THE SEROTONIN RECEPTOR ANTAGONISTS NAN-190, METHIOTHEPIN, METHYSERGIDE, MIANSERIN OR ONDANSETRON PROTECT AGAINST METHAMPHETAMINE-INDUCED HYPERTHERMIA IN MICE

Omer T. Ginawi*, Aymen K. Al-Suwailem and Abdulhakeem A. Al-Majed

The effect of some serotonin receptor antagonists was investigated on the body temperature of male mice. These antagonists produced significant reductions of body temperature in methamphetamine-induced hyperthermic male mice. Thus, the hyperthermia induced by methamphetamine (11 -tmol/kg, i.p.) was antagonized by the previous i.p. administration of 1.06 gmol/kg NAN-190 (5-HT1A receptor antagonist), 22.1 ftmol/kg methiothepin (5-HT1C receptor antagonist), 2.13 ptrnol/kg methysergide (5-HT2A/2B receptor antagonist), 3.3 ~.tmol/kg mianserin (5-HT2C and u2 adrenoceptor antagonist) or 0.003 ~moll/kg ondansetron (5-HT3 receptor antagonist). The present results indicate that central serotonin receptors may be involved in the hyperthermic effect of methamphetamine. Ondansetron, being effective at a relatively small dose (0.003 ~tmol/kg) as compared to other antagonists used in this study, may be a potential antidote for methamphetamine-induced hyperthermia.

Key words: Methamphetamine, serotonin (5-HT), hyperthermia, Nan-190, methiothepin, methysergide, mianserin, ondansetron.

Introduction

Some aspects of locomotor activity and the stereotyped behavior induced by methamphetamine, a known drug of abuse worldwide, are probably a consequence of the release of dopamine from dopaminergic nerve terminals, particularly in the neostriatum. With higher doses of methamphetamine, disturbances of perception and overt psychotic behavior occur. These effects may be due to release of 5-hydroxytryptamine (5-HT, serotonin) from tryptaminergic neurons and of dopamine in the mesolimbic system (1). Other suggested mechanisms for the actions of methamphetamine include reuptake inhibition of biogenic amines and inhibition of monoamine oxidase. Both mechanisms may result in increased concentrations of the biogenic amines centrally and peripherally (1). Neurochemical studies indicate that methamphetamine increased central serotonin levels more markedly than other psychomotor stimulants such as amphetamine or cocaine. However, several studies indicate that 5-HT agonists and uptake inhibitors did not produce any methamphetaminelike stimulus effects when administrated alone, but if taken together with methamphetamine; the methamphetamine effects were significantly potentiated (2).

In addition to these mechanisms, methamphetamine may exert direct effects on central receptors for biogenic amines. Previously it was difficult to investigate such drug-receptor interactions, especially with serotonergic systems. However with the newly proposed serotonin receptor subtypes (3) and with the innovation of new selective agonist and antagonist drugs at these receptors, it became possible to try to investigate the receptor subtypes involved in the actions of methamphetamine.

Methamphetamine can induce hyperthermia (4) especially if it was used in overdose. Methamphetamine-induced hyperthermia may have serious consequences on the user such as rhabdomyolysis, culminating in fatal acute renal failure (5).

There is no known specific antidote for methamphetamine-induced hyperthermia. Haloperidol, the dopamine antagonist, has been used to reduce many of the behavioural actions induced by methamphetamine. However, haloperidol precipitates serious extrapyramidal side effects in the user. Therefore, search for a suitable treatment of the serious outcomes of poisoning with methamphetamine, such as rhabdomyolysis, is encouraged. Serotonin plays an important role in thermoregulation. Methamphetamine releases serotonin especially when used at high
poisoning doses and causes hyperthermia. Therefore, the present study was designed to identify some of the serotonin receptors involved in the hyperthermic action of methamphetamine, by using some of the available selective 5-HT antagonists.

