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Effects of WAY 100635 and (−)-5-Me-8-OH-DPAT, a novel 5-HT$_{1A}$ receptor antagonist, on 8-OH-DPAT responses

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Abstract

The neurochemical profile at both post and presynaptic 5-HT$_{1A}$ receptors of a novel 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) analog, 5-methyl-8-hydroxy-2-(di-*n*-propylamino)tetralin {(±)-5-Me-8-OH-DPAT} and its stereoisomers was determined and compared to that of the highly selective 5-HT$_{1A}$ receptor antagonist, *N*-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide (WAY 100635). We evaluated their effects on 8-OH-DPAT-induced decrease in cAMP production, on 8-OH-DPAT-induced decrease in rat ventral hippocampal extracellular 5-hydroxytryptamine (5-HT$_{ext}$) levels and in body temperature in mice. Both (±)- and (−)-5-Me-8-OH-DPAT blocked the 8-OH-DPAT-induced inhibition of forskolin-stimulated cAMP production. Moreover, while having no significant effect when injected alone, (±)-, (−)-5-Me-8-OH-DPAT and WAY 100635 antagonized the 8-OH-DPAT-induced decrease in 5-HT$_{ext}$ in rats and hypothermia in mice. By contrast, the (+) isomer inhibited the cAMP synthesis and did not modify the 8-OH-DPAT response on 5-HT$_{ext}$ in ventral hippocampus. These data suggest that (±)-5-Me-8-OH-DPAT acts selectively, its activity residing in the (−) enantiomer, this latter compound acting similarly to WAY 100635 as a full, selective and silent 5-HT$_{1A}$ antagonist. © 1998 Elsevier Science B.V.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT$_{1A}$ receptor antagonist; cAMP; Microdialysis; Hypothermia

1. Introduction

Among the fourteen 5-HT receptor sub-types currently recognized (Hoyer et al., 1994), the 5-HT$_{1A}$ receptor sub-type has been extensively studied due to the early identification of a high affinity 5-HT$_{1A}$ receptor binding ligand with agonist properties for this site, 8-hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT (Hjorth et al., 1982; Gozlan et al., 1983; Middlemiss and Fozard, 1983). Behavioral and neurochemical effects of 5-HT$_{1A}$ receptor agonists appear to depend on the stimulation of 5-HT$_{1A}$ receptors located presynaptically or postsynaptically. The former 5-HT$_{1A}$ receptor is located on cell bodies and dendrites of serotoninergic neurones in the midbrain raphe nuclei, while the latter 5-HT$_{1A}$ receptor is mainly present in terminal projection areas of these neurones such as the cortex and hippocampus (Radja et al., 1992; Vergé et al., 1985). Activation of the postsynaptic 5-HT$_{1A}$ receptors leads, among other responses, to the 5-HT behavioral syndrome and to the inhibition of forskolin-stimulated adenylyl cyclase activity implying a decrease in cAMP concentrations (Fletcher et al., 1993b). Systemic administration to rats of 8-OH-DPAT, by stimulating presynaptic 5-HT$_{1A}$ receptors, inhibited the 5-HT synthesis, the firing of 5-HT neurones in the raphe nuclei and consequently the 5-HT release at serotoninergic nerve terminals assessed by in vivo microdialysis (Hjorth and Sharp, 1991). When administered to mice, 8-OH-DPAT decreased body temperature (Martin et al., 1992; Forster et al., 1995), this latter response being abolished by lesioning central serotoninergic neurones by the intracerebroventricular infusion

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of the neurotoxin 5,7-dihydroxytryptamine (Bill et al., 1991). These results suggest that this response to 8-OH-DPAT is mediated by the activation of inhibitory somatodendritic 5-HT_{1A} autoreceptors located in the raphe nuclei.

However, the progress in the physiological and functional role of this receptor has been precluded by the lack of availability of highly selective 5-HT_{1A} receptor antagonists, which are both selective and full ('silent'). Indeed, some β-adrenoceptor antagonists {(−)-pindolol, (−)-penbutolol, (−)-tertatolol} that bind with a low affinity to 5-HT_{1A} receptors thus inhibiting various 8-OH-DPAT-induced effects (Middlemiss, 1986), are not selective since they also bind with high affinity to β-adrenoceptor receptors. (*S*)-UH-301, an enantiomer of 5-fluoro-8-OH-DPAT, presents a 5-HT_{1A} receptor antagonist profile, but is also non-selective as it binds to dopamine D_2 sites (Hillver et al., 1990). The phenylpiperazine derivatives NAN 190 and BMY 7378, which first were claimed to be selective 5-HT_{1A} receptor antagonists (Chaput and De Montigny, 1988; Glennon et al., 1988), displayed antagonist properties only in postsynaptic 5-HT_{1A} receptor models and have been shown to evoke agonist-like responses at presynaptic somatodendritic 5-HT_{1A} receptors, thereby inhibiting raphe neuronal firing (Sharp et al., 1990; Hjorth and Sharp, 1990). (+)-WAY 100135 has been described as displaying a high degree of selectivity for 5-HT_{1A} sites and being an antagonist at both pre- and postsynaptic 5-HT_{1A} receptors (Fletcher et al., 1993a), but its inhibitory influence on the firing of 5-HT neurones seems to reflect the blockade of α_1-adrenoreceptors (Lanfumey et al., 1993). WAY 100635 appears to be the only highly selective, full 5-HT_{1A} receptor antagonist that is without intrinsic activity in several models studying its effects at pre- and postsynaptic receptors and being able to block the effects of the standard 5-HT_{1A} receptor agonist, 8-OH-DPAT in all 5-HT_{1A} receptor functional models studied so far (Fletcher et al., 1994; Khawaja et al., 1995).

