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(+)-WAY 100135, a partial agonist, at native and recombinant 5-$HT_{1B/1D}$ receptors

[1]Colin Davidson, *Michael Ho, *Gary W. Price, *Brian J. Jones & [2]Jonathan A. Stamford

Anaesthetics Unit (Neurotransmission Laboratory), St Bartholomew's and the Royal London School of Medicine and Dentistry, Alexandra Wing, Royal London Hospital, Whitechapel, London E1 1BB and *SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex, CM19 5AW

1 We have studied the effects of the purportedly selective 5-HT_{1A} receptor antagonist (+)-WAY100135 on electrically stimulated 5-hydroxytryptamine (5-HT) efflux in the ventrolateral geniculate nucleus (vLGN), and its affinity at human 5-HT_{1B} and 5-HT_{1D} receptors stably expressed in Chinese hamster ovary (CHO) cells.

2 On short 'pseudo single pulse' stimulations (20 pulses at 100 Hz, 190 ms train duration), (+)-WAY100135 (1.0 μM) decreased 5-HT efflux in the vLGN to 68±8% of pre-drug values ($P<0.01$). This decrease could be blocked by the 5-$HT_{1D/1B}$ receptor antagonist GR 127935 (50 nM). Conversely, when long stimulations (20 pulses at 20 Hz, 950 ms train) were used, (+)-WAY100135 had no effect on 5-HT efflux (84±8% of pre-drug values) although both methiothepin (200nM) and GR 127935 (50 nM) caused significant increases (to 175±18 and 130±10% of pre-drug values, respectively).

3 Paroxetine (100 nM), the selective 5-HT reuptake inhibitor, increased stimulated 5-HT efflux and re-uptake half-life (to 145±18% and 649±121%, respectively) on pseudo single pulse stimulations. When (+)-WAY 100135 was added in combination with the uptake blocker, the effect of paroxetine on stimulated 5-HT efflux was potentiated to 282±48% ($P<0.01$) without further effect on the 5-HT re-uptake half-life.

4 The affinity and intrinsic activity of (+)-WAY 100135 were determined at recombinant human 5-HT_{1B} and 5-HT_{1D} receptors expressed in CHO cells, by use of radioligand binding and [^{35}S]-GTPγS binding. (+)-WAY 100135 was a partial agonist at human 5-HT_{1B} and 5-HT_{1D} receptors with moderately high affinity for 5-HT_{1D} receptors ($pEC_{50}=7.61$).

5 In conclusion, (+)-WAY 100135 was found to be not a selective 5-HT_{1A} autoreceptor antagonist but may act as a partial agonist at the 5-$HT_{1B/1D}$ receptor, displaying agonist or antagonist properties depending on the stimulation protocol used and the resultant 5-HT 'tone' at the receptor.

Keywords: Lateral geniculate nucleus; 5-hydroxytryptamine (5-HT); 5-$HT_{1B/1D}$ autoreceptor; (+)-WAY100135; fast cyclic voltammetry; partial agonist; [^{35}S]-GTPγS binding

Introduction

Until relatively recently, 5-HT_{1A} and 5-HT_{1B} receptors were thought to be the main somatodendritic and terminal autoreceptors respectively, controlling synthesis, turnover and efflux of 5-hydroxytryptamine (5-HT) in the rat brain (see Hoyer *et al*., 1994 for review). However, more recently, it has been shown that the rat also possesses mRNA for the 5-HT_{1D} receptor (Hamblin *et al*., 1992). Furthermore, recent data from this laboratory and others have demonstrated the existence of functional 5-HT_{1D} autoreceptors in rat 5-HT cell body (Davidson & Stamford, 1995; Pineyro *et al*., 1995) and terminal regions (Limberger *et al*., 1991).

We have studied a 5-HT terminal region, the ventral lateral geniculate nucleus (vLGN). The vLGN is a pivotal relay station in the visual system. It receives afferents from both the retina and visual cortex (Brauer *et al*., 1984), sends efferent projections to the SCN, the circadian pacemaker (Ribak & Petera, 1975), and may even show circadian properties itself (Mason, 1986). The vLGN also has an extremely dense 5-HT innervation from 5-hydroxytryptaminergic cell bodies in the dorsal raphe nucleus (Brauer *et al*., 1984).

