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Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-hydroxytryptamine levels: (*S*)-WAY100135, but not WAY100635, has partial agonist properties

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Abstract

In vivo microdialysis measuring 5-hydroxytryptamine (5-HT) levels in the ventral hippocampus of chloral hydrate-anaesthetised rats was used to characterise further the recently described 5-HT_{1A} receptor antagonists (*S*)-WAY100135 ((*S*)-*N*-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide) and WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide). In addition, binding experiments were performed to determine the affinity of the compounds for 5-HT_{1A} receptors and for α_1-adrenoceptors. Both (*S*)-WAY100135 and WAY100635 exhibited high affinity for 5-HT_{1A} receptors and moderate affinity for α_1-adrenoceptors. The effects of (*S*)-WAY100135 (0.63–20 mg/kg) and of WAY100635 (0.0025–0.16 mg/kg) on 5-HT levels were examined alone, and in combination with the 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Both compounds dose-dependently reversed the 8-OH-DPAT-induced decrease in extracellular 5-HT levels with ED_{50} values of approximately 3.3 and 0.03 mg/kg, respectively. When given alone, WAY100635 did not alter 5-HT levels. (*S*)-WAY100135, however, induced, by itself, a transient but significant and dose-dependent decrease in 5-HT levels. WAY100635 (0.16 mg/kg) prevented the decrease induced by (*S*)-WAY100135 (10 mg/kg), but did not reverse the decrease induced by the α_1-adrenoceptor antagonist, prazosin (0.16 mg/kg). These results are further evidence that (*S*)-WAY100135 may modulate the release of 5-HT by acting as a partial agonist at somatodendritic 5-HT_{1A} receptors. In contrast, WAY100635 acts as a potent and selective 5-HT_{1A} receptor antagonist.

Keywords: 5-HT_{1A} receptor antagonist; 5-HT (5-hydroxytryptamine, serotonin) level, extracellular; Microdialysis; (*S*)-WAY100135; WAY100635

1. Introduction

Amongst the 5-HT receptor subtypes, 5-HT_{1A} receptors are of particular interest because of their potential role as therapeutic targets in the treatment of certain psychiatric disorders (e.g. Deakin, 1993). These receptors are located both presynaptically at the somatodendritic level in the raphe nuclei, and postsynaptically in 5-hydroxytrypytamine (5-HT) terminal regions. Many 5-HT_{1A} receptor ligands that act as partial agonists or as antagonists at postsynaptic receptors behave as full agonists at somatodendritic autoreceptors probably because of a greater receptor reserve (Meller et al., 1990). The activation of somatodendritic receptors has been shown to decrease the firing of serotonergic neurons in the raphe nucleus (Sprouse and Aghajanian, 1987; Blier and Demontigny, 1987) and to decrease the release of 5-HT in terminal regions (e.g. Sharp et al., 1989b). Recently, WAY100135 (*N*-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide) and WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide) have been proposed to be selective 5-HT_{1A} receptor antagonists not only at postsynaptic, but also at somatodendritic receptors in the raphe (Fletcher et al., 1993; Forster et al., 1995). WAY100135 has been shown to inhibit the effects of 5-HT_{1A} receptor agonists on (1) the firing of 5-HT neurons in the raphe nucleus (Fletcher et al., 1993; Mundey et al., 1994a), and (2) hippocampal 5-HT release as assessed by in vivo microdialysis in freely moving rats (Routledge et al., 1993). Although WAY100135, when given alone, was reported not to affect 5-HT release (Routledge et al., 1993), electrophysiological evidence showed WAY100135 to decrease the firing of 5-HT neurons in the raphe nucleus (Escandon et al., 1994; Lanfumey et al., 1993; Fornal et al., 1994). WAY100635 has