Following the synthesis of (±)-5-Me-8-OH-DPAT, a C5-methyl substituted analog of 8-OH-DPAT (Langlois et al., 1993), both isomers were separated and binding studies revealed that (+) and (−)-5-Me-8-OH-DPAT had good affinity for 5-HT_{1A} receptors ($K_i = 32.9 \pm 0.8$ and 45.6 ± 2 nM, respectively). Affinities for D_2 receptors are 570 and 3500 nM for (+)- and (−)-5-Me-8-OH-DPAT, respectively and > 1000 nM for 5-HT_{1B} receptors for both isomers, suggesting high selectivity of the compounds for the 5-HT_{1A} site. The purpose of the present study was to characterize the effects of (±)-5-Me-8-OH-DPAT and its enantiomers at both post and presynaptic 5-HT_{1A} receptors. We examined the effects of these drugs on various 8-OH-DPAT-induced responses: 1-) inhibition of forskolin-stimulated adenylyl cyclase activity in the rat hippocampal homogenates as a model of postsynaptic 5-HT_{1A} receptor activation (De Vivo and Maayani, 1986) 2-) decrease in extracellular 5-HT levels (5-HT_{ext}) as measured by in vivo microdialysis in the ventral hippocampus of freely moving rats (Sharp et al., 1989) and 3-) hypothermia in mice (Goodwin et al., 1985), these last two tests reflecting presynaptic 5-HT_{1A} receptor activation. In all the tests performed so far, we compared the neurochemical and behavioral effects of the drugs to those of WAY 100635.

2. Materials and Methods

2.1. Animals

cAMP assays and microdialysis studies were carried out in male Sprague–Dawley rats (200–300 g, Charles River, France). Animals were allowed to have free access to food and water and housed singly for the experiments. For body temperature studies, female 129/Sv mice (25–30 g, bred in our animal facility, Châtenay-Malabry, France) were housed in groups of eight at an ambient temperature of 21 ± 0.5°C for at least 2 h before the first measurement of body temperature and drug administration.

2.2. Drugs and treatment

The following drugs and chemicals were used in this study: 8-Hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT, Tris, EGTA, NaCl, sucrose, $MgSO_4$, 3-isobutyl-1-methylxanthine (IBMX), Forskolin, GTPγS, 5-HT from Sigma (L'Isle d'Abeau, France); GTP, ATP, creatine phosphate, creatine kinase, cAMP from Boehringer (Les Ulis, France); [^{3}H]8-OH-DPAT, [^{32}P]α-ATP, [^{3}H]cAMP from Dupont-NEN (Les Ulis, France); {*N*-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide, 3HCl, WAY 100635 from Weyth Research (Maidenhead, UK), 5-methyl-8-Hydroxy-2-(di-*n*-propylamino)tetralin, (±)-5-Me-8-OH-DPAT, (−)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT were synthetized by Drs. Mathé-Allainmat and Langlois, CNRS BIOCIS URA 1843 (Châtenay-Malabry, France).

2.3. Adenylyl cyclase experiments

Rat hippocampi were dissected on ice immediately after death by decapitation and homogenized in 50 volumes (v/w) of 2 mM ice-cold Tris–maleate containing 2 mM ethylene glycol bis β-aminoethyl-ether tetraacetic acid (EGTA) and 0.3 M sucrose, pH = 7.2, using an Arthur H. Thomas tissues homogenizer. Homogenates were centrifuged at $500 \times g$ for 5 min at 4°C, and the supernatants were collected for a further centrifugation at $35\,000 \times g$ for 10 min at 4°C. The sedimented material was then suspended in the same volume of the isotonic Tris–maleate buffer (as above), and aliquots (40 μl) were used for the adenylyl cyclase assays (De Vivo and Maayani, 1986) with forskolin (10 μM), GTP (10 μM) and NaCl (0.1 M)

added to the assay mixture (25 mM Tris–maleate, 1 mM $MgSO_4$, 0.1 mM IBMX, 0.5 mM ATP plus 1 μCi of [^{32}P]α-ATP, [^{3}H]cAMP (about 10 000 cpm), 20 mM creatine phosphate and 0.2 mg/ml of creatine kinase, 1 mM cAMP, pH = 7.2 and with various concentrations of 8-OH-DPAT (10 μM to 0.2 nM) or (+)-5-Me-8-OH-DPAT (10 μM to 10 nM) in the presence or the absence of (±)-5-Me-8-OH-DPAT (10 μM) or (−)-5-Me-8-OH-DPAT (1 to 10 μM) or WAY 100635 (0.2 μM), (total volume 0.1 ml). Incubation proceeded for 20 min at 30°C, and was stopped by adding 0.1 ml of a mixture containing 50 mM Tris–HCl, 5 mM ATP, 2 mM cAMP and 1% sodium dodecylsulphate, pH = 7.4. Newly synthetized [^{32}P]cAMP was finally extracted by ion exchange and absorption chromatography as described by Salomon (1979). Proteins were determined according to the method with a Micro BCA protein assay reagent kit (Pierce) using bovine serum albumin as the standard. Adenylyl cyclase activity is expressed as pmol [^{32}P]cAMP synthesized per mg of protein per minute at 30°C.

