.3. Measurement of body temperature

Temperature measurements were made at room temperature (22 ± 1°C). The range of body temperature for all animals used in this experiment was 37—37.8°C; other animals lying outside this temperature range were rejected. Measurement of body temperature before and after drug administration (30 and 90 min post-injection) was done. Rectal temperature was measured by gently holding the mouse and inserting about 2 cm of a lubricated temperature probe (Model YSI 400, France) into the mouse rectum for a period of 30 s. The probe was attached to a digital thermometer (Aplex, Ph033 I, Panlab, France), which recorded the body temperature of the animal. Increases or decreases in body temperature were calculated by subtraction of the post-injection from the pre-injection rectal temperature readings.

2.4.4. Measurement of antinociceptive activity

The writhing test was used. An irritant agent (acetic acid 0.75%) was injected intraperitoneally to the mouse at a dose of 10 mL/kg. Writhing or stretching syndrome is characterized by a wave of contractions of the abdomen musculature followed by the extension of the hind limb. The numbers of stretching movements were counted for 20 min after acetic acid administration. The percentage inhibition of writhing for the test drug in comparison with the control group was calculated.

2.4.5. Statistical analysis

Statistical analysis of the results was performed by using Kruskal—Wallis one-way ANOVA. For significant results, a post hoc comparison between the means was done by Mann—Whitney U test. The level of significance adopted was at P=0.05.

3. Results

3.1. The effect of NA N-i 90 on methamphetamine-induced locomotor activity

From preliminary experiments, methamphetamine at a dose of 1 mg/kg did not modify the spontaneous locomotor activity of male mice throughout the 2 h observation period. A dose of 1.5 mg/kg of methamphetamine, however, significantly increased the spontaneous locomotor activity of mice; the latter dose was selected for challenge with NAN-190.

The effect of various doses of NAN-190 on methamphetamine-induced locomotor activity in male mice was explored. A dose of 4 mg/kg of NAN-190 was the least possible dose that significantly reduced methamphetamine-induced locomotor activity throughout the 2 h observation period (P<0.01), as seen in Fig. 1. This figure has previously been published by our group (Ginawi et al., 2004).

It is also clear from Fig. 1 that NAN-190 (4 mg/kg), when used alone (NAN 4+ saline groups), did not modify the spontaneous locomotor activity of control mice (saline + saline groups) during the time period observed.

3.2. The effect of NA N-i 90 on methamphetamine-induced anorectic activity

From preliminary experiments, methamphetamine at a dose of 2 mg/kg caused significant anorectic activity in food-deprived male mice, therefore, that dose was selected for challenge with NAN-190. It
was observed that methamphetamine-induced anorexia persisted only during the first 2 h following food presentation to the mice. Two hours after food presentation, the food intake of methamphetamine-treated mice was only about 9% of that of animals, which were not administered methamphetamine (Fig. 2). Methamphetamine-treated animals started to feed during the third hour of the observation period. By the end of the third hour, their food intake was remarkably increased to about 35% of that of mice not treated with methamphetamine, indicating reduction or loss of methamphetamine-induced anorexia at 2—3 h after its administration. For that reason, the evaluation of the results of the anorexia experiment was performed only during the first 2 h of the observation period.

From a series of doses of NAN-190 that significantly \( P < 0.01 \) antagonized the food intake of methamphetamine-treated mice throughout the first 2 h observation period was 2mg/kg, as shown in Fig. 2. It was also observed that NAN-190 (2mg/kg) did not modify food intake of food-deprived male mice.

### 3.3. The effect of NA N-i 90 on methamphetamine-induced hyperthermia

Methamphetamine (2mg/kg) significantly increased the normal body temperature of male mice throughout the 90 min observation period (Fig. 3). From a series of doses of NAN-190, the smallest dose that significantly \( P < 0.01 \) decreased the hyperthermic effect of methamphetamine throughout the 90 min observation period was 0.5mg/kg. That dose of NAN-190 (i.e., 0.5mg/kg) did not modify the normal body temperature of mice when used alone.