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been reported to be a more potent 5-HT_{1A} receptor antagonist than WAY100135 (Gurling et al., 1994; Forster et al., 1995) and to increase rather than decrease the firing of 5-HT neurons (Fornal et al., 1994; Mundey et al., 1994b). The present work was aimed at studying the effects of (*S*)-WAY100135 (the active enantiomer of the compound) and of WAY100635, when given alone, and in combination with the prototypic 5-HT_{1A} receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), on extracellular 5-HT levels in the ventral hippocampus of the anaesthetised rat using in vivo microdialysis. When given alone, (*S*)-WAY100135, but not WAY100635, transiently decreased 5-HT levels. Because both compounds have been shown to have affinity for α_1-adrenoceptors (Fletcher et al., 1993; Forster et al., 1995), and because the α_1-adrenoceptor antagonist, prazosin, has been reported to decrease 5-HT levels (Rouquier et al., 1994; Hjorth et al., 1995), binding experiments were carried out in rat cerebral cortex to determine, in the same experimental conditions, the affinities of (*S*)-WAY100135, WAY100635 and prazosin for 5-HT_{1A} receptors and for α_1-adrenoceptors. Finally, the ability of WAY100635 to antagonise the effects of (*S*)-WAY100135 and of prazosin on 5-HT levels was examined. Part of this work has been reported in a preliminary form at the 6th International Conference on in vivo Methods (Assié and Koek, 1994), and at the 25th Annual Meeting of the Society for Neuroscience (Assié and Koek, 1995).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Ico: OFA SD (I.O.P.S. Caw); Iffa Credo, France), weighing 260–340 g, were used in the microdialysis studies. Upon arrival, the animals were group housed (three rats per cage) in the animal-keeping facilities, under controlled conditions (illumination 12/12 light/dark cycle: lights on 07.00 a.m.; ambient temperature 21 ± 1°C; humidity 55 ± 5%), with rat food (AO4, UAR, France) and filtered (0.2 μm) tap water available ad libitum. The animals were housed under these conditions for at least 5 days before they were used in the experiments. The experimental procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1985) and with the French legislation, and were approved by the institutional Protocol Review Committee (protocol 069).

2.2. Binding experiments

Frozen brains of male Sprague Dawley rats were purchased from Iffa Credo, France (see Section 2.1 for specifications) and were stored at −70°C prior to use in binding assays. 5-HT_{1A} receptor and α_1-adrenoceptor binding experiments were carried out in cerebral cortex using the conditions described in Table 1. For the 5-HT_{1A} receptor binding assay, the tissue was homogenised in 20 vols. of ice cold Tris-HCl (50 mM, pH 7.4 at 25°C). The homogenate was centrifuged at 39 000 × *g* for 10 min, the pellet was resuspended in the same volume of buffer and was recentrifuged as before. Following a further resuspension, the tissue was incubated for 10 min at 37°C and centrifuged as before. The final pellet was then resuspended in 80 vols. of a 5-HT assay buffer of Tris-HCl (50 mM, pH 7.4 at 25°C) containing pargyline (10 μM), $CaCl_2$ (4 mM) and ascorbic acid (0.1%). For the α_1-adrenergic binding assay, the tissue was homogenised in 40 vols. of ice cold Tris-HCl (50 mM, pH 7.4 at 25°C). The homogenate was centrifuged at 1000 × *g* for 10 min, the supernatant was then centrifuged at 30 000 × *g* for 10 min, the pellet was suspended in the same volume of buffer and recentrifuged as before. The final pellet was suspended in 160 vols. of buffer.

2.3. Microdialysis procedure

The method used in the present experiments was similar to that described by Sharp et al. (1989a). The rats were anaesthetised with chloral hydrate (400–500 mg/kg i.p., plus supplementary doses of approximately 80 mg/kg per h) and were mounted in a stereotaxic apparatus (David Kopf). The skull was exposed and a hole was drilled to implant a microdialysis probe (CMA/12, 2 mm length, 0.5 mm diameter, CMA, Microdialysis) into the left hippocampus (stereotaxic coordinates: rostral −4.8 mm, lateral +4.6 mm, ventral −7.5 mm, from bregma and dura surface according to Paxinos and Watson (1986)). The probe was continuously perfused (1.1 μl/min) with artifi-

Table 1
Experimental details for 5-HT_{1A} receptor and α_1-adrenoceptor binding assays

	5-HT_{1A} receptor	α_1-Adrenoceptor
^{3}H-Ligand (nM)	[^{3}H]8-OH-DPAT (0.2)	[^{3}H]Prazosin (0.1)
Final concentration of tissue	10 mg/ml	5 mg/ml
Incubation time	30 min	30 min
Incubation temperature	23°C	23°C
Nonspecific ligand (μM)	Serotonin (10)	Phentolamine (50)
Reference	Sleight and Peroutka (1991)	Hornung et al. (1979)

cial cerebrospinal fluid (140 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 0.27 mM NaH_2PO_4, 7.2 mM glucose) containing 1 μM of the selective 5-HT uptake inhibitor citalopram, via FEP teflon tubing connected to a 1 ml syringe mounted on a microinfusion pump (CMA/100, CMA, Microdialysis). Starting approximately 2 h after implantation (see Sharp et al., 1989a), when the 5-HT levels became relatively constant (S.E.M. of the first four samples less than 20% of the mean), perfusates were collected every 20 min in small polypropylene tubes inverted over the outlet cannula and were analysed immediately for 5-HT content using high performance liquid chromatography with electrochemical detection (HPLC-EC).