2.4. In vivo microdialysis

Concentric dialysis probes were made of polyacrilonitril fibers (Hospal AN69, France) and constructed as described previously (Gardier et al., 1994). The size of the dialysis membranes was 4 mm long × 0.30 mm OD. The probes were tested for in vitro recovery of 5-HT on the day before use to verify that recoveries were within a desired range (around 20% for 5-HT). Animals were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame. Rats were implanted with a probe in the left ventral hippocampus following coordinates (in mm) taken from bregma and top of the skull (Paxinos and Watson, 1986): A −4.8, L −4.8, V −7.5. The probe was cemented in place. The animals were allowed to recover from the surgery for approximately 20 h, and then, the probe was continuously perfused with an artificial CSF (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.0, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, pH 7.4 ± 0.2) at a flow rate of 1.3 μl/min using a CMA/100 pump (Carnegie Medicin, Stockholm, Sweden). At the end of each experiment, the brain was removed, fixed in NaCl (0.9%)/paraformaldehyde solution (30%), cut on a Leitz cryomicrotome, and on serial coronal sections placement of microdialysis probes was verified. Only data obtained from rats with properly implanted probes were included in the results.

Dialysate samples were collected every 15 min in Eppendorf tubes and were immediately analyzed for 5-HT by high performance liquid chromatography (HPLC), using LC-4B amperometric detector (Bioanalytical System), as previously described (Malagié et al., 1995). The limit of sensitivity was about 1 fmol/sample for 5-HT. Usually four to five basal fractions were collected to obtain basal level (means ± S.E.M.) before peripheral administration of the drugs.

2.5. Drug administration

Animals received a first subcutaneous (s.c.) injection of either saline or WAY 100635 (1 mg/kg) or (±)-5-Me-8-OH-DPAT or (−)-5-Me-8-OH-DPAT or (+)-5-Me-8-OH-DPAT all at 10 mg/kg followed by a second s.c. injection, 30 min later, of either saline or 8-OH-DPAT (0.1 mg/kg).

2.6. 8-OH-DPAT-induced hypothermia

The procedures used for these studies are based on those described by Bill et al. (1991). Body temperature was measured in gently restrained mice with a thermistor probe (M-99 electro-therm, Phymep, France) inserted 2 cm into the rectum. (−)-5-Me-8-OH-DPAT (1–10 mg/kg) or WAY 100635 (0.01–1 mg/kg) or vehicle were administered subcutaneously 30 min before the s.c. injection of standard challenge dose of 8-OH-DPAT (0.1 mg/kg). Temperatures were measured immediately before each drug administration and at 15 and 30 min after 8-OH-DPAT injection. The hypothermic response to 8-OH-DPAT was measured as the maximal decrease in body temperature recorded in this latter period.

2.7. Data analysis

All the statistical analyses were performed by the use of the computer software Statview 4.02 (Abacus concepts, Berkeley, CA, USA), significance level was set at $P < 0.05$.

2.7.1. Microdialysis experiments

5-HT_{ext} (not corrected for in vitro recovery) were expressed as percentage of basal value (means ± S.E.M.). To compare 5-HT_{ext} to the respective basal value in each group of treated animals, statistical analysis was carried out using a one-way analysis of variance (ANOVA) for repeated measures on the time followed by Fisher Protected Least Significant Difference (PLSD) post-hoc test. Furthermore, using percentage data, net changes in dialysate 5-HT were determined by means of the area under the curve (AUC) for a 0–165 min period. Statistical comparisons of these AUCs were made by applying a one-way ANOVA followed by Fisher PLSD post-hoc test.

2.7.2. Hypothermia study

Maximal decreases in body temperature were analysed by a one-way ANOVA followed by a Fisher PLSD post-hoc test.

3. Results

3.1. Effect of (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT, (−)-5-Me-8-OH-DPAT on the 8-OH-DPAT-induced inhibition of forskolin-stimulated cAMP production

8-OH-DPAT inhibited forskolin-stimulated adenylyl cyclase activity over a concentration range of 1 nM–0.1 μM with a maximal inhibition of total cAMP production of −25% at 3 μM (Fig. 1A and B). The racemic mixture as well as (−)-5-Me-8-OH-DPAT did not significantly affect forskolin-stimulated adenylyl cyclase activity over a range of 1 nM–10 μM (Fig. 1D). Furthermore, both (±)-5-Me-8-OH-DPAT (10 μM, Fig. 1A) and (−)-5-Me-8-OH-DPAT (1 μM, Fig. 1B) shifted to the right the concentration–effect curve for the inhibition by 8-OH-DPAT of forskolin-stimulated adenylyl cyclase activity. The pA2 value for the antagonism by (−)-5-Me-8-OH-DPAT of the 8-OH-DPAT-induced inhibition of adenylyl cyclase activity was 7.6. By contrast, the (+) enantiomer induced a marked inhibition of forskolin-stimulated cAMP production (Fig. 1C), the maximal inhibition being about −15% at 10 μM. This inhibitory response was completely abolished by WAY 100635 (0.2 μM, Fig. 1C). This latter drug had, at this concentration, no effect on its own (data not shown).

