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Research report

Serotonergic involvement in methamphetamine-induced locomotor activity: A detailed pharmacological study

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ABSTRACT

The mechanism by which the psychostimulant methamphetamine (METH) increases locomotor activity may be attributable to indirect activation of serotonin (5-HT) and dopamine (DA) receptors. In the present study, the ability of the serotonin reuptake inhibitor fluvoxamine, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptor antagonists WAY100635, GR127935, M100907 and SB242084, and the 5-HT_{2C} receptor agonists WAY163909 and Ro 60-0175 or the 5-HT synthesis inhibitor para-chlorophenylalanine (pCPA) to alter METH-induced hyperactivity was analysed. Further, for comparative purposes, the involvement of the DA D_1 and D_2 receptor antagonists SCH23390 and haloperidol, D_2 partial agonists terguride, (−)3PPP and aripiprazole and finally clozapine were assessed. Doses of pCPA that attenuated 5-HT levels reduced METH activity. The 5-HT_{1B} antagonist GR127935 had no effect on METH-induced locomotor activity but blocked that induced by MDMA. The 5-HT_{1A} antagonist WAY100635 reduced activity but this did not reach significance. In contrast, M100907 (minimal effective dose; MED = 0.125 mg/kg), WAY163909 (MED = 3 mg/kg), Ro 60-0175 (MED = 3 mg/kg), haloperidol (MED = 0.1 mg/kg), clozapine (MED = 5 mg/kg), aripiprazole (MED = 1 mg/kg), (−)3PPP (MED = 3 mg/kg), terguride (MED = 0.2 mg/kg) and SCH23390 (MED = 0.001325 mg/kg) attenuated METH-induced locomotor activity. Administration of 20 mg/kg fluvoxamine attenuated, while SB242084 (MED = 0.25 mg/kg) potentiated METH-induced activity.

These results contribute significantly to the understanding of the mechanism of action of this psychostimulant and suggest for the first time, that METH-induced locomotor stimulation is modulated by 5-HT_{2A} and 5-HT_{2C} receptors, but demonstrate that 5-HT_{1B} receptors are not directly involved. The involvement of the dopaminergic system was also demonstrated.

1. Introduction

Methamphetamine (METH) is a potent and highly addictive psychostimulant [1] which is epidemically abused with significant socio-economic consequences worldwide (United Nations Office on Drugs and Crime Global Survey, 2003). Chronic METH abuse has been shown to induce long-term behavioural changes such as severe motor and cognitive impairment and psychosis. Given its high propensity for abuse-liability and the devastating consequences of chronic METH abuse, an increased understanding of the pharmacology of METH is needed [2,3].

METH is primarily a serotonin (5-hydroxytryptamine; 5-HT) and dopamine (DA) releaser, liberating and inhibiting reuptake via the reversal of the 5-HT and DA transporters (5-HTT and DAT, respectively; [4–7]). A considerable body of literature exists which supports the role of 5-HT and DA neurotransmission in the mediation of METH effects *in vivo*. For example, microdialysis studies demonstrate significant increases in extracellular DA and 5-HT following METH administration [8,9] and conversely inhibition of DA synthesis and antagonism of DA receptors have been shown to attenuate METH-induced changes in dopaminergic transmission [10]. In addition to the neurochemical effects, behavioural studies confirm the involvement of both DA and 5-HT in METH-mediated behaviours, as the DA release inhibitor, CGS-10746B, antagonised METH-induced locomotor hyperactivity [11] and the antagonism of METH-induced behaviour by DA D_1 and D_2 antagonists suggest both receptor sub-types may be critical for the mediation of METH-induced locomotor activation [12–16]. With regards to the role of 5-HT in the mediation of METH effects, a novel 5-HT_{1A} receptor agonist, JB-788, has recently been shown to dose-dependently inhibit METH-induced locomotor activity [17]. Similarly, pre-treatment

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with a 5-HT_{2A} receptor antagonist also attenuated locomotor activity induced by METH, although the compounds used possessed DA D_2 and other pharmacological properties [16,18]. The mechanism of action of METH, therefore, might rely on its dual 5-HT and DA properties [7]; a postulation endorsed for the behavioural effects of other psychostimulants such as MDMA [19–22]. Incidentally, a number of these findings are also true for the psychostimulants MDMA, cocaine and amphetamine, which suggest a mechanism of action, at least in part, common to all these drugs (for recent reviews see [19,23–31,69]). Even so, data shows that the magnitude and extent of the contribution of 5-HT upon the behavioural effects of METH has not yet been categorically identified and the contribution of different 5-HT receptor subtypes to METH-induced locomotor hyperactivity requires further investigation.

