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A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635

Elaine A. Forster [a], Ian A. Cliffe [c], David J. Bill [a], Gillian M. Dover [b], Deborah Jones [d], Yvonne Reilly [b], Allan Fletcher [a,*]

[a] *Department of Neuropharmacology, Wyeth Research (UK) Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire SL6 0PH, UK*
[b] *Department of Molecular Pharmacology, Wyeth Research (UK) Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire SL6 0PH, UK*
[c] *Department of Medicinal Chemistry, Wyeth Research (UK) Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire SL6 0PH, UK*
[d] *Central Nervous System Pharmacology, Wyeth-Ayerst Research, CN8000, Princeton NJ 08540, USA*

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Abstract

WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) is an achiral phenylpiperazine derivative that binds with high affinity and selectivity to the 5-HT_{1A} receptor. WAY-100635 displaced specific binding of the 5-HT_{1A} radioligand, [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), to rat hippocampal membranes with a pIC_{50} of 8.87. This represented a greater than 100-fold selectivity relative to binding at other 5-HT receptor subtypes and major neurotransmitter receptor, reuptake and ion channel sites. In functional assays, WAY-100635 was a potent 5-HT_{1A} receptor antagonist, with no evidence of any 5-HT_{1A} receptor agonist or partial agonist activity. In the isolated guinea-pig ileum WAY-100635 was a potent and, at high concentrations, an insurmountable antagonist of the 5-HT_{1A} receptor agonist action of 5-carboxamidotryptamine, with an apparent pA_2 value (at 0.3 nM) of 9.71. WAY-100635 blocked the inhibitory action of 8-OH-DPAT on dorsal raphe neuronal firing in the anaesthetised rat at doses which had no inhibitory action per se. In behavioural models, WAY-100635 itself induced no overt behavioural changes but potently antagonised the behavioural syndrome induced by 8-OH-DPAT in the rat and guinea-pig (minimum effective dose = 0.003 mg/kg s.c. and ID_{50} = 0.01 mg/kg s.c., respectively). WAY-100635 also blocked the hypothermia induced by 8-OH-DPAT in the mouse and rat with ID_{50} values of 0.01 mg/kg s.c. These data indicate that WAY-100635 will be used as a standard antagonist in further studies of 5-HT_{1A} receptor function.

Keywords: 5-HT_{1A} receptor; WAY-100635 ; 5-HT_{1A} receptor antagonist

1. Introduction

The neurophysiology of serotonin (5-HT; 5-hydroxytryptamine) is complex, with four main classes of receptor (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4) mediating its role(s) as a neurotransmitter, and three further classes (5-HT_5, 5-HT_6 and 5-HT_7) having been classified recently by molecular biology techniques (Humphrey et al., 1993; Matthes et al., 1993; Monsma et al., 1993; Lovenberg et al., 1993). Of the various 5-HT_1 receptor subtypes the function(s) and pharmacological properties of the 5-HT_{1A} receptor have been studied extensively (e.g. Dourish et al., 1987; Wilkinson and Dourish, 1991). However, although selective 5-HT_{1A} receptor agonists and partial agonists have been available for several years, further characterisation of 5-HT_{1A} receptor function has been hindered by the lack of highly selective 5-HT_{1A} receptor antagonists. Until recently, the only antagonists available were either non-selective or displayed partial agonist activity in several 5-HT_{1A} receptor functional models (for review of this area see Fletcher et al., 1993a). Some advances have been made in recent years with the use of (*S*)-UH-301 (an enantiomer of 5-fluoro-8-hydroxy-2-(di-*n*-propylamino)tetralin; Hillver et al., 1990) and WAY-100135 (*N-tert*-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropionamide; Fletcher et al., 1993b), two 5-HT_{1A} receptor ligands which have displayed antago-

* Corresponding author. Tel. 1628 414739, fax 1628 666587.