Four baseline control samples were collected before the first drug was given. When two drugs were used, the second was given 40 min after the first. Samples were collected for 140 min after administration of the last drug. Using an injection volume of 10 ml/kg, (*S*)-WAY100135 and WAY100635 were administered s.c., and 8-OH-DPAT and prazosin were administered i.p.. The dose of 8-OH-DPAT used here (i.e., 0.31 mg/kg, i.p.) was based on previous dose-response studies showing the effects of this dose on 5-HT levels to be submaximal under the present experimental conditions (Assié and Koek, 1996).

At the end of the experiment, the animal was killed by decapitation and the brain was removed, frozen and cut in a cryomicrotome (Jung Frigocut 2800) to verify the placement of the probe.

2.4. HPLC-EC analysis of 5-HT

The HPLC-EC method used to analyse 5-HT in hippocampal dialysates was similar to that of Sleight et al. (1988). 5-HT was separated from 5-hydroxyindole-3-acetic acid (5-HIAA) by ion-pair, reverse phase chromatography. Separation took place on a column (Merck, Lichrocart 125-2, Superspher 100 RP-18, length 119 mm, internal diameter 2 mm, granulometry 4 μm). The mobile phase (0.15 M NaH_2PO_4, 0.1 mM EDTA, 0.5 mM 1-octanesulphonic acid sodium salt, 16% methanol) was pumped through the column at a rate of 0.2 ml/min (Beckman, HPLC-116). 5-HT eluted from the column (retention time 7–9 min) was measured with a glassy carbon working electrode maintained at a potential of +0.64 V (Decade detector, Antec Leyden). The concentrations of 5-HT were estimated with reference to external standards, run daily, using a Beckman Gold system; the limit of detection (three times baseline noise) was approximately 1–2 fmol/20 μl sample.

2.5. Chemicals

[^{3}H]8-OH-DPAT (TRK 850, specific activity 160–240 Ci/mmol) and [^{3}H]prazosin (TRK 843, specific activity 65–85 Ci/mmol) were obtained from Amersham France (Les Ulis, France). 5-HT creatinine sulphate, 5-HIAA, phentolamine mesylate and prazosin hydrochloride were purchased from Sigma (Saint Quentin Fallavier, France), 8-OH-DPAT from RBI (Bioblock Scientific, Illkirch, France), and chloral hydrate from Acros (Geel, Belgium). Citalopram was kindly donated by Lundbeck (Copenhagen, Denmark). (*S*)-WAY100135 hydrochloride and WAY100635 dihydrochloride were synthesised by the chemistry department of the Pierre Fabre Research Centre (Castres, France). The doses of compounds were expressed as the base. Prazosin was dissolved with lactic acid and NaOH 1 M to pH 4.5; all other compounds were dissolved in distilled water.

2.6. Data analyses

Binding data were analysed using the nonlinear, least square curve-fitting EBDA/LIGAND program (Biosoft). Results are expressed as pK_i values (mean ± S.E.M. of three determinations).

The perfusate levels of 5-HT are expressed as percent of the mean amount of 5-HT collected in the four pre-injection control samples (basal level). Animals for which the 5-HT levels were too variable (S.E.M. of the four pre-injection samples greater than 20% of the mean) were not included (two animals). Because the intrinsic effects of (*S*)-WAY100135 were most apparent from 20 to 80 min after its administration, the percent area under the curve (AUC) for this period of time was used to establish the dose-response curves of the antagonists when given alone. The 5-HT_{1A} receptor antagonist effects of (*S*)-WAY100135 and WAY100635 were expressed as percent AUC for the 140 min period following the administration of 8-OH-DPAT. Treatment effects on percent AUC values were analysed either by means of the Student's *t* test, or by a one-way analysis of variance (followed by Dunnett's test), where appropriate. ED_{50} values were estimated by linear interpolation (saline control as 100% and 8-OH-DPAT alone as 0% for antagonists, vehicle control as 0% and maximal effect of the compound as 100% for prazosin).