Interestingly, the vLGN has a relatively high density of 5-HT_{1D} binding sites (Bruinvels *et al*., 1993) and we have shown that this translates into the existence of functional 5-HT_{1D} autoreceptors in this nucleus (Davidson & Stamford, 1996) as well as 5-HT_{1B} sites. We found CP 93129 (300 nM), the 5-HT_{1B} agonist (Hoyer *et al*., 1994) to inhibit 5-HT efflux. This inhibition was antagonized by the 5-HT_{1B} antagonist, isamoltane (500 nM) and the 5-$HT_{1D/1B}$ antagonist, GR 127935 (50 nM). The partially selective 5-HT_{1D} agonist sumatriptan (500 nM) was able to inhibit 5-HT efflux in the vLGN and the effect was antagonized by GR 127935 (50 nM) but not by isamoltane (500 nM). Conversely, as expected, we found no evidence of 5-HT_{1A} receptors in the vLGN.

WAY 100135 has been proposed to be a selective 5-HT_{1A} receptor antagonist in the rat (Fletcher *et al*., 1993) at both pre- and post-synaptic 5-HT_{1A} receptors, with very low affinity at other 5-HT receptors: binding studies show that is has p*K*i values of 7.6 and <5 at 5-HT_{1A} and 5-HT_{1B} receptors, respectively, and that the dextrorotatory enantiomer is the more active form (Fletcher *et al*., 1993).

During preliminary experiments with (+)-WAY 100135 in the vLGN, we were surprised to find that (+)-WAY 100135 appeared to modify stimulated 5-HT efflux, despite the absence of any significant number of 5-HT_{1A} receptors in this nucleus (Pazos & Palacios, 1985; Bruinvels *et al*., 1993), suggesting an interaction with another component of the control of 5-HT efflux. In the present study we therefore attempted to characterize the activity of (+)-WAY 100135 at the 5-HT autoreceptor in the rat vLGN. To support our findings, we also assessed its affinity and intrinsic activity at human cloned 5-HT_{1B} and 5-HT_{1D} receptors expressed in Chinese hamster ovary (CHO) cells.

[1]Present address: School of Psychology, University of St Andrews, Fife, KY16 9JU.
[2]Author for correspondence at: Anaesthetics Unit (Neurotransmission Laboratory), Royal London Hospital, Whitechapel, London E1 1BB.

Methods

We used two types of experiment to determine the action of (+)-WAY 100135 at 5-HT$_{1B/1D}$ receptors. In the first set of experiments, we studied the effects of (+)-WAY 100135 on the autoreceptor control of electrically stimulated 5-HT efflux in rat brain slices of the vLGN. In the second experiment, we investigated the effect of (+)-WAY 100135 on radioligand binding and [^{35}S]-GTPγS binding in Chinese hamster ovary (CHO) cells stably expressing human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors.

Brain slices

All brain slice experiments were conducted in tissue from male Wistar rats (100–150 g). The animals were housed 6 per cage under controlled conditions of ambient temperature (21±2°C), and a 12:12 h light/dark cycle (lights on at 6 h 30 min), with standard rat chow and tap water freely available. Rats were killed by cervical dislocation. The brain was swiftly chilled in ice-cold artificial cerebrospinal fluid (ACSF) and carefully removed from the cranial vault. A small tissue block containing the vLGN was trimmed rostrocaudally until the desired coronal section was prepared (at ~+4.6 mm versus the interaural line: Paxinos & Watson, 1986). At this level, a 350 μm section was taken, transferred to a superfusion-type brain slice chamber and superfused with oxygenated ACSF (32°C) at 1.2 ml min^{-1} throughout the experiment. The ACSF consisted of the following salts (in mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $NaHCO_3$ 25, $CaCl_2$ 2 and (+)-glucose 11. The ACSF was gassed at 32°C with 95% O_2/5% CO_2 for 1 h before use. The vLGN slice was allowed to equilibrate in the chamber for at least an hour before any stimulation was conducted. 5-HT efflux was evoked and recorded in the pars magnocellularis of the nucleus (Davidson & Stamford, 1996).