3.2. Effect of (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT, (−)-5-Me-8-OH-DPAT on basal extracellular 5-HT in ventral hippocampal dialysates

Basal extracellular levels of 5-HT (in fmol/20 μl) in the ventral hippocampus of rats treated with saline, (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT and (−)-5-Me-8-OH-DPAT were 7.0 ± 1.7 ($n = 5$), 3.5 ± 0.4 ($n = 7$); 6.7 ± 2.8 ($n = 8$) and 6.8 ± 1.2 ($n = 10$), respectively. (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT and (−)-5-Me-8-OH-DPAT all at a dose of 10 mg/kg injected subcutaneously had no significant effect on hippocampal 5-HT_{ext} when compared either to the respective basal value (Fig. 2A) or to the control group (Fig. 2B).

3.3. Effect of (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT, (−)-5-Me-8-OH-DPAT on the 8-OH-DPAT-induced decrease in ventral hippocampal 5-HT dialysates

The selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (0.1 mg/kg) induced a significant maximal decrease in 5-HT_{ext} either by −48% ($P < 0.001$) when compared to the basal value (Fig. 3A) or by −39% ($P < 0.01$) when AUC value was compared to that of the saline-control group (Fig. 3B). (+)-5-Me-8-OH-DPAT at a dose of 10 mg/kg did not significantly block the 8-OH-DPAT re-

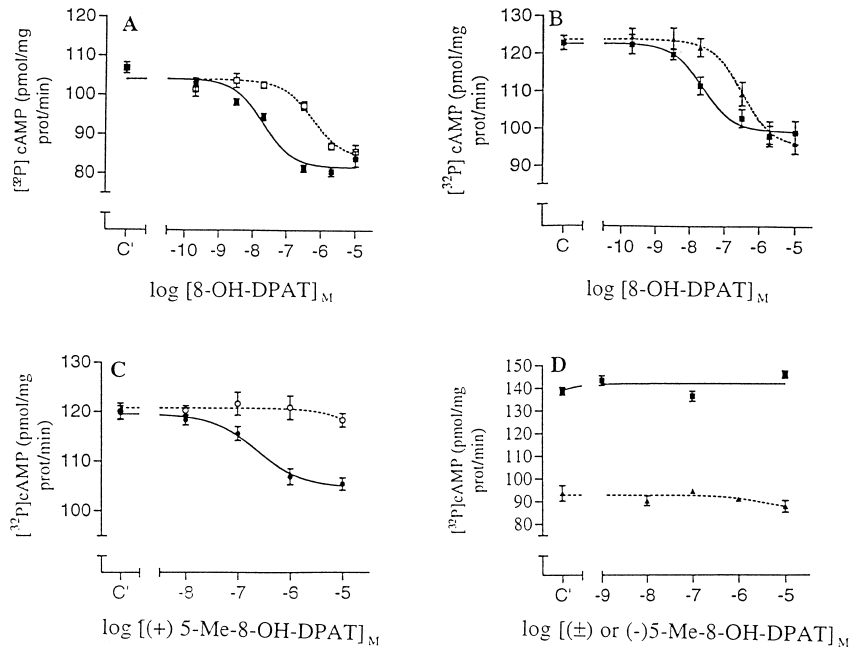

Fig. 1. Effect of (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT and (−)-5-Me-8-OH-DPAT on forskolin-stimulated cAMP production and on 8-OH-DPAT-induced inhibition of forskolin-stimulated cAMP production. (A) experiments were performed with 8-OH-DPAT in the absence (■) or the presence (□) of (±)-5-Me-8-OH-DPAT 10 μM, (B) experiments were performed with 8-OH-DPAT in the absence (■) or the presence (▲) of (−)-5-Me-8-OH-DPAT 1 μM and (C) experiments were performed with (+)-5-Me-8-OH-DPAT in the absence (●) or the presence (○) of WAY 100635 0.2 μM. (D) experiments were performed in the presence (▲) of (±)-5-Me-8-OH-DPAT or (■) (−)-5-Me-8-OH-DPAT. Adenylyl activity is expressed in pmol [^{32}P]cAMP synthesized per mg protein per min at 30°C. Each point is the mean ± S.E.M. of triplicate determinations in 2–3 separated experiments. C′ on abscissa: assays without 5-HT_{1A} receptor agonist.