3. Results

3.1. Binding results

(*S*)-WAY100135 and WAY100635 exhibited high affinity for 5-HT_{1A} receptors (pK_i ± S.E.M. 8.35 ± 0.05 and 9.02 ± 0.02, respectively), but only moderate affinity for α_1-adrenoceptors (pK_i 6.64 ± 0.01 and 7.24 ± 0.03, respectively). Prazosin displayed high affinity for α_1-adrenoceptors (pK_i 9.86 ± 0.13), but did not show any detectable affinity for 5-HT_{1A} receptors ($pIC_{50} < 5$).

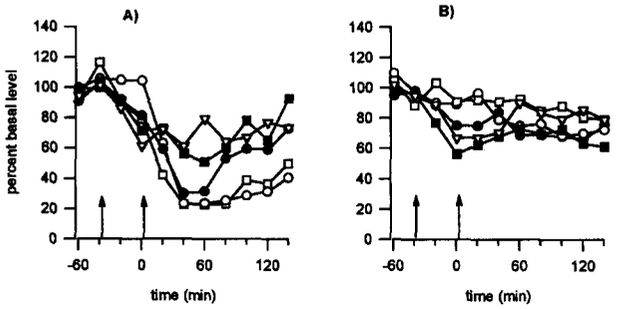

Fig. 1. Effects of (*S*)-WAY100135 (A) in combination with 8-OH-DPAT (0.31 mg/kg, i.p.) and (B) alone, on 5-HT levels in rat ventral hippocampus expressed as a percent of the mean absolute amount of 5-HT in the four samples collected before the first injection. (○) 8-OH-DPAT or saline, (□) (*S*)-WAY100135 0.63 mg/kg, (●) (*S*)-WAY100135 2.5 mg/kg, (■) (*S*)-WAY100135 10 mg/kg, (▽) (*S*)-WAY100135 20 mg/kg. The first arrow indicates administration of saline or (*S*)-WAY100135, the second indicates administration of 8-OH-DPAT or saline. Data shown are means for five animals per group.

3.2. Microdialysis results

Mean basal levels of 5-HT in the rat ventral hippocampus were 32.0 ± 0.9 fmol/20 μl sample ($n = 155$) in the presence of the uptake inhibitor citalopram (1 μM).

3.2.1. Effects of (S)-WAY100135 and WAY100635, in combination with 8-OH-DPAT and alone

8-OH-DPAT, at the submaximal dose of 0.31 mg/kg, i.p., induced a large and long-lasting decrease in 5-HT levels (Fig. 1A and Fig. 2A). (*S*)-WAY100135 (0.63–20 mg/kg, s.c.) dose-dependently prevented this decrease (Fig. 1A). The antagonist effects of (*S*)-WAY100135 reached statistical significance at 10–20 mg/kg (Fig. 3C), and the ED_{50} value for these effects was approximately 3.3 mg/kg. Similarly, WAY100635 (0.0025–0.16 mg/kg, s.c.) dose-dependently prevented the decrease in 5-HT levels produced by 8-OH-DPAT (Fig. 2A). The antagonist

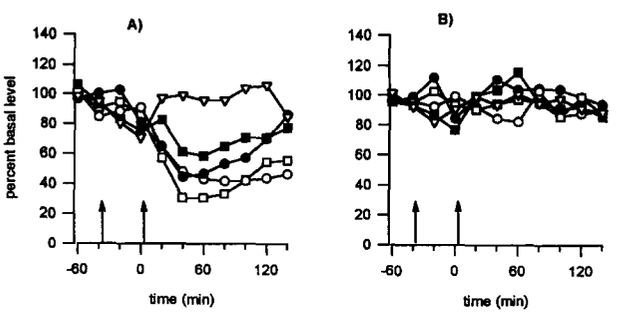

Fig. 2. Effects of WAY100635 (A) in combination with 8-OH-DPAT (0.31 mg/kg, i.p.) and (B) alone, on 5-HT levels in rat ventral hippocampus expressed as a percent of the mean absolute amount of 5-HT in the four samples collected before the first injection. (○) 8-OH-DPAT or saline, (□) WAY100635 0.0025 mg/kg, (●) WAY100635 0.01 mg/kg, (■) WAY100635 0.04 mg/kg, (▽) WAY100635 0.16 mg/kg. The first arrow indicates administration of saline or WAY100635, the second indicates administration of 8-OH-DPAT or saline. Data shown are means for five animals per group.