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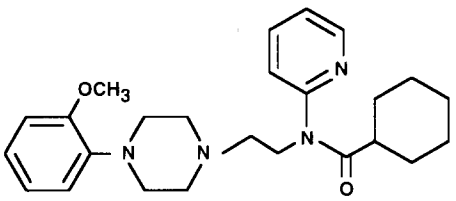

Fig. 1. The chemical structure of WAY-100635.

nist actions in a range of 5-HT_{1A} receptor functional models. However, (*S*)-UH-301 displays only a moderate binding selectivity relative to the dopamine D_2 site (Hillver et al., 1990), where it acts as an agonist (Arborelius et al., 1993), and WAY-100135 has been reported to depress raphe neuronal firing in vivo at high doses (Fletcher et al., 1993b). It is unclear whether the latter effect is due to weak α_1-adrenoceptor antagonist activity (Lanfumey et al., 1993) or to a residual weak partial agonist action. WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexane carboxamide trihydrochloride; Fig. 1) is an achiral analogue of WAY-100135 which is significantly more potent and selective than the latter compound and which displays unequivocal 5-HT_{1A} receptor antagonist activity in a range of pre- (somatodendritic) and postsynaptic functional models. Preliminary reports describing these properties of WAY-100635 were presented to a meeting of the British Pharmacological Society (Fletcher et al., 1994; Gurling et al., 1994; Hartley et al., 1994; Mundey et al., 1994). It has also been reported that radiolabelled WAY-100635 is the first selective antagonist radioligand for both in vitro (Khawaja, 1995; Khawaja et al., 1995; Gozlan et al., 1995) and in vivo (Hume et al., 1994; Laporte et al., 1994) 5-HT_{1A} receptor binding studies.

2. Materials and methods

2.1. Radioligand binding assays

As described previously (Fletcher et al., 1993b), standard radioligand binding assays were used to determine the pIC_{50} values of WAY-100635 for displacement of specific [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) binding (at a radioligand concentration of 1 nM) to rat hippocampal 5-HT_{1A} receptors, and for displacement of specific radioligand binding at other receptor sites. In addition to these primary binding assays, a NovaScreen wide binding profile was determined in order to assess WAY-100635 displacement of specific radioligand binding at a further 40 receptor, reuptake and ion channel sites, at three concentrations of WAY-100635 (10^{-5}, 10^{-7} and 10^{-9} M).

Data were analysed using a 4-parameter non-linear least squares regression analysis programme (ALLFIT) which yields affinity constants (IC_{50}) and slope factors. Binding displacement at each concentration of test compound was determined in triplicate, and at least three separate concentration-response curves were constructed for the estimation of IC_{50} values.

2.2. Isolated guinea-pig ileum

The antagonist action of WAY-100635 at 5-HT_{1A} receptors in the isolated guinea-pig ileum was examined using the method described previously (Fletcher et al., 1993b). Briefly, male guinea-pigs (350–600 g, supplied by Tuck) were killed by cervical dislocation, and segments 1.5–2.0 cm long were cut from the terminal ileum at least 10 cm from the ileo-caecal junction. The segments were mounted on tissue holders fitted with two parallel bipolar platinum electrodes 6–8 mm apart, such that one electrode was positioned within the length of the ileal lumen. Following a short equilibration period (15–20 min) in an organ bath, the tissues were placed under a resting tension of 1 g and stimulated at 0.05 Hz with square wave pulses of 1 ms duration at supramaximal current (17–20 mA). Tissue responses to stimulation were measured isometrically. Routinely, four ileum segments from the same animal were studied simultaneously. When the responses to stimulation became constant the tissues were washed with Krebs solution containing 3 μM mesulergine. The use of 3 μM mesulergine to block a non-5-HT_{1A} receptor-mediated muscle relaxant effect at high concentrations of 5-carboxamidotryptamine (5-CT) has been reported previously (Bill et al., 1990). The tissues were then stimulated at 7 min intervals with trains of six pulses at 0.05 Hz pulse width and supramaximal current. When the response to trains of pulses was constant the test compound was added to the organ baths of two of the tissues and equilibrated for 1 h. Antagonist evaluation was carried out by constructing concentration-response curves for 5-CT in the presence or absence of test compound. Antagonist pA_2 values were calculated from concentration ratios obtained at the IC_{50} level from concentration-response curves obtained in paired preparations.