Electrical stimulation procedures

Constant current electrical stimulations were generated with Neurolog modules and applied via an NL 800 optical isolator to a parallel bipolar tungsten stimulating electrode. Two monopolar microelectrodes, 125 μm diameter (A-M Systems, Seattle) were joined to form a bipolar electrode with a tip separation of 125 μm. This stimulating electrode was inserted approximately 80 μm into the tissue at a location approximately 200 μm ventral to the carbon fibre microelectrode in a configuration whereby the tips formed an isosceles triangle with the working electrode.

Two different types of stimulation paradigms were used: (a) A short 'pseudo-single-pulse' (PSP) stimulation train (Singer, 1988) consisting of 20 pulses (0.2 ms duration, 100 Hz, 190 ms train duration) applied every 10 min. This protocol was designed to minimize autoreceptor activation by endogenously released 5-HT and enabled the effects of autoreceptor agonists to be observed. (b) A longer 20 pulse train (0.2 ms pulses, 20 Hz, 950 ms train duration, applied every 10 min) where the 5-HT released in the earlier part of the train could stimulate the autoreceptors and induce autoinhibition during the course of the stimulation. This protocol was designed to study the effects of autoreceptor antagonists. In each, there were 3 control (pre-drug) stimulations followed by addition of the drug to the ACSF for 1 or 2 h. Drug effects on 5-HT efflux are expressed as a percentage of the mean 5-HT efflux on the 3 pre-drug stimulations.

Voltammetric electrodes

The working electrode was a glass-encased carbon fibre (8 μm diameter, 50 μm length) microelectrode (Armstrong & Millar, 1979). The auxiliary electrode consisted of a stainless steel wire attached to a binding post on the chamber and the reference electrode was a silver/silver chloride (Ag/AgCl) cylinder. Both working and stimulating electrodes were positioned 80 μm below the brain slice surface in the magnocellular layer of the vLGN, immediately ventral to the intergeniculate leaflet. Reference and auxiliary electrodes were positioned at a convenient location in the chamber away from the tissue.

Measurement of 5-HT efflux and uptake by fast cyclic voltammetry

Stimulated 5-HT efflux was monitored with fast cyclic voltammetry (FCV: see Stamford, 1990). The potentiostat input comprised 1½ cycles of a triangular waveform (−1.0 to +1.4 V vs AG/AgCl, 480 Vs^{-1} scan rate) applied every 500 ms. The current output and voltage input of the working electrode were displayed on a Nicolet 310DD digital storage oscilloscope. Background current signals before stimulation were subtracted from those obtained after a stimulus. The difference comprised the faradaic current derived from 5-HT oxidation and subsequent reduction. A sample and hold circuit monitored 5-HT oxidation current at the peak potential (+570 mV vs Ag/AgCl). Its analogue output was digitised and stored on a microcomputer by use of CED (Cambridge Electronic Design) Chart software. Changes in oxidation current were converted to 5-HT concentrations on the basis of post-experiment calibrations.

Following cessation of stimulation, released 5-HT was removed from the extracellular space by uptake. The time taken for the extracellular concentration of 5-HT to fall by ½ ($t_{½}$) was used as a measure of the 5-HT uptake process.

Receptor expression and membrane preparation

The methods used for 5-HT$_1$ receptor expression and membrane preparation were essentially those of Watson *et al.* (1996). Briefly, a CHO (ACC098) cell line was stably transfected with the human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptor gene by use of electroporesis. Both cell lines of non-clonal origin were cultured in suspension in a medium devoid of serum and nucleosides. Cells were harvested by centrifugation, resuspended in HEPES buffer (20 mM) containing EDTA (10 mM) and homogenized with an Ultra-Turrax. The membranes were washed (HEPES 20 mM, EDTA 0.1 mM), centrifuged and stored as frozen aliquots. These membranes were used in both radioligand binding and [^{35}S]-GTPγS binding assays.