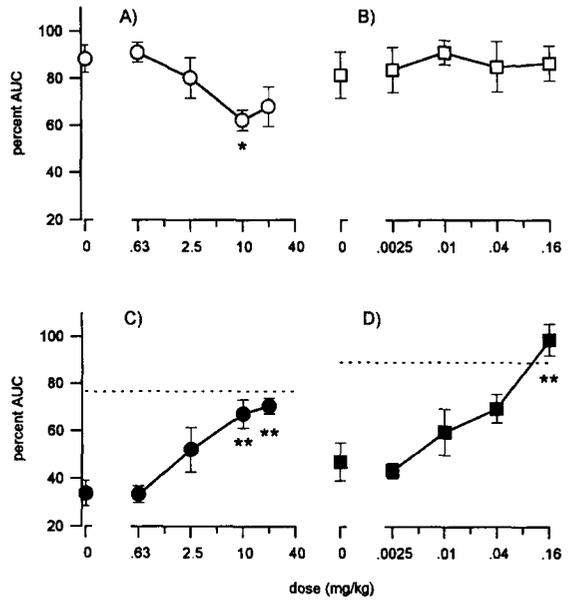

Fig. 3. Effects of (*S*)-WAY100135 (○) and WAY100635 (□) on 5-HT levels in rat ventral hippocampus. A and B: intrinsic effects expressed as percent AUC for the 20–80 min period after injection of the compounds (open symbols); C and D: antagonism of the 8-OH-DPAT-induced decrease in 5-HT levels expressed as percent AUC for the 0–140 min period after injection of 8-OH-DPAT (filled symbols); the dotted line depicts percent AUC value for control animals (saline + saline treatment). Results are means ± S.E.M. of five animals per dose. Significant differences from dose 0 are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$ (one-way analysis of variance followed by Dunnett's test).

effects of WAY100635 reached statistical significance at 0.16 mg/kg (Fig. 3D), and the ED_{50} value for these effects was approximately 0.03 mg/kg.

When given alone, (*S*)-WAY100135, moderately but significantly decreased 5-HT levels, in a dose dependent manner, 20–80 min after its administration (Fig. 1B and Fig. 3A). The intrinsic effects of (*S*)-WAY100135 reached statistical significance at 10 mg/kg (Fig. 3A), a dose at

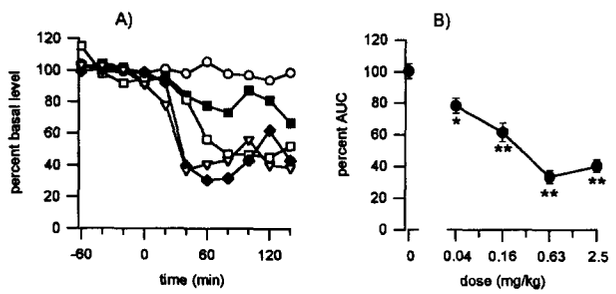

Fig. 4. Effects of prazosin (i.p.) on 5-HT levels in rat ventral hippocampus. Data shown are means for five animals per group. A: 5-HT levels are expressed as a percent of the mean absolute amount of 5-HT in the four samples collected before drug injection (time 0); (○) vehicle, (■) 0.04 mg/kg, (□) 0.16 mg/kg, (◆) 0.63 mg/kg, (▽) 2.5 mg/kg. B: dose response curve for the inhibitory effect of prazosin. The values of 5-HT represent the mean percent area under the curve (± S.E.M.) for the 20–80 min post injection period. Significant differences from dose 0 are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$ (one-way analysis of variance followed by Dunnett's test).