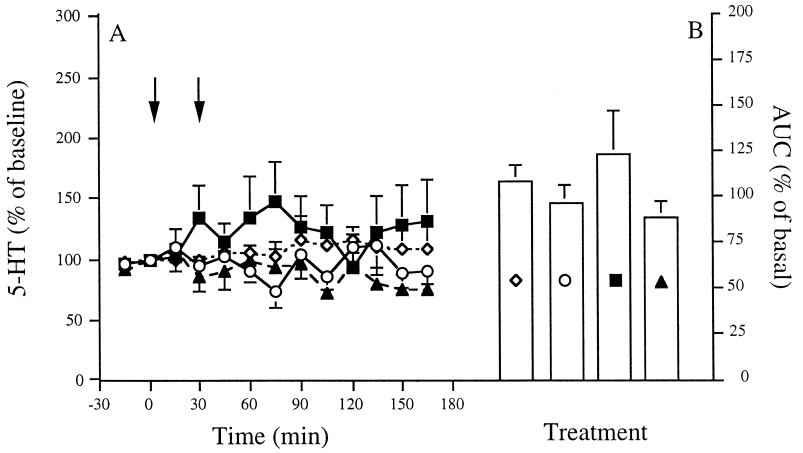

Fig. 2. Effect of (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT and (−)-5-Me-8-OH-DPAT all at a dose of 10 mg/kg (s.c.) on extracellular 5-HT levels in rat ventral hippocampal dialysates. (A) data shown are expressed as % of 5-HT baseline and (B) as the area under the curve, AUC (% of 5-HT, 0–165 min). Values are means ± S.E.M. for 5 to 10 animals per treatment group, see results for 5-HT basal levels. First arrow denotes subcutaneous administration of vehicle or drug, the second denotes subcutaneous administration of saline. Vehicle controls (◇), (+)-5-Me-8-OH-DPAT (○), (−)-5-Me-8-OH-DPAT (■), (±)-5-Me-8-OH-DPAT (▲).

sponse (Fig. 3A and B). By contrast, (±) and (−)-5-Me-8-OH-DPAT at a dose of 10 mg/kg completely blocked ($P < 0.01$ and $P < 0.001$, respectively) the 8-OH-DPAT (0.1 mg/kg)-induced decrease in 5-HT_{ext} (Fig. 3A and B).

3.4. Effect of WAY 100635 on the 8-OH-DPAT-induced decrease in ventral hippocampal 5-HT dialysates

Basal extracellular levels of 5-HT (in fmol/20 μl) in rats treated with saline, WAY 100635, 8-OH-DPAT or 8-OH-DPAT + WAY 100635 were 7.0 ± 1.7 ($n = 5$), 4.6 ± 1.3 ($n = 5$), 12 ± 3 ($n = 4$) and 4.2 ± 0.4 ($n = 6$), respectively. WAY 100635 at a dose of 1 mg/kg had no significant effect on 5-HT_{ext} when compared either to the basal value (Fig. 4A) or to the saline-control group (Fig. 4B). By contrast, 8-OH-DPAT decreased 5-HT_{ext} either by −62% ($P < 0.001$) when compared to the basal value or by −50% ($P < 0.01$) when AUC value was compared to that of the saline-control group. Furthermore, WAY 100635 (1 mg/kg) completely blocked ($P < 0.01$) the 8-OH-DPAT (0.1 mg/kg)-induced decrease in 5-HT_{ext} (Fig. 4B).

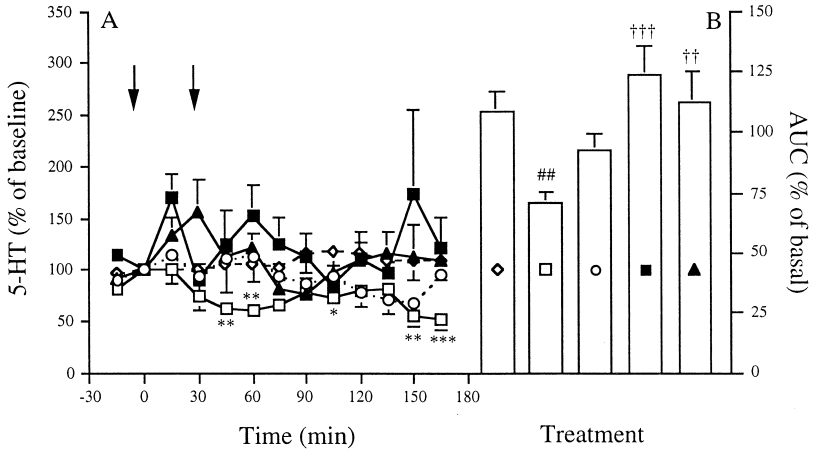

Fig. 3. Effect of (±)-5-Me-8-OH-DPAT, (+)-5-Me-8-OH-DPAT and (−)-5-Me-8-OH-DPAT all at a dose of 10 mg/kg (s.c.) on the 8-OH-DPAT-induced decrease in extracellular 5-HT in rat ventral hippocampal dialysates. (A) data shown are expressed as % of 5-HT baseline and (B) as the area under the curve, AUC (% of 5-HT, 0–165 min). Values are means ± S.E.M. for 5 to 6 animals per treatment group, see results for 5-HT basal levels. First arrow denotes subcutaneous administration of vehicle or (−), (+), (±)-5-Me-8-OH-DPAT (10 mg/kg), the second one denotes subcutaneous administration of 8-OH-DPAT (0.1 mg/kg). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, ANOVA for repeated measures and Fisher PLSD multiple comparison test when compared to the respective basal value. ## $P < 0.01$ one-way ANOVA followed by Fisher PLSD multiple comparison test vs. control group, †† $P < 0.01$; ††† $P < 0.001$ vs. 8-OH-DPAT group. Vehicle controls (◇), 8-OH-DPAT (0.1 mg/kg) (□), (+)-5-Me-8-OH-DPAT 10 mg/kg + 8-OH-DPAT (○), (−)-5-Me-8-OH-DPAT 10 mg/kg + 8-OH-DPAT (■), (±)-5-Me-8-OH-DPAT + 8-OH-DPAT (▲).