2.3. In vivo recording of dorsal raphe neuronal firing

Recording procedures were essentially the same as those of Haigler and Aghajanian (1974) and have been described in detail elsewhere (Fletcher et al., 1993b). Male Sprague-Dawley rats (200–300 g; Charles River) were initially anaesthetized with chloral hydrate (400 mg/kg i.p.) and given subsequent i.v. maintenance injections as needed. The animals were placed in a stereotaxic frame in the orientation of König and Klip-

pel (1963) and a micropipette was lowered through a burr hole in the skull to a depth of 1.0 mm above the dorsal raphe nucleus (vertical coordinate 4.5 mm, on the midline and −0.6 mm from interaural zero). The recording electrode was further advanced into the recording site by means of a hydraulic microdrive. Neurones in the dorsal raphe nucleus were identified from their characteristic waveform and slow rhythmic activity (Haigler and Aghajanian, 1974). Once these criteria were satisfied, spontaneous activity was monitored for at least 3–5 min to establish baseline firing rate. Drugs were then administered i.v. via a lateral tail vein cannula. Only one cell was studied in each rat to avoid residual drug effects. ID_{50} values (dose required to reduce firing to 50% of baseline; $n = 5$) were calculated using non-linear inverse regression analysis. At the termination of the experiment, pontamine sky blue dye was deposited for histological confirmation of the recording site and reconstruction of the electrode track. Data from recording sites not contained within the histological boundaries of the dorsal raphe nucleus were discarded. The effects of WAY-100635 alone on firing rates were statistically analysed using a repeated measures models in which drug effects were compared with (pre-drug) baseline data by a *t*-test in conjunction with Satterthwaite's formula to determine degrees of freedom. ID_{50} values were calculated by parallel logistic regression and statistically compared using a weighted analysis of variance.

2.4. Antagonism of 8-OH-DPAT-induced behavioural syndrome

Male Sprague-Dawley rats (250–400 g) were used. In order to assess agonist activity, drugs were administered intravenously up to a dose of 10 mg/kg and the animals placed in circular Perspex observation chambers for a period of 5 min (or until the onset of seizure activity, when the animals were immediately killed). For antagonist evaluation, groups of at least ten animals received vehicle or test compound subcutaneously 30 min before the intravenous administration of 8-OH-DPAT. An ED_{50} for 8-OH-DPAT to induce a behavioural syndrome (forepaw treading, extended flat body posture, hyperlocomotion) was determined in each treatment group using a sequential, up/down technique (Kimball et al., 1957) as described previously (Fletcher et al., 1993b). The 8-OH-DPAT syndrome was assessed by an observer 'blind' to drug pretreatments, as present (definite syndrome response) or absent (equivocal or no syndrome response) during the period 0–5 min immediately following the intravenous administration of 8-OH-DPAT. ED_{50} values (with 95% confidence limits) were calculated from these quantal responses by a modified probit analysis as described by Kimball et al. (1957). ED_{50} values were considered to be significantly different if the confidence limits did not overlap. The antagonist potency of WAY-100635 in this model was expressed as a minimum effective dose (MED; lowest dose to significantly increase the 8-OH-DPAT ED_{50}).

In male Dunkin-Hartley albino guinea-pigs (450–600 g) a single submaximal challenge dose of 8-OH-DPAT (0.5 mg/kg s.c.) was administered to groups of eight animals pretreated 10 min previously with either vehicle or various doses of WAY-100635. Twenty minutes later the intensity of the behavioural syndrome induced by 8-OH-DPAT was scored (by an observer unaware of the drug pretreatments administered to each animal) using an arbitrary rating scale. The main components of the syndrome (hyperactivity, forepaw treading, and head-weaving) were rated on a scale of 0–3 according to intensity (0 = absent, 1 = equivocal, 2 = unequivocal, 3 = severe) and tremor was rated as absent (0) or present (1). This yielded a maximum possible score of 10 for each animal.