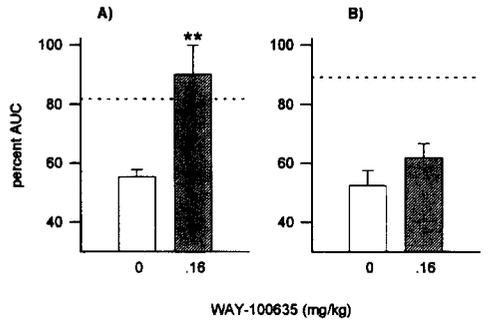

Fig. 5. Effects of WAY100635 (0.16 mg/kg) on the decrease in 5-HT levels induced by: (A) (*S*)-WAY100135 10 mg/kg, and (B) prazosin 0.16 mg/kg, on 5-HT levels in rat ventral hippocampus expressed as percent AUC for the 20–80 min period after administration of the compounds. The dotted line depicts percent AUC value for control animals (saline + vehicle treatment). Results are means ± S.E.M. of five animals per dose. Significant differences between treatments are indicated as ** $P < 0.01$ (Student's *t* test).

which the compound also significantly blocked the effects of 8-OH-DPAT (Fig. 3C). The decrease in 5-HT levels induced by 8-OH-DPAT, however, was large and long-lasting, whilst the intrinsic effects of (*S*)-WAY100135 were moderate and transient. In contrast, WAY100635 did not affect 5-HT levels when given alone (Fig. 2B and Fig. 3B).

3.2.2. Effects of prazosin

The α_1-adrenoceptor antagonist prazosin (0.04–2.5 mg/kg i.p.) dose-dependently decreased 5-HT levels (Fig. 4A). The ED_{50} for this effect, during the time period from 20 to 80 min after administration, was approximately 0.12 mg/kg (Fig. 4B).

3.2.3. Effects of WAY100635 on the decrease in 5-HT levels induced by (S)-WAY100135 and prazosin

The decrease in 5-HT levels induced by 10 mg/kg (*S*)-WAY100135 between 20 and 80 min after its injection (Fig. 5A), was of a magnitude similar to that found in the previous experiment (Fig. 3A), and was completely abolished by pretreatment with 0.16 mg/kg WAY100635 (Fig. 5A). In contrast, the decrease in 5-HT levels induced by 0.16 mg/kg prazosin, which was of the same magnitude as that induced by 10 mg/kg (*S*)-WAY100135, was not significantly altered by pretreatment with 0.16 mg/kg WAY100635 (Fig. 5B).

4. Discussion

The present data, showing that (*S*)-WAY100135 and WAY100635 block the ability of 8-OH-DPAT to decrease 5-HT release in the hippocampus, provide further evidence that both compounds act as antagonists at somatodendritic 5-HT_{1A} receptors. In addition, (*S*)-WAY100135, but not WAY100635, was found to decrease hippocampal 5-HT levels when given alone. Evidence was obtained suggesting that these intrinsic effects of (*S*)-WAY100135 involve its partial agonist properties at somatodendritic 5-HT_{1A} receptors.

Until recently, all compounds used as antagonists at somatodendritic 5-HT_{1A} receptors lacked selectivity for, or exerted intrinsic effects at these receptors. Compounds such as penbutolol, tertatolol and (−)-pindolol, have 5-$HT_{1A/1B}$ receptor antagonist and β-adrenoceptor blocking properties; in microdialysis studies, these compounds have been reported to antagonise the 8-OH-DPAT-induced decrease in 5-HT levels, but also to increase 5-HT levels when given alone, probably by acting at 5-HT_{1B} receptors (Hjorth and Sharp, 1993; Assié and Koek, 1996). Further, spiperone, which has been reported to act as a 5-HT_{1A} receptor antagonist in the dorsal raphe nucleus (Lum and Piercey, 1988), has high affinity for 5-HT_{2A} receptors, dopamine D_2 receptors and α_1-adrenoceptors. Finally, a number of other compounds, such as BMY 7378 and NAN-190, have been claimed to be 5-HT_{1A} receptor antagonists, but these compounds have subsequently been shown to act as agonists at somatodendritic 5-HT_{1A} receptors (Hjorth and Sharp, 1990; Sharp et al., 1990). The present finding that both (*S*)-WAY100135 and WAY100635 dose-dependently prevented the decrease in 5-HT levels induced by 8-OH-DPAT in chloral hydrate-anaesthetised rats are in agreement with previous data, obtained in freely moving rats (Routledge et al., 1993; Gurling et al., 1994). The doses necessary to abolish completely the effects of 8-OH-DPAT were 0.16 mg/kg WAY100635 and 20 mg/kg (*S*)-WAY100135; these results confirm the greater potency of the former compound and parallel its higher affinity for 5-HT_{1A} receptors. Indeed, WAY100635 has been shown to display potent 5-HT_{1A} receptor antagonist properties, in vitro in isolated guinea pig ileum, and in vivo in electrophysiological studies and behavioural studies (Fornal et al., 1994; Mundey et al., 1994b; Forster et al., 1995).