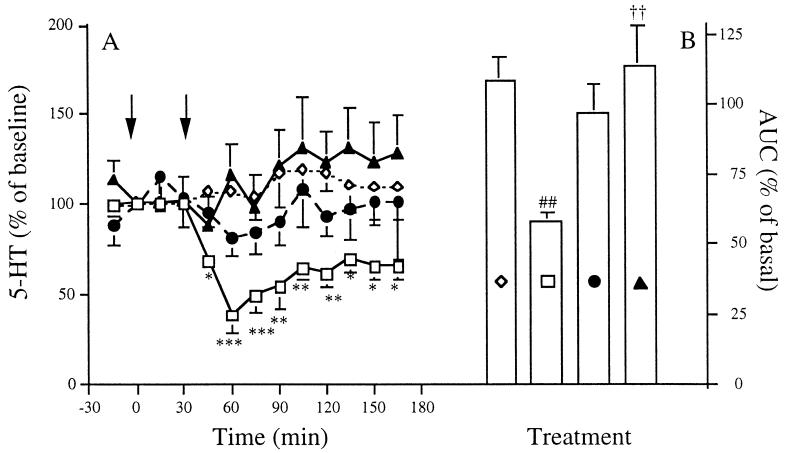

Fig. 4. Effect of WAY 100635 (1 mg/kg, s.c.) on extracellular 5-HT levels in rat ventral hippocampal dialysates and on the 8-OH-DPAT-induced decrease in extracellular 5-HT in rat ventral hippocampal dialysates. (A) data are expressed as percentage of baseline levels and (B) as the area under the curve, AUC (0–165 min). Values are means ± S.E.M. for 4 to 6 animals per treatment group, see results for 5-HT basal levels. The selective 5-HT_{1A} autoreceptor antagonist WAY100635 was administered at t = 0 (First arrow) and 8-OH-DPAT (0.1 mg/kg, s.c.) 30 min later (second arrow). $* P < 0.05$; $** P < 0.01$; $*** P < 0.001$, ANOVA for repeated measures and Fisher PLSD multiple comparison test when compared to the respective basal value. $## P < 0.01$ one-way ANOVA followed by Fisher PLSD multiple comparison test vs. control group, $†† P < 0.01$ one-way ANOVA followed by Fisher PLSD multiple comparison test vs. 8-OH-DPAT group. Vehicle controls (◇), 8-OH-DPAT (□), WAY 100635 (●), WAY 100635 + 8-OH-DPAT (▲).

3.5. Effect of (−)-5-Me-8-OH-DPAT on 8-OH-DPAT-induced decrease in ventral hippocampal 5-HT dialysates

To further characterize the (−)-5-Me-8-OH-DPAT-induced blockade of the 8-OH-DPAT response on dialysate 5-HT, a dose-effect study was performed. In order to compare properly the different doses of (−)-5-Me-8-OH-DPAT and to compare the 3 compounds the same 8-OH-DPAT group was used (Fig. 3A and B). (−)-5-Me-8-OH-DPAT 10 mg/kg ($P < 0.001$), 3 mg/kg ($P < 0.01$) and 1 mg/kg ($P < 0.01$) significantly attenuated the effects of 8-OH-DPAT in a all or none manner (Fig. 5A and B). Thus, (−)-5-Me-8-OH-DPAT displays similar antagonist property at 5-HT_{1A} receptors to that of WAY 100635, although being less potent than this latter compound.

3.6. Effect of (−)-5-Me-8-OH-DPAT and WAY 100635 on 8-OH-DPAT-induced hypothermia

8-OH-DPAT (0.1 mg/kg s.c.) induced a significant decrease (−1.2°C, $P < 0.001$) in body temperature compared to saline group (Fig. 6A and B).

(−)-5-Me-8-OH-DPAT (1–10 mg/kg s.c., Fig. 6A) as well as WAY 100635 (0.01–1 mg/kg s.c., Fig. 6B)