Materials and Methods

Drugs:

Drugs used in this study were: methamphetamine hydrochloride (Merck AG. Darmstadt, Germany), NAN—190 hydrobromide (Sigma Chemical Co., St.Louis, USA), methiothepin (Winlab, Leicestershire, UK), methysergide (Research Biochemicals Inc., Wayland, USA), mianserin hydrochloride (Sigma Chemical Co., St. Louis, USA) and ondansetron hydrochloride dihydrate (Glaxo Laboratories Ltd, Greenford, UK). All drugs were dissolved in 0.9% NaCl solution. Doses are expressed as μmol/kg of the salt. Doses were administered in a volume of 10 ml/kg, intraperitoneally.

Animals

Male Swiss albino mice (obtained from the Animal Care Center, College of Pharmacy, King Saud University) weighing 25-30 grams were used. The animals were housed, 10 mice per cage, under conditions of constant room temperature (22 ± 1°C) humidity and light cycle (7 am. to 7 p.m.). They were given access to food (standard lab chow, Grain Silos and Flour Mills Organization, Riyadh) and water ad libitum. Each mouse was used only once.

Treatment protocol

In each of the experimental procedures planned, two groups (each group consisting of 10 mice) were designated for each antagonist study. The first group was for antagonist + saline study, and the second group was for antagonist + methamphetamine study. In addition, two groups (each group consisting of 10 mice) were designated for control purposes. These were saline + saline group and saline + methamphetamine group.

The first drug (i.e. saline or antagonist) was given 30 mm before the second drug (saline or methamphetamine).

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). Measurement of body temperature was performed before any drug administration and at 30 and 90 minutes after injection of the second drug. Rectal temperature was measured by gently inserting a lubricated temperature probe (model YSI 400, France) 2 cm into the rectum of the mouse for 30 seconds. The probe was connected to a digital thermometer (Aplex, Pb033 1, Panlab, France). Increases or decreases in body temperature were calculated by subtraction of the post-injection from the pre-injection rectal temperature readings.

Statistics:

Statistical analysis of the results was performed by using one way ANOVA. For significant results, a post-hoc comparison between the means was done by Tukey-krammer test. Significance was accepted at P<0.05.

Results

Appropriate dosages for methamphetamine and ~ for each of the 5-HI antagonists were determined g. from pilot experiments, conforming with doses of ~ these drugs commonly employed in previous studies cited in the literature.

Methamphetamine at a dose of 11 μmol/kg, i.p. (2 mg/kg), significantly increased the normal body ~ temperature of male mice during an observation ~ period of 90 mm. This dose of methamphetamine was selected for challenge with 5-HI antagonists in all experiments of this study.

The effect of NAN-190 on methamphetamine-induced hyperthermia in male mice:

Escalating doses of NAN-190 were tested against methamphetamine-induced hyperthermia in male mice. Practically, the least possible dose of NAN-190, which significantly (P< 0.001) abolished the hyperthermia-induced by methamphetamine during the 90 mm observation period, was 1.06 μmol/kg, i.p. ( 0.5 mg/kg), as shown in Fig 1. This dose of NAN-190 did not alter the normal body temperature of mice when used alone.

The effect of methiothepin on methamphetamine-induced hyperthermia:

The least possible dose of methiothepin, which significantly (P< 0.001) abolished the hyperthermia induced by methamphetamine during the 90 mm observation period was 22.1 μmol/kg, i.p.( 0.1 mg/kg), as shown in Fig 2. However, this dose of methiothepin caused a significant reduction in normal body temperature of male mice when used alone (P< 0.05).
**Time effect of methysergide on methamphetamine-induced hyperthermia:**

Various doses of methysergide were tested against methamphetamine-induced hyperthermia in male mice. The least possible dose of methysergide, which significantly \( P<0.001 \) abolished the hyperthermia induced by methamphetamine during the 90 min observation period (Fig 3.) was 2.13 \( \mu \)mol/kg, i.p. (1 mg/kg). This dose of methysergide did not affect the normal body temperature of mice when used alone.

**The effect of mianserin on methamphetamine-induced hyperthermia:**

The least possible dose of mianserin, which significantly \( P<0.001 \) abolished the hyperthermia induced by methamphetamine during the 90 min observation period was 3.3 j mol/kg, i.p. (1 mg/kg), as shown in Fig 4. This dose of mianserin did not affect the normal body temperature of mice when used alone.