Although (*S*)-WAY100135 was able to attenuate the effects of 8-OH-DPAT on 5-HT levels, it decreased 5-HT levels when given alone. This latter effect, though moderate, was dose-dependent and maximal at 10 mg/kg. A previous report (Routledge et al., 1993) failed to obtain evidence that WAY100135 decreases hippocampal 5-HT levels when given alone. Unlike this previous report, the present study involved the use of anaesthetised animals and the presence of the 5-HT uptake inhibitor, citalopram in the perfusion medium. Such procedural differences may perhaps account in part for the discrepancy (e.g. Kreiss et al., 1993). The present finding that 5-HT levels were decreased, however, is compatible with recent electrophysiological data. WAY100135 decreased 5-HT cell firing in vivo in cat dorsal raphe nucleus (Fornal et al., 1994; Escandon et al., 1994) and in vitro in rat dorsal raphe nucleus (Lanfumey et al., 1993). Two hypotheses have

been advanced to account for the observation that WAY100135 decreased the firing of 5-HT neurons: Escandon et al. (1994) suggested this decrease to result from partial agonist activity at somatodendritic 5-HT_{1A} receptors, whereas Lanfumey et al. (1993) suggested that 5-HT_{1A} autoreceptors were probably not involved in the decrease, because it could not be antagonised by the non selective 5-HT_{1A} receptor antagonist, (−)tertatolol. Instead, the latter authors suggested that the (*S*)-WAY100135-induced decrease in 5-HT cell firing may involve α_1-adrenoceptor blocking properties of the compound, as the decrease was attenuated by increasing the α_1-adrenergic tone. Indeed, it has been shown that α_1-adrenoceptor antagonists suppress the firing of 5-HT neurons in the raphe nuclei which is dependent on a tonically active adrenergic system (Baraban and Aghajanian, 1980). In agreement with previous data (Rouquier et al., 1994; Hjorth et al., 1995), the present work showed the α_1-adrenoceptor antagonist, prazosin, to decrease hippocampal 5-HT levels. This decrease, however, could not be reversed by the 5-HT_{1A} receptor antagonist, WAY100635, which is consistent with a recent report (Hjorth et al., 1995). In contrast, WAY100635 reversed the (*S*)-WAY100135-induced decrease in 5-HT levels. In agreement with previous work (Fletcher et al., 1993; Forster et al., 1995), (*S*)-WAY100135 and WAY 100635 had only moderate affinity for α_1-adrenoceptors. (*S*)-WAY100135 and WAY100635 had α_1-adrenoceptor affinities that were about 51 and 60 times lower, respectively, than their affinities for 5-HT_{1A} receptors, indicating that the separation between their α_1-adrenoceptor and 5-HT_{1A} receptor affinities is similar for both compounds. Thus, assuming a role for α_1-adrenoceptors in the intrinsic effects of (*S*)-WAY100135 observed here, such intrinsic effects would be expected to be produced as well by doses of WAY100635 that are equivalent to those of (*S*)-WAY100135 in terms of 5-HT_{1A} receptor antagonist properties. At such doses of WAY100635, however, no effects on 5-HT levels were observed. This observation, together with the aforementioned finding that the intrinsic effects of (*S*)-WAY100135 could be blocked by WAY100635, suggest that the ability of (*S*)-WAY100135 to decrease 5-HT levels does not involve its possible α_1-adrenoceptor antagonist properties, but results from its partial agonist activity at 5-HT_{1A} receptors.

In summary, the present study provides further evidence of the potent 5-HT_{1A} receptor antagonist properties of WAY100635. When given alone and at the doses examined here, WAY100635 did not affect hippocampal 5-HT levels, unlike (*S*)-WAY100135, which decreased these levels. Because the decrease in 5-HT levels induced by (*S*)-WAY100135, but not by prazosin, could be antagonised by WAY100635, it appears likely that (*S*)-WAY100135 decreased 5-HT levels in the hippocampus because of its partial 5-HT_{1A} receptor agonist properties and not because of its possible α_1-adrenoceptor antagonist properties. Thus, WAY100635 appears to be more suitable than (*S*)-WAY100135 for use as an antagonist in studies of 5-HT_{1A} receptor function.

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