Radioligand binding assays

CHO cell membranes expressing the human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors were resuspended in Tris buffer (50 mM), $MgCl_2$ (10 mM), ascorbate (6 mM) and pargyline (0.5 μM). Membranes (1×10^6 cells) were incubated for 45 min at 37°C in a final volume of 0.5 ml containing [^{3}H]-5-HT(4 nM). Non-specific binding was determined by the addition of 10 μM 5-HT. The reaction was stopped by filtration through Whatman GF/B filters followed by 5 brief washes with ice-cold Tris buffer. Radioactivity was determined by liquid scintillation spectrometry.

[^{35}S]-GTPγS binding studies

[^{35}S]-GTPγS binding studies in CHO cells expressing the human 5-HT$_{1B}$ or 5-HT$_{1D}$ receptor were performed as previously described (Thomas *et al.*, 1995). In brief, 1×10^6 cells were preincubated at 30°C for 30 min in HEPES buffer (20 mM), $MgCl_2$ (3 mM), NaCl (100 mM), ascorbate (0.2 mM), containing GDP (10 μM), with or without test compounds. The reaction was started by the addition of 10 μl of [^{35}S]-GTPγS (100 pM) followed by a further 30 min incubation at 30°C. Non-specific binding was determined by addition of unlabelled GTPγS (10 μM) before the addition of cells. The reaction was stopped by rapid filtration with Whatman GF/B grade filters followed by five washes with ice-cold HEPES buffer. Radioactivity was determined by liquid scintillation spectrometry.

Drugs

The following drugs were obtained from the sources stated: (+)-WAY 100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piper-

azin-1-yl)-2-phenylpropionamide dihydrochloride), a gift from Wyeth Research Ltd, paroxetine and GR 127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide, SmithKline Beecham Pharmaceuticals); methiothepin mesylate (RBI Inc). Paroxetine, methiothepin and (+)-WAY 100135 were initially dissolved in distilled water to make stock solutions of 1 mM. A few drops of dimethylsulphoxide were used to dissolve the GR 127935.

5-HT$_1$ receptor nomenclature

In the light of the recent recommendations from the Serotonin Club Nomenclature Committee (Hartig *et al*., 1996), we have used the following terminology throughout the manuscript: 5-HT$_{1B}$ refers, in addition to those receptors already named 5-HT$_{1B}$ in rat, also to those classed as 5-HT$_{1D\beta}$ in other species. 5-HT$_{1D}$ is used to define those receptors previously called 5-HT$_{1D}$ in rat and 5-HT$_{1D\alpha}$ in other species. Where the receptors used are the human forms, we have used the terms h5-HT$_{1B}$ and h5-HT$_{1D}$ as appropriate.

Data analysis and statistics

Statistical comparisons between groups were made by one-way analysis of variance (ANOVA) with *post hoc* application of the Student-Newman-Keuls test (Graphpad Instat). Where only two groups were to be compared, 2 way ANOVA was used. Binding data were analysed by a four parameter logistic equation by use of GRAFIT (Erithacus Software Ltd).

Results

(+)-WAY 100135 decreases 5-HT efflux on PSP stimulations in the vLGN

Figure 1 shows the effect of (+)-WAY 100135 upon stimulated 5-HT efflux. Under pseudo-single-pulse (PSP) stimulation conditions (20 pulses, 0.2 ms, 100 Hz), (+)-WAY 100135 (1.0 μM) decreased stimulated 5-HT efflux by 32% in the vLGN (Figure 1a). This decrease was significant ($P<0.001$: 2 way ANOVA) and was observed within 20 min of addition of the drug to the ACSF. Pretreatment of vLGN slices for 1 h with the 5-HT$_{1B/D}$ receptor antagonist, GR 127935 (50 nM), abolished the effect of subsequent challenge with (+)-WAY 100135 (Figure 1b).