2.5. 8-OH-DPAT-induced hypothermia

The procedures used for these studies are based on those described in Bill et al. (1991). Female T/O mice (20–27 g; Tuck) or male Sprague-Dawley rats (190–220 g) were housed in groups of eight or four, respectively, at an ambient temperature of 20.0 ± 0.5° C for at least 2 h before the measurement of body temperature and drug administration. Body temperature was measured in gently restrained animals using a thermistor probe (connected to a digital 2001 thermometer) inserted to a depth of 2 cm into the rectum (mice) or 4 cm into the oesophagus (rat). WAY-100635 or vehicle were administered s.c. to groups of eight animals per treatment 20 min before the s.c. injection of standard challenge doses of 8-OH-DPAT (0.5 mg/kg for mice; 0.25 mg/kg for rats). Temperatures were measured immediately before each drug injection, and at 15 and 30 min after injection of 8-OH-DPAT. The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature recorded in this latter period. Treatment groups receiving vehicle/vehicle, vehicle/8-OH-DPAT and the highest dose of the test compound followed by vehicle were included in all experiments. In further experiments, the effects of WAY-100635 on apomorphine or UK14304 (both at 0.5 mg/kg s.c.)-induced hypothermia in the mouse were examined using the same protocol.

2.6. Drugs

Drugs were administered as solutions in isotonic saline at dose volumes of 2 ml/kg (rats) or 10 ml/kg (mice) and doses refer to mg/kg of base. The drugs used in these studies, together with their sources were

as follows: WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride), 5-carboxamidotryptamine, 8-OH-DPAT (hydrobromide) and UK14304 (tartrate) were synthesised at Wyeth Research (UK) Ltd.; mesulergine (Sandoz), apomorphine hydrochloride (Sigma).

3. Results

3.1. Radioligand binding assays

The pIC_{50} value for WAY-100635 at 5-HT_{1A} sites was 8.87 ± 0.14. The highest plC_{50} value for WAY-100635 at other sites tested was 6.64 ± 0.04 at the α_1-adrenoceptor site (0.2 nM ^{3}[H]prazosin radioligand in rat cortical membranes; values are means ± S.E.M. of three displacement curves). WAY-100635 was > 100-fold selective for 5-HT_{1A} sites at all other sites tested: 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2C}, 5-HT_3, 5-HT_4, α_2- and β-adrenoceptors, dopamine (D_1, D_2, D_4), $GABA_A$, $GABA_B$, histamine(H_1, H_2, H_3), muscarinic (M_1, M_2, M_3), nicotinic, NMDA, kainate, quisqualate, central benzodiazepine, opiate (μ, δ, κ), adenosine (A_1, A_2), reuptake sites (dopamine, noradrenaline, 5-HT, GABA) and ion channels (Ca^{2+} N, T and L; Cl^-, Na^+ (sites 1 and 2) and K^+ (ATP; voltage-dependent; apamin-sensitive)).

3.2. Isolated guinea-pig ileum

In the isolated guinea-pig ileum WAY-100635 potently antagonised the 5-HT_{1A} receptor mediated inhibition of electrically evoked twitch induced by 5-CT, with an apparent pA_2 value of 9.71 (9.3–10.2) at a WAY-100635 concentraion (0.3 nM) which did not significantly reduce the maximum response to 5-CT (Fig. 2). The calculated (ALLFIT) maximum responses (means ± S.E.M.) for the WAY-100635 (0.3 nM) concentration-response curve and its own control curve were, respectively, $32.6 \pm 2.3\%$ and $35.9 \pm 14\%$ (both $n = 6$). At higher concentrations the antagonist action of WAY-100635 was insurmountable, depressing the maximum response to 5-CT.