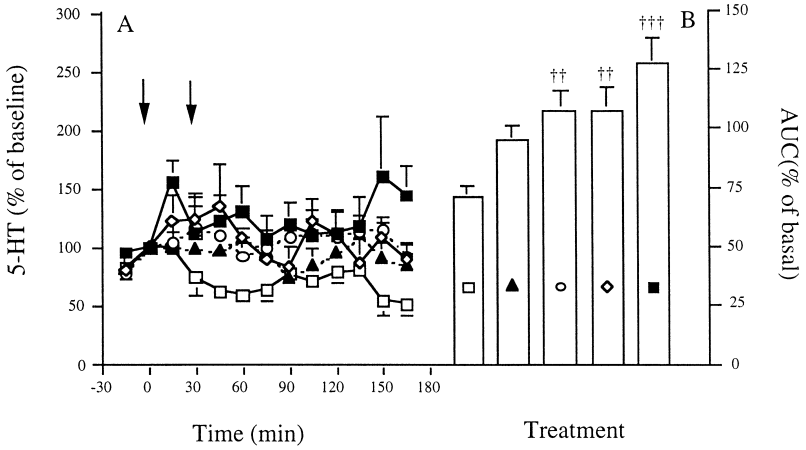

Fig. 5. Effect of (−)-5-Me-8-OH-DPAT at a dose of 0.3, 1, 3 and 10 mg/kg on the 8-OH-DPAT-induced decrease in extracellular 5-HT in rat ventral hippocampal dialysates. (A) data shown are expressed as % of 5-HT baseline and (B) as the area under the curve, AUC (% of 5-HT, 0-165 min). Values are means ± S.E.M. for 5 to 6 animals per treatment group, see results for 5-HT basal levels. First arrow denotes subcutaneous administration of vehicle or (−)-5-Me-8-OH-DPAT, the second denotes subcutaneous administration of 8-OH-DPAT (0.1 mg/kg). The 8-OH-DPAT group is the one already used in Fig. 3. $†† P < 0.01$; $††† P < 0.001$ one-way ANOVA followed by Fisher PLSD multiple comparison test vs. 8-OH-DPAT group. 8-OH-DPAT (□), (−)-5-Me-8-OH-DPAT 0.3 mg/kg + 8-OH-DPAT (▲), (−)-5-Me-8-OH-DPAT 1 mg/kg + 8-OH-DPAT (○), (−)-5-Me-8-OH-DPAT 3 mg/kg + 8-OH-DPAT (◇), (−)-5-Me-8-OH-DPAT 10 mg/kg + 8-OH-DPAT (■).

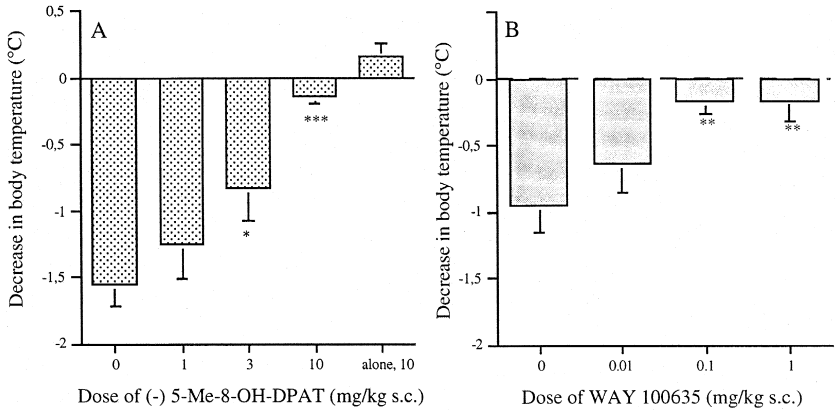

Fig. 6. Antagonism of 8-OH-DPAT-induced hypothermia in the mouse by (−)-5-Me-8-OH-DPAT or WAY 100635. (−)-5-Me-8-OH-DPAT (1, 3, 10 mg/kg) or WAY 100635 (0.01–1 mg/kg) was administered subcutaneously 30 min before 8-OH-DPAT (0.1 mg/kg s.c.) or saline. Body temperatures were recorded immediately before each drug administration, and 15 and 30 min following 8-OH-DPAT injection. The hypothermic response was measured as the maximum fall in body temperature following 8-OH-DPAT administration. $* P < 0.05$; $** P < 0.01$; $*** P < 0.001$ ANOVA followed by PLSD *t*-test vs. control group (NaCl-8-OH-DPAT injected group).

markedly and dose-dependently antagonised the 8-OH-DPAT-induced hypothermia, with ED50 (dose require to reduce the hypothermic effect by 50%) of 2.85 and 0.05 mg/kg s.c., respectively. In all experiments, control animals pretreated with (−)-5-Me-8-OH-DPAT or WAY 100635 alone showed no significant change in body temperature (data not shown).

4. Discussion

The present data provide neurochemical and behavioral evidences that (±)-5-Me-8-OH-DPAT, similarly to WAY 100635, but acting at higher doses is a full and selective 5-HT_{1A} receptor antagonist. At postsynaptic 5-HT_{1A} sites, (±)-5-Me-8-OH-DPAT, and the (−) isomer have no effect on their own on forskolin-stimulated cAMP production and induced a dose-dependent shift to the right of the 8-OH-DPAT-induced decrease in this second messenger synthesis in rat hippocampal homogenates. This data demonstrate a competitive interaction between these two drugs and 8-OH-DPAT for the same site, i.e., 5-HT_{1A} receptor site and suggest that both ligands, (±) and (−)-5-Me-8-OH-DPAT, display a postsynaptic 5-HT_{1A} receptor antagonist profile. Furthermore, (+)-5-Me-8-OH-DPAT induced a dose-dependent inhibition of forskolin-stimulated cAMP production which was completely blocked by the highly selective 5-HT_{1A} receptor antagonist, WAY 100635, suggesting a 5-HT_{1A} receptor agonist profile for this enantiomer.