(+)-WAY 100135 potentiates the effect of paroxetine on 5-HT efflux in the vLGN

Figure 2a shows the ability of (+)-WAY 100135 to potentiate the effect of paroxetine on stimulated 5-HT efflux evoked by pseudo single pulse trains (20 pulses, 0.2 ms, 100 Hz). 5-HT efflux, 120 min after paroxetine (100 nM), a selective 5-HT reuptake inhibitor (SSRI), was 145±18% of pre-drug values. However, when given in combination with (+)-WAY 100135 (1.0 μM), the effect of paroxetine on 5-HT efflux was significantly ($P<0.01$) potentiated to 282±48% of the control period. (+)-WAY 100135 had no effect on 5-HT uptake (Figure 2b) and, furthermore, did not potentiate the effect of paroxetine on uptake half-life (684±134% and 604±140% of pre-drug values with and without (+)-WAY 100135, respectively).

(+)-WAY 100135 has no effect on 5-HT efflux by long stimulations

Figure 3 shows the effects of three 5-HT$_1$ antagonists on 5-HT efflux on long stimulations (20 pulses, 0.2 ms, 20 Hz). Methiothepin, the non-selective 5-HT$_1$ receptor antagonist (200 nM), increased 5-HT efflux to 175±18% ($P<0.001$) at maximum relative to controls. The selective 5-HT$_{1D/B}$ receptor antagonist, GR 127935 (50 nM), also significantly ($P<0.05$) increased efflux to 130±10% at maximum. In contrast, (+)-WAY 100135 (1.0 μM) did not significantly increase stimulated 5-HT efflux under these conditions.

(+)-WAY 100135 has affinity for h5-HT$_{1D}$ receptors

(+)-WAY 100135 bound to stably expressed h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors. (+)-WAY 100135 had only modest affinity at