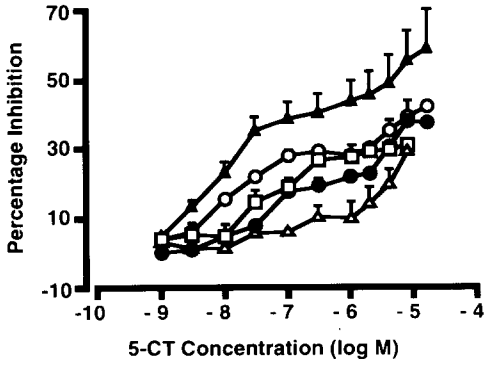

Fig. 2. 5-HT_{1A} receptor antagonist action of WAY-100635 in the guinea-pig ileum. Concentration-response curves for inhibition of electrically evoked twitch were constructed for 5-carboxamidotryptamine (5-CT) alone (▲; $n = 10$) and in the presence of various concentrations of WAY-100635: 3×10^{-10} M (○; $n = 6$); 10^{-9} M (●; $n = 6$); 3×10^{-9} M (□; $n = 4$) and 10^{-8} M (△; $n = 4$). Vertical bars represent the S.E.M.

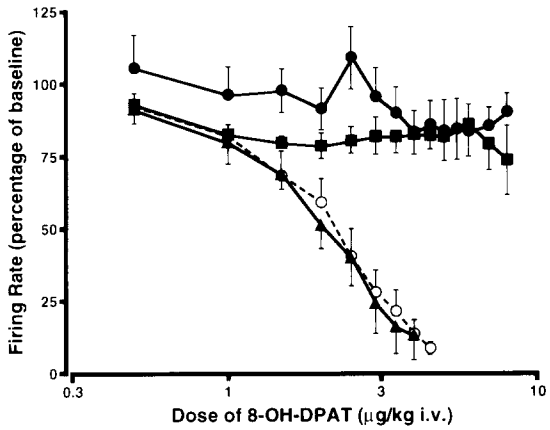

Fig. 3. Antagonism of 8-OH-DPAT-induced inhibition of dorsal raphe neuronal firing in the anaesthetised rat. Cumulative intravenous dose-response curves for the inhibition of neuronal firing by 8-OH-DPAT were determined in rats pretreated intravenously with saline (○) or WAY-100635 at doses of 1 (▲), 10 (■) or 100 (●) μg/kg. Values are means (±S.E.M., represented by vertical bars; $n = 6$–8 rats) firing rates expressed as a percentage of the baseline firing rate recorded before administration of vehicle or drug. WAY-100635 alone had no significant effect on raphe neuronal firing rate.

3.3. In vivo recording of dorsal raphe neuronal firing

The effects of WAY-100635 on the inhibition of dorsal raphe nucleus 5-HT neuronal firing induced by 8-OH-DPAT are shown in Fig. 3. At doses of 10 and 100 μg/kg, WAY-100635 blocked the inhibition of firing induced by 8-OH-DPAT. Importantly, the administration of WAY-100635 alone, over the dose range 5–100 μg/kg i.v., did not attenuate neuronal firing. There was a tendency for WAY-100635 to increase firing rate (the greatest apparent effects were 25% and 21% increases at 40 and 60 μg/kg i.v., respectively), although this effect did not achieve statistical significance at any dose of WAY-100635.

3.4. Antagonism of 8-OH-DPAT-induced behavioural syndrome

Following intravenous administration up to a dose of 10 mg/kg in rats WAY-100635 did not evoke any component of the '5-HT syndrome' elicited by selective and non-selective 5-HT_{1A} receptor agonists. The ED_{50} values (with 95% confidence limits, in μg/kg i.v.) for

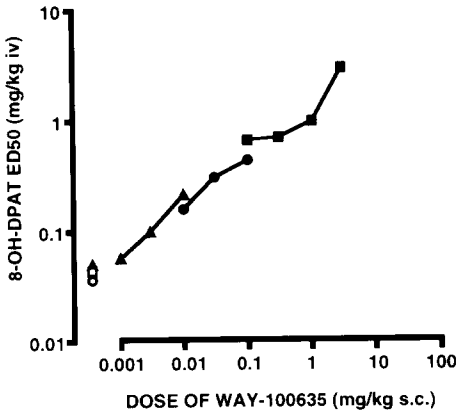

Fig. 4. Antagonism of 8-OH-DPAT-induced syndrome in the rat by WAY-100635. WAY-100635 was administered 30 min before the intravenous administration of 8-OH-DPAT. The results of three separate experiments (each experiment including a group of vehicle-treated controls, represented by the open symbols) are shown, encompassing a dose range of 0.001–3.0 mg/kg s.c. of the antagonist. ED_{50} values (mg/kg i.v. with 95% confidence limits) for 8-OH-DPAT to induce the syndrome in treatment groups of ten animals were determined. WAY-100635, at doses of 0.003 mg/kg s.c. or greater, significantly ($P < 0.05$) increased the ED_{50} of 8-OH-DPAT.