### 3.4. The effect of NAN-i 90 on methamphetamine-induced antinociception

Methamphetamine (1 and 2 mg/kg) significantly inhibited writhing induced by acetic acid in male mice during the 20 min observation period. The latter doses caused more than 65% inhibition of writhes. The dose of methamphetamine selected for challenge with NAN-190 was 1 mg/kg. Also, from a series of doses of NAN-190, the lowest dose of NAN-190 that significantly \( P < 0.01 \) decreased the % inhibition of writhing induced by methamphetamine was 1 mg/kg (from about 65% to less than 5% inhibition of writhing), as seen in Fig. 4. However, NAN-190 (1 mg/kg) did not modify the number of writhes of male mice elicited by acetic acid (0.75%) when tised alone.

### 4. Discussion

In this study, a known selective receptor antagonist for 5-HTIA receptor had been used prior to methamphetamine treatment, in some of the major activities induced by methamphetamine in mice. Prior treatment with NAN-190 caused inhibition of locomotor activity, anorexia, hyperthermia, and antinociception, which are tially induced by methamphetamine as well as other amphetamines. However, NAN-190 did not modify any of the studied activities when used alone. From previous stidies, we have indicated a role for 5-HTI’ in some actions of methamphetamine (Ginawi et al., 1980, 2004). Therefore, it is not tunial for 5-HTI’ antagonists to abolish at least some of the actions of methamphetamine. Moreover, in a recent report by Munzar and Laulbrt (1999) it has been shown that methamphetamine markedly increased central 5-HTI’ levels.

The present findings suggest that the 5-HTIA receptor subtype is involved in the mediation of methamphetamine-induced hyperactivity, anorexia, hyperthermia, and antinociception. However, the mechanism underlying this involvement is not clear, but may be related to modulation of the presynaptic inhibitory autoreceptors, which are believed to redice 5-HTI’ release when they are stimulated. 5-HTIA receptors reveal a dual localization:

that is, they are situated both post-synaptically to serotonergic neurons, for example, in the spinal cord, hypothalamus, hippocampus, and cortex as well as presynaptically as inhibitory autoreceptors on dendrites of raphe-localized serotonergic perikarya (Millan et al., 1993).

These results may suggest a direct agonistic effect of methamphetamine on presynaptic 5-HTIA receptors. The presynaptic 5-HTIA autoreceptors are more sensitive in nature to both agonists and antagonists when compared with the post-synaptic receptors (Cox and Ennis, 1982; Luscutthe et al., 1993). The inhibitory effect of NAN-190 towards methamphetamine-induced activities is mediated pharmacologically rather than being a result of non-specific sedation, since NAN-190 alone did not decrease any of these activities of control mice (Watson et al., 1992).

Abuse of the amphetamines causes severe medical and social problems throughout the world. The dopamine DL antagonist haloperidol has been used in the management of amphetamine poisoning or
overdose. However, it is not ideal for that purpose, because it is known to precipitate in the patient extrapyramidal side effects. Therefore, the search for more appropriate antagonist(s) for the amphetamines continues. Glutamate antagonists, like WBQX and NPC12626, for example, were shown to block the locomotor stimulant effects of methamphetamine in mice (Witkin, 1993). Similarly, dopamine D4 receptor antagonists, such as NRAO190 and clozapine, antagonized locomotor hyperactivity and stereotyped behavior induced by methamphetamine in mice (Okuyama et al., 1999).

However, the above-mentioned antagonists for glutamate or dopamine D4 receptors were only effective at high doses.

In conclusion, this study proposes a useful antagonist for the amphetamines illicit group of drugs. The 5-HT1A antagonist NAN-190 was found to reverse the major adverse characteristics of methamphetamine. Hence NAN-190 could be a more reliable drug than haloperidol in the management of amphetamine poisoning or overdose.

References

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