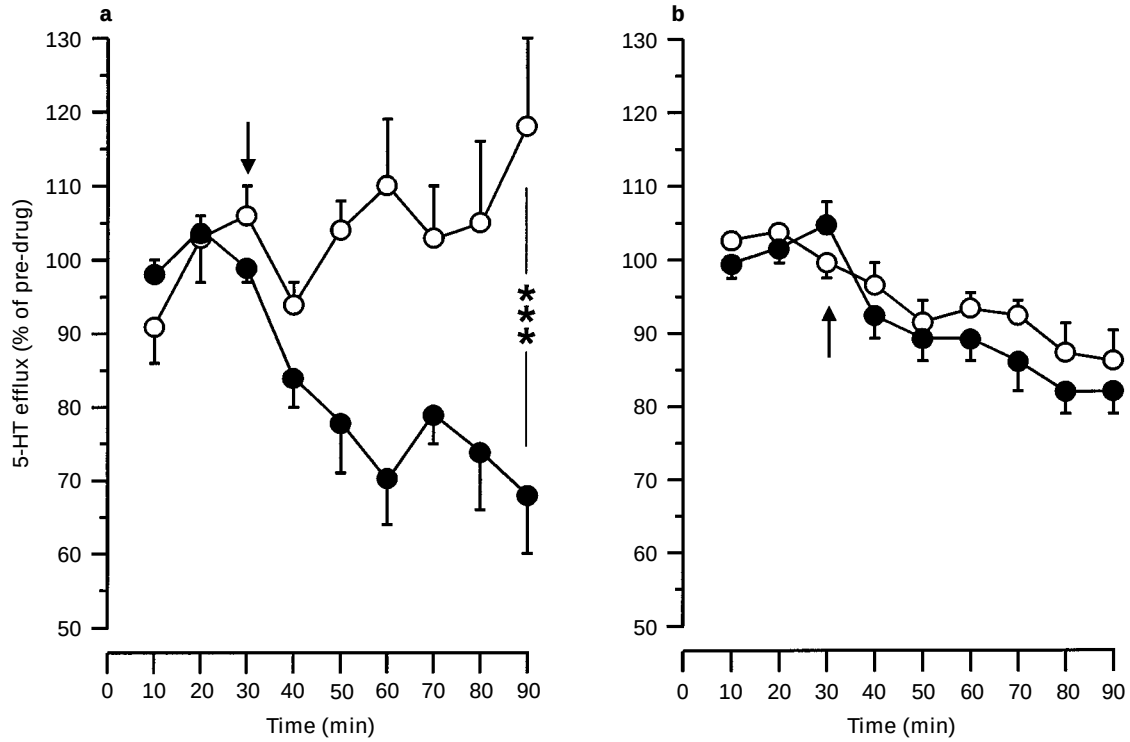

Figure 1 (+)-WAY 100135 decreases 5-HT efflux on pseudo single pulse stimulations in the vLGN. 5-HT efflux in the vLGN as a function of time in controls (○) and following (+)-WAY 100135 (1 μM) (●), given at the arrow, in untreated brain slices (a) and in the presence of 50 nM GR 127935 (b). ***$P<0.001$ vs controls (2 way ANOVA). Values are means and vertical lines show s.e.mean ($n=4-6$).

h5-HT$_{1B}$ receptors (pK_i = 5.8 ± 0.3) but had nearly 100 fold greater affinity at h5-HT$_{1D}$ receptors (pK_i = 7.6 ± 0.2; see Table 1). (+)-WAY 100135 therefore has virtually the same affinity for h5-HT$_{1D}$ receptors as it does for 5-HT$_{1A}$ receptors.

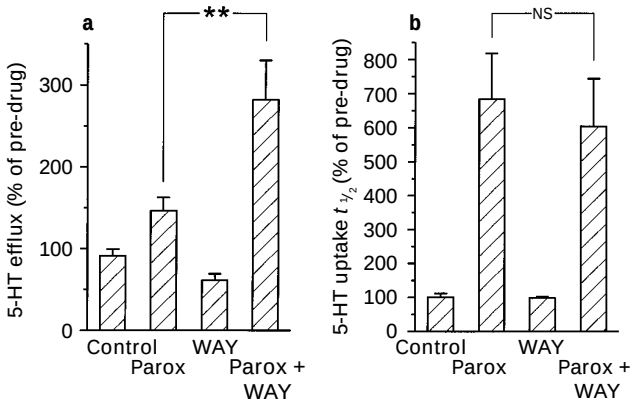

Figure 2 (+)-WAY 100135 potentiates the effect of paroxetine on 5-HT efflux in the vLGN. 5-HT efflux (a) and uptake $t_{½}$ (b) in controls and 120 min following addition of paroxetine (Parox, 100 nM), (+)-WAY 100135 (WAY 1 μM) or the combination. *P<0.05; NS: no significant difference between columns (Student-Newman-Keuls test). Values are means ± s.e.mean (n = 4).

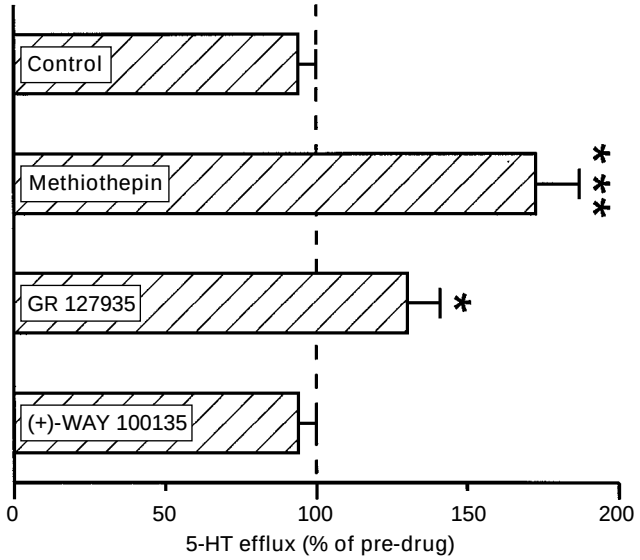

Figure 3 (+)-WAY 100135 has no effect on 5-HT efflux by long stimulations. 5-HT efflux (% of pre-drug values) on long stimulus trains (20 pulses, 20 Hz) in controls and 120 min following methiothepin (200 nM), GR 127935 (50 nM) or (+)-WAY 100135 (1 μM). *P<0.05, ***P<0.001 vs controls (Student-Newman-Keuls test). Values are means ± s.e.mean (n = 4).