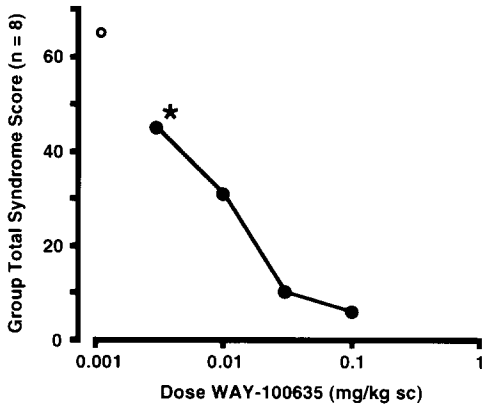

Fig. 5. Antagonism of 8-OH-DPAT-induced syndrome in the guinea-pig by WAY-100635. The syndrome response to a challenge dose of 8-OH-DPAT (0.5 mg/kg s.c.) was determined in groups of eight animals pretreated with either saline vehicle (open symbol) or various doses of WAY-100635. The behavioural response to 8-OH-DPAT was significantly ($P < 0.01$) reduced by doses of WAY-100635 of 0.003 mg/kg s.c. or greater (Mann-Whitney U-test).

8-OH-DPAT to induce the behavioural syndrome in saline-pretreated animals and in animals pretreated with 1, 3 or 10 μg/kg s.c. of WAY-100635 were, respectively: 50 (37–68), 58 (35–96), 100 (80–130)* and 220 (190–260)* [*$P < 0.05$ relative to vehicle controls on the basis that 95% confidence limits do not overlap]. Fig. 4 summarises the results of three separate experiments examining the effects of a wider range of WAY-100635 doses on 8-OH-DPAT-induced syndrome.

In the guinea-pig WAY-100635, at doses of 0.003 mg/kg s.c. or greater, also significantly and markedly inhibited the behavioural syndrome induced by a single challenge dose of 8-OH-DPAT (0.5 mg/kg s.c.; Fig. 5). The ED_{50} of WAY-100635 in this model was 0.01 mg/kg s.c.

3.5. Antagonism of 8-OH-DPAT-induced hypothermia

WAY-100635 potently and dose dependently antagonised the hypothermic response to 8-OH-DPAT in