**Discussion**

In this study, methamphetamine produced a significant increase in body temperature. The hyperthermia induced by methamphetamine have previously been explained to be due to methamphetamine’s increases of oxidative stress, which may mediate the toxic effects of this drug. Systemic methamphetamine increases hydroxyl radical formation and leads to protein modification in the striatum (6-8). In addition, energy use is increased after systemic administration of methamphetamine, as evidenced by an immediate and sustained increase in the extracellular concentrations of lactate in the striatum (9). Furthermore, adenosine triphosphate (ATP) concentrations are rapidly lost in brain slices from the mouse striatum after methamphetamine pretreatment (10).

Methamphetamine-induced hyperthermia is also related to the toxicity of methamphetamine on both serotonin and dopamine (DA) neurons. Following an initial massive release of these neurotransmitters, methamphetamine causes long-lasting decreases in tissue concentrations of 5-Hi and DA (11-13) as well as decreases in the activities of their respective rate-limiting enzymes, tryptophan hydroxylase and tyrosine hydroxylase, respectively (14).

Serotonin is a biogenic amine that is distributed widely in various body tissues and cell types and possesses a diversity of pharmacological effects at both central and peripheral sites. Serotonin is involved in the control of temperature regulation, cardiovascular function, muscle contraction and endocrine regulation. Seven major families of 5-HT receptors have been identified, all of which have distinct pharmacological properties, regional distributions and physiological functions. The physiological effects of 5-HT are mediated by at least four groups of 5-HT receptors, which have been distinguished pharmacologically according to the second messenger systems to which they are coupled (15).

In the present study, and with the exception of methiothepin, all 5-HT antagonists used (i.e. NAN190, methysergide, mianserin, and ondansetron) did not alter the body temperature when used alone. However, all the 5-HT antagonists tested in this study, including methiothepin abolished methamphetamine-induced hyperthermia. These findings may be related to the ability of these antagonists to act directly or indirectly to block the actions of released 5-HT on the central neuronal pathways involved in the temperature regulation and therefore prevent any temperature elevation (16-18).

NAN-190 is not a pure selective antagonist at 5-HTIA receptors. Recently, NAN-190 has been reported to be a partial agonist of presynaptic and an antagonist of postsynaptic 5-HTIA receptors and alpha-adrenoceptors (19). Therefore, the results of NAN-190 in the present study should be cautiously interpreted. The same investigators, however (19), studied the effects of MM77 and MP245 (agonists of postsynaptic 5-HTA receptors), as well as 8-OH-DPAT (a 5-HTIA agonist) and prazosin (an alpha-adrenoceptor antagonist) on the body temperature of mice. All of these agents induced a dose-dependent hyperthermia in mice, indicating the involvement of their respective receptors in thermoregulation.

The results of the present study indicate the possible involvement of 5-HT12 receptors (or some of their subtypes) in methamphetamine-induced hyperthermia. Thus, 5-HTIA receptors (which are blocked by NAN-190), 5-HTIB/ID receptors (which are blocked by methiothepin), 5-HT2A/2B (which are blocked by methysergide), 5-HT3, (which are blocked by mianserin) and 5-HT3 (which are blocked by ondansetron) are involved in methamphetamine-induced hyperthermia. A relatively small dose of ondansetron as compared to the other 5-HT antagonists used in this study, has successfully reversed methamphetamine-induced hyperthermia. This observation suggests that 5-HT3 receptors are a possible site of action in methamphetamine-induced hyperthermia. However, this is not to exclude 5-HT4 receptors, which were not investigated in this study.

Little is known about the role of 5-HT in skeletal muscle cells, the site for rhabdomyolysis. However, 5-HT exerts direct effects at 5-HT receptors in skeletal muscles. It has been shown that administration of 5-HI produced an increase in the intracellular concentration of cyclic 3,5-AMP in skeletal muscle preparations via specific 5-HT receptors on skeletal muscle (20). The 5-HT3 receptor is a ligand-gated ion channel and activation of this receptor leads to increased adenyl cyclase activity and hence muscle contraction. Therefore, it is not unusual that blockers of this receptor may...
antagonize muscle contraction and may protect against hyperthermia and rhabdomyolysis.

References