(±)-5-Me-8-OH-DPAT completely blocked the decrease in 5-HT_{ext} in the rat ventral hippocampus induced by the potent and selective 5-HT_{1A} receptor agonist 8-OH-DPAT, while having no effect on 5-HT_{ext} when administered alone systemically. It has been demonstrated that decrease in 5-HT release, in nerve terminal regions, is a consequence of the decrease in the firing of 5-HT neurones mediated by the stimulation, by 5-HT_{1A} receptor agonists, of somatodendritic 5-HT_{1A} receptors located on raphe serotoninergic neurones (De Montigny et al., 1984; Sprouse and Aghajanian, 1986; Sharp et al., 1989, 1990). Thus, the lack of effect of (±)-5-Me-8-OH-DPAT on 5-HT_{ext} at hippocampal serotoninergic nerve terminals when administered alone demonstrate that this compound, similarly to WAY 100635 (Gurling et al., 1994; Malagié et al., 1996) is devoid of intrinsic activity at the somatodendritic 5-HT_{1A} receptors. The stereoisomers of (±)-5-Me-8-OH-DPAT, (−)-5-Me-8-OH-DPAT and (+)-5-Me-8-OH-DPAT, also did not affect hippocampal 5-HT_{ext} in rats. Moreover, (−)-5-Me-8-OH-DPAT, at lower dose than the racemic mixture one, completely blocked the effects of 8-OH-DPAT on hippocampal 5-HT_{ext}, suggesting that this compound displays a 5-HT_{1A} autoreceptor antagonist property. By contrast, the (+) isomer failed to block the 8-OH-DPAT response. Taken together, these in vivo data demonstrate that the racemic mixture (±)-5-Me-8-OH-DPAT acts stereoselectively at somatodendritic 5-HT_{1A} receptors, the activity residing in the (−) enantiomer. This stereoselectivity is consistent with in vitro data showing that (±) and (−)-5-Me-8-OH-DPAT were able to antagonize the 8-OH-DPAT-induced decrease in forskolin-stimulated cAMP production in the rat hippocampus. In contrast, the (+) isomer showed partial 5-HT_{1A} agonist effect in this model of postsynaptic 5-HT_{1A} receptor activation. In the microdialysis experiments (+)-5-Me-8-OH-DPAT is devoid of 5-HT_{1A} agonist properties this can be explain by the weak partial agonist properties of this isomer therefore presynaptic 5-HT_{1A} autoreceptors are not sufficiently stimulated to induce a decrease in $[5\text{-HT}]_{ext}$.

The mechanism by which 8-OH-DPAT induces hypothermia in the rat is controversial (Bill et al., 1991; O'Connell et al., 1992). However, in the mouse, there is good evidence that the 5-HT_{1A} receptors mediating 8-OH-

DPAT-induced hypothermia are located presynaptically, since 5,7-dihydroxytryptamine lesions or pCPA-induced 5-HT depletion abolished the hypothermic response to 8-OH-DPAT (Goodwin et al., 1985). In the present study, (−)-5-Me-8-OH-DPAT had no effect on its own on body temperature in mice suggesting that this compound does not have agonist activity in this behavioral model of somatodendritic 5-HT_{1A} receptor activation. Moreover, both (−)-5-Me-8-OH-DPAT and WAY 100635 antagonized dose-dependently the 8-OH-DPAT-induced hypothermia. These results are consistent with those already reported for WAY 100635 (Forster et al., 1995), and confirm that (−)-5-Me-8-OH-DPAT is likely to have an antagonist profile at somatodendritic 5-HT_{1A} receptors.

The majority of the new drugs currently characterized as 5-HT_{1A} receptor antagonists (NAN 190, BMY 7378, MDL 73005EF, SDZ-216,525, (±) WAY 100135) appeared to be 5-HT_{1A} receptor antagonists in functional models involving activation of postsynaptic 5-HT_{1A} receptor sites, while having partial agonist properties in various models studying 5-HT_{1A} somatodendritic autoreceptor functions (Fletcher et al., 1993b). For example, SDZ-216,525 was able to antagonize the 8-OH-DPAT-induced decrease in forskolin-stimulated cAMP formation and the 8-OH-DPAT-induced behavioural syndrome (Schoeffter et al., 1993). However, SDZ-216,525 induced a decrease in 5-HT_{ext} as measured by in vivo microdialysis as well as an hyperphagia in rats, thus demonstrating its agonist activity at 5-HT_{1A} somatodendritic receptors (Gurling et al., 1993; Sharp et al., 1993). This latter property could be due to the much larger receptor reserve associated with the presynaptic location of this receptor sub-type as opposed to postsynaptic ones (Hoyer et al., 1994).

Taken together, our data demonstrate that several in vitro and in vivo responses to 8-OH-DPAT performed either in the rat or in the mouse are similarly and dose-dependently blocked by WAY 100635 and (−)-5-Me-8-OH-DPAT suggesting that this latter compound also exerts a selective and silent antagonist action at 5-HT_{1A} receptors. However, in these tests (−)-5-Me-8-OH-DPAT was less effective than WAY 100635 in blocking the 8-OH-DPAT-induced responses. Indeed, this new compound antagonized the neurochemical and behavioral effects of 8-OH-DPAT at both pre and postsynaptic sites when administered systemically at higher doses than WAY 100635. Thus, due to the lack of availability of 5-HT_{1A} receptor antagonists, (−)-5-Me-8-OH-DPAT could be an useful tool for studying the involvement of 5-HT_{1A} receptors in the mechanism of action of indirect serotoninergic agonists such as antidepressant drugs (Artigas et al., 1996).

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