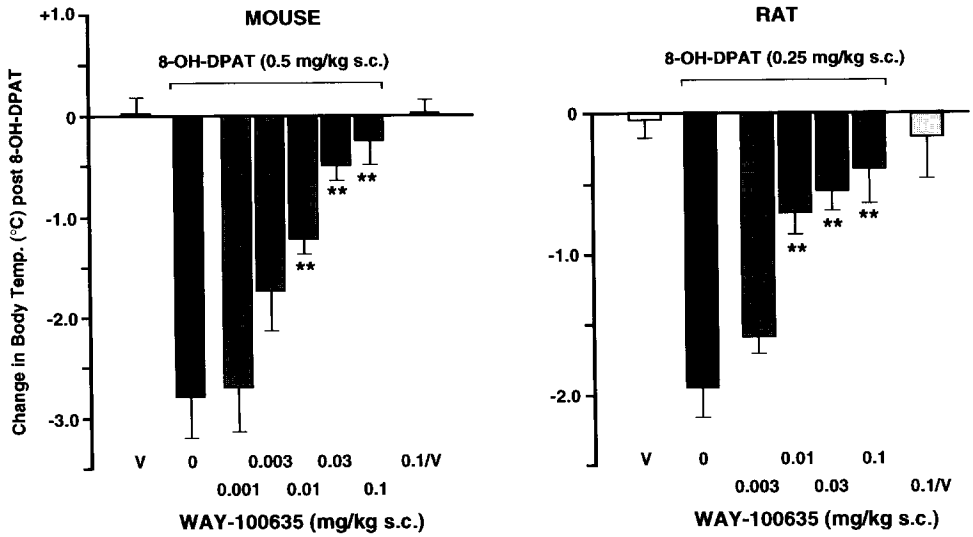

Fig. 6. Antagonism of 8-OH-DPAT-induced hypothermia by WAY-100635. The dose-related antagonism of 8-OH-DPAT-induced hypothermia in the mouse (left) and rat (right) is illustrated. Open bars represent means (±S.E.M. represented as vertical bars) body temperature changes in control animals receiving vehicle only (V), and the black bars show the effect of 8-OH-DPAT alone. Shaded bars show the response to 8-OH-DPAT in animals receiving prior doses of WAY-100635, and the bar at the extreme right of each histogram illustrates the response to the top dose of WAY-100635 alone, administered to vehicle (V) control animals. In both species WAY-100635 potently antagonised the hypothermic response to 8-OH-DPAT with an ED50 value of 0.01 mg/kg s.c. ** $P < 0.01$ relative to 8-OH-DPAT alone (one-way ANOVA followed by *t*-test).

both the mouse and rat (Fig. 6). The ED_{50} values (with 95% confidence limits) of WAY-100635 (doses required to reduce the hypothermic response by 50%) were 0.01 (0.004–0.022) and 0.01 (0.003–0.025) mg/kg s.c. in the mouse and rat, respectively. In contrast, WAY-100635, at a dose of 1 mg/kg s.c., had no effect on the hypothermic responses to either the dopamine D_1/D_2 receptor agonist, apomorphine, or the α_2-adrenoceptor agonist, UK14304 (both at 0.5 mg/kg s.c.) in the mouse (data not shown).

4. Discussion

Owing to the differences between presynaptic (somatodendritic) and postsynaptic 5-HT_{1A} receptor populations (in terms of receptor reserve and receptor-effector coupling) several 5-HT_{1A} receptor partial agonists act as antagonists of postsynaptic receptors but can activate presynaptic receptors. This has led to several selective 5-HT_{1A} receptor ligands being described as antagonists on the basis of data derived from postsynaptic functional models. In the majority of cases such compounds have been shown to be 5-HT_{1A} receptor partial agonists when examined in models of somatodendritic 5-HT_{1A} receptor function (inter alia Fletcher et al., 1993b). In order to clearly distinguish true antagonists from partial agonists, the term 'silent' 5-HT_{1A} receptor antagonist has been employed. Although some non-selective ligands (e.g. spiperone, (−)-propranolol) have consistently displayed antagonist activity in a range of pre- and postsynaptic models of 5-HT_{1A} receptor function, the development of selective 'silent' 5-HT_{1A} receptor antagonists has proved to be surprisingly difficult. Several compounds shown to display 5-HT_{1A} receptor antagonist properties in models of postsynaptic 5-HT_{1A} receptor function (e.g. BMY7378, NAN-190, SDZ-216,525) are now known to be 5-HT_{1A} receptor partial agonists (Fletcher et al., 1993a). Significant advances in this area were made with the syntheses of the aminotetralin, (*S*)-UH-301 (Hillver et al., 1990) and the phenylpiperazine derivative, (*S*)-WAY-100135 (Cliffe et al., 1993; Fletcher et al., 1993b). However, (*S*)-UH-301 is not a highly selective ligand (Hillver et al., 1990) and (*S*)-WAY-100135, despite displaying antagonist actions in models of both presynaptic and postsynaptic 5-HT_{1A} receptor function, has also evoked responses which may be consistent either with α_1-adrenoceptor antagonist activity at high concentrations (Lanfumey et al., 1993) or a weak 5-HT_{1A} receptor partial agonist action (Fletcher et al., 1993b). WAY-100635 appears to be the first highly selective and 'silent' 5-HT_{1A} receptor antagonist that has displayed only antagonist activity in all 5-HT_{1A} receptor functional models examined to date. In addition to the binding data reported in this paper we have also received data showing that WAY-100635 (at a concentration of 100 nM), unlike several other 5-HT_{1A} receptor ligands (Lovenberg et al., 1993), does not induce significant displacement of specific radioligand binding to the rat 5-HT_7 site (Hamblin, unpublished observations). The selectivity of WAY-100635 for 5-HT_{1A} relative to 5-HT_7 sites, therefore, is at least 74-fold.