The purpose of the present studies was therefore to gain further insight into the contribution of 5-HT and of 5-HT receptors on the behavioural effects of METH-induced locomotor activity with specific focus on 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors. Additionally, for comparative purposes, effects were compared to those of dopaminergic receptor ligands.

2. Materials and methods

2.1. Animals

Experimentally naïve male Wistar rats ($n = 8$ per treatment group; Harlan, The Netherlands) weighing 175–199 g at the beginning of experimental procedures were used. The rats were housed three per cage in a colony room maintained at constant temperature (19–21 °C) and humidity (50–70%) for at least 5 days before experimentation. Food and water were available *ad libitum* except during experimental sessions. Lighting was maintained on a 12 h light/dark cycle (lights on 07:00–19:00). All measurements of locomotor activity were carried out during the light phase (08:30–17:00) and rats were acclimatized to the testing room the night before experimentation. All treatments were balanced across the experimentation period in a randomized order.

All procedures were conducted under the auspices and guidance of the institutional animal care and use committee guidelines (*Dieren Ethisch Commissie, DEC*, Number (0107-024)) and in accordance with Dutch Law.

2.2. Apparatus

Locomotor activity was monitored using a commercially available locomotor activity system (San Diego Instruments, San Diego, USA). Each test chamber ($21 \times 36 \times 18$ cm) was housed within a sound-attenuated enclosure and placed between 7 horizontal photobeams. Interruptions of the photobeams resulted in counts of ambulatory activity. The control software (Photobeam Activity Software, San Diego Instruments, San Diego, USA) was used to analyse data, which were stored for subsequent statistical analyses.

2.3. Behavioural procedures

2.3.1. General behavioural procedure

In all cases, rats were placed in the locomotor activity chambers for 30 min prior to dosing with vehicle (2 mL/kg) or the serotonin uptake inhibitor (SSRI) fluvoxamine (5, 10 and 20 mg/kg intraperitoneally (ip)), the 5-HT_{1A} antagonist WAY100635 (0.1, 0.3, 1 and 3 mg/kg sub-cutaneously (sc)), the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (0.1, 0.625, 1.25, 2.5 mg/kg sc), the selective 5-HT_{2A} receptor antagonist M100907 (0.0625, 0.125, 0.25 and 0.5 mg/kg ip), the 5-HT_{2C} receptor antagonist SB242084 (0.25, 0.5, 1 and 2 mg/kg ip), the 5-HT_{2C} receptor agonists WAY163909 (1, 3, 10 and 30 mg/kg ip) and Ro 60-0175 (0.1, 0.3, 1 and 3 mg/kg ip), the DA D_2 antagonist haloperidol (0.03 and 0.1 mg/kg ip), the atypical antipsychotic clozapine (5, 10 mg/kg ip), the D_2/D_3 partial agonist and atypical antipsychotic aripiprazole (1, 3, 10, 30 mg/kg ip), the DA D_2 receptor partial agonists (−)3PPP (0.3, 1, 3, 6 mg/kg sc) and terguride (0.05, 0.1, 0.2 and 0.4 mg/kg sc) or the D_1 receptor antagonist SCH23390 (3.125, 6.25, 12.5 and 25 μg/kg sc). Thirty minutes later rats were injected sc with 0.8 mg/kg METH or saline (0.9%) vehicle and returned to the test chamber. Recording began immediately in 5 min time epochs and continued for 120 min following METH administration.

Selection of drug doses was based on previous experience with these ligands and from reference to the literature i.e. WAY100635: [32]; GR127935: [22]; M100907: [31,33]; SB242084: [34,35]; WAY163909: [36]; Ro 60-0175: [37]; Haloperidol: [24]; Clozapine: [28]; Aripiprazole: [38]; (−)3PPP: [39]; Terguride: [39]; SCH23390: [19,30]). The 0.8 mg/kg (sc) test dose of METH was based on preliminary dose-effect studies in our laboratory (not shown).

In addition, using the same protocol described above, GR127935 (0.1, 0.3, 1.25, 2.5 mg/kg sc) was tested for its ability to attenuate (+)-MDMA-induced locomotor activity (3 mg/kg sc; [22]).

Notably, naïve rats were only tested once in the behavioural paradigms preventing sensitization to the effects of METH (and MDMA) and, therefore, ensuring only the acute effects of the psychostimulants, and no context-specific conditioning effects, were characterized.