Our in vivo studies clearly demonstrated that WAY-100635 lacks agonist activity in several physiological and behavioural models of central 5-HT_{1A} receptor activation. However, in all models and species examined (both of presynaptic (somatodendritic) and postsynaptic 5-HT_{1A} receptor function), WAY-100635 was a potent antagonist of responses evoked by the standard 5-HT_{1A} receptor agonist, 8-OH-DPAT. Thus, WAY-100635 blocked the 5-HT_{1A} receptor agonist action of 5-CT in the guinea-pig isolated ileum, 8-OH-DPAT-induced 5-HT syndrome in the rat and guinea-pig, hypothermia in the mouse and rat, and inhibition of raphe 5-HT neuronal firing in the rat. Although the effect did not achieve statistical significance, there was a tendency for WAY-100635 alone to increase the firing rates of 5-HT neurones in the dorsal raphe nucleus, possibly suggesting that these neurones are under tonic inhibitory control by release of endogenous 5-HT. In the conscious cat WAY-100635 unequivocally and significantly increased raphe 5-HT neuronal cell firing (Fornal et al., 1994) indicating that these cells are under a tonic inhibitory control by endogenous 5-HT. WAY-100635 has also been shown to block the inhibitory effect of 8-OH-DPAT on dorsal raphe nucleus 5-HT neuronal firing in the guinea-pig (Mundey et al., 1994). Several additional in vivo responses to 8-OH-DPAT in the rat are also potently and dose dependently blocked by WAY-100635, i.e. inhibition of hippocampal 5-HT release (Gurling et al., 1994), elevations in plasma ACTH (Critchley et al., 1994) and the 8-OH-DPAT discriminative cue (Piesla and Marquis, 1994).

Since 5-HT_{1A} receptors are thought to be involved in several psychiatric and neurological disorders (e.g. Lesch and Osterheider, 1991; Bowen et al., 1993) it is feasible that potent and selective 5-HT_{1A} receptor antagonists such as WAY-100635 may have therapeutic actions (Fletcher et al., 1993a). WAY-100635 and other 5-HT_{1A} receptor antagonists have been reported to display anxiolytic-like activity in the mouse with potencies correlated with their functional in vivo 5-HT_{1A} receptor antagonist activity in the same species (Bill and Fletcher, 1993). It is also feasible that 5-HT_{1A} receptor antagonists may ameliorate the symptomatology of dementia by facilitating glutamate release (Bowen et al., 1993) and thereby compensate to some extent for the loss of cortical glutamatergic neurones thought to occur in this illness (Bowen et al., 1992).

In addition to the utility of WAY-100635 in characterising 5-HT_{1A} receptor-mediated functional responses, this ligand has also been shown to be of great importance in receptor binding studies, since the tritium-labelled WAY-100635 molecule displays a high level of specific 5-HT_{1A} receptor binding both in vitro (Khawaja, 1995; Khawaja et al., 1995; Gozlan et al., 1995) and in vivo (Hume et al., 1994; Laporte et al., 1994) and is now being used as the first antagonist 5-HT_{1A} receptor radioligand in binding studies. Of particular importance with regard to future clinical studies Pike et al. (1994) were the first to report that the positron-emitting [^{11}C]WAY-100635 radioligand displays excellent in vivo binding characteristics in the rat (confirmed by Mathis et al. (1994) in the rhesus monkey) and is currently under evaluation as the first positron emission tomography (PET) radioligand for imaging central 5-HT_{1A} receptors in Man (McCarron et al., 1995; Pike et al., 1995). Therefore, in addition to its use as a standard antagonist in studies of 5-HT_{1A} receptor function, WAY-100635 will also be employed as the first 5-HT_{1A} receptor radioligand both for preclinical binding studies and imaging of 5-HT_{1A} receptor populations in the living human brain.

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