2.3.2. The Effect of the 5-HT synthesis inhibitor pCPA on METH-induced locomotor activity

In order to test the hypothesis that 5-HT is pivotally involved in the locomotor stimulating properties of METH, rats were pretreated with the 5-HT synthesis inhibitor para-chlorophenylalanine (pCPA). Rats ($n = 7$ per treatment group) were treated with vehicle (0.9% saline; 2 mL/kg) or pCPA (150 mg/kg ip, bi-daily; administered at 09:00 and 17:00 h) for 3 days. Approximately 16 h after the last administration of pCPA or vehicle, the rats were habituated to the locomotor testing apparatus for 60 min prior to receiving METH (0.8 mg/kg sc) or vehicle and locomotor activity was recorded for 120 min in 5 min time epochs. Satellite groups were used to assess the impact of the pCPA vs. vehicle treatment on whole-brain 5-HT and DA levels. This was not tested in the same groups of rats used in the behavioural experiment as METH-induced 5-HT and DA release would result in functional depletion and confound interpretation. However, these satellite groups were treated parallel to the other animals and were euthanized on the same day of behavioural testing, following the initiation of the behavioural studies.

2.3.3. Tissue preparation for analysis of DA and 5-HT levels from pCPA studies

Following cervical dislocation and decapitation, brains were rapidly dissected (4 °C), the cerebella removed, and immediately frozen at −80 °C for later analyses. High performance liquid chromatography (HPLC) was employed to determine DA and 5-HT content (whole-brain less cerebellum). Frozen brain samples were weighed and immediately homogenized with 0.4N perchloric acid (containing 100 ng/mL methyl serotonin and 0.1% cysteine). Homogenates were centrifuged at 12000 rpm for 2 min at room temperature (21 ± 2 °C) and the supernatant injected into an HPLC column with electrochemical detection for 5-HT and DA. The mobile phase (ammonium dihydrogen phosphate (0.05 M), $NaClO_4$ (0.1 M), octane sulphonic acid (1.5 mM), Na_2EDTA (0.1 mM) and propanol (2%)) was delivered through a Supelcosil column (LC-8-DB, 150×4.6 mm, 3 μm, Supelco, PA, USA) at a flow rate of 1 mL/min (Hewlett Packard pump, Amsterdam, the Netherlands). The column was maintained at 25 °C and pH 2.5. Volumes of perchloric acid solution (μL) were calculated from the weight of the samples (weight × 500/50). Cerebella tissue was used for calibration purposes.

2.4. Statistical analyses

Ambulatory counts were summed for each individual rat across the measurement period. The data are presented as mean ambulatory activity counts (+the standard error of the mean; +SEM) for the first 60 min following METH administration, since this generated the maximal effect. One-way analysis of variance (ANOVA) was used to determine overall treatment effects on locomotor activity across the test session. Dunnett's test was used for post-hoc analysis in all instances following a significant ANOVA ($p \leq 0.05$) to assess the difference between treatment groups and a pre-selected control group. Simultaneous comparisons were made between all treatment groups and the vehicle – vehicle control and against the vehicle – METH treatment groups in most cases. In all experiments, the minimal effective dose (MED) was defined as the minimal dose at which statistically different results were seen. Statistical analyses of tissue levels of 5-HT and DA following saline vehicle and pCPA pretreatment were assessed using Student's *t*-test.

2.5. Drugs

WAY100635 (*N*-[2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl]-7*V*-2-pyridinyl) cyclohexanecarbonate trihydrochloride]), GR127935 ((2′-methyl-4′-(5-methyl-(1,2,4)oxadiazol-3-yl)biphenyl-4-carboxylic acid (4-methoxy)-3-(4-methylpiperazin-1-yl)phenyl)amide), M100907 (R-(+)-α-2,3-dimethoxyphenyl)-1-(2-(4-fluorophenylethyl))-4-piperidine-methanol), SB242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl] indoline), Ro 60-0175 (2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine) fumarate), WAY163909 ((7b*R*,10a*R*)-1,2,3,4,8,9,10,10a-octahydro-7b*H*-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole), fluvoxamine maleate, aripiprazole maleate, were synthesized by Abbott Healthcare Products B.V. Terguride malate, clozapine HCl, and pCPA (methylester hydrochloride) and (−)3PPP HCl were supplied by Sigma–Aldrich, Zwijndrecht, The Netherlands. Haloperidol (Siegfried Zofingen, Switzerland) and (+)-MDMA (Sigma, St. Louis, USA), Methamphetamine HCl (Siegfried Zofingen, Switzerland), WAY100635, (−)3PPP, pCPA, SCH23390 maleate (Schering Corporation, Bloomfield, NJ, USA) and fluvoxamine were dissolved in 0.9% saline. Haloperidol and clozapine were dissolved in 25% hydroxypropyl β-cyclodextrin (HPβCD). MDMA was prepared in 1% methylcellulose. All other compounds were prepared in 40% HPβCD with mild acidification and gentle heating and pHs were adjusted to pH 6.5–7.5. Verification that compounds were dissolved was made visually. All doses refer to the weight of the salt and all drugs and vehicles were administered at a constant volume of 2 mL/kg. Doses and routes of administration were based upon those previously reported in the literature as detailed above. METH was injected sc with reference to Gentry et al. [40].