In [^{35}S]-GTPγS binding experiments, (+)-WAY 100135 is a partial agonist at h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors

In both h5-HT$_{1B}$ and h5-HT$_{1D}$ receptor-expressing cell lines, 5-HT stimulated [^{35}S]-GTPγS binding in a concentration-dependent manner (Figure 4). 5-HT had similar potency at 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (pEC_{50} = 8.05 and 8.35, respectively) but produced a greater stimulation at h5-HT$_{1B}$ receptors (E_{max} = 260% of basal) than at h5-HT$_{1D}$ receptors (E_{max} = 134% of basal). (+)-WAY 100135 was a partial agonist at both receptor subtypes, with intrinsic activities of 0.46 and 0.44, respectively, relative to the 5-HT response in h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors. The pEC_{50} for (+)-WAY 100135 at h5-HT$_{1D}$ receptors correlated closely with its receptor binding affinity (Figure 4 and Table 1). WAY 100135 produced no response (neither stimulation nor inhibition of basal binding) in CHO cells which were not transfected with either 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors.

Discussion

The rat vLGN receives its main 5-hydroxytryptaminergic innervation from the dorsal raphe nucleus (Brauer *et al*., 1984). Although it is generally held that the 5-HT terminal autoreceptor in the rat is of the 5-HT$_{1B}$ subtype, it has recently been shown that the rat dorsal raphe nucleus contains significant 5-HT$_{1D}$ mRNA (Hamblin *et al*., 1992) and it is possible that some of this reflects receptors that may be expressed at the level of the terminals. In a recent study, we have shown that stimulated 5-HT efflux in the vLGN is under the control of both 5-HT$_{1B}$ and 5-HT$_{1D}$ autoreceptors (Davidson & Stamford, 1996). 8-Hydroxy-(-2-(di-n-propylamino)tetralin (8-OH-DPAT) and WAY 100635, selective 5-HT$_{1A}$ agonist and antagonist, respectively, have no effect (Davidson & Stamford, unpublished data), confirming, as expected, that 5-HT$_{1A}$ receptors do not exist on 5-HT terminals in the vLGN. WAY 100635 has no effect at the native 5-HT$_{1B/1D}$ autoreceptors in the vLGN nor at the recombinant h5-HT$_{1B}$ or h5-HT$_{1D}$ receptors used in this study (data not shown).

In the present study we used different stimulation parameters to unveil agonist and/or antagonist properties of (+)-WAY 100135 at the rat terminal 5-HT$_{1B/D}$ autoreceptor. On short stimulations (<200 ms duration), the 5-HT efflux event is effectively over before the 5-HT released can cause autoreceptor-mediated inhibition of further 5-HT efflux. This is 'pseudo single pulse' stimulation (Singer, 1988) and it is ideal for examining agonist effects. Conversely, stimulations long enough that the 5-HT released can act on the autoreceptor to inhibit further efflux may allow antagonist effects to be observed, but reduce the efficacy of agonists which must now compete with the endogenous ligand. We have also used an SSRI (paroxetine) which increases the concentration of 5-HT in the synapse causing tonic activation of the autoreceptor.

The present study strongly suggests that (+)-WAY 100135 may have partial agonist properties at the rat terminal 5-HT$_{1B/D}$ autoreceptor in the vLGN. Depending on the choice of stimulation parameters and conditions, (+)-WAY 100135 either has no effect or may behave in a manner similar to a 5-HT$_{1B/D}$ agonist or antagonist.

Table 1 Affinities and intrinsic activities of WAY 100135 on human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors

	h5-HT$_{1B}$ receptors			*h5-HT$_{1D}$ receptors*		
	Binding	*[^{35}S]-GTPγS*		*Binding*	*[^{35}S]-GTPγS*	
	pK_i	pEC_{50}	E_{max} (%)	pK_i	pEC_{50}	E_{max} (%)
5-HT	8.47 ± 0.02	8.05 ± 0.09	260 ± 30	8.42 ± 0.02	8.35 ± 0.09	134 ± 5
(+)-WAY 100135	5.82 ± 0.27	6.28 ± 0.15	174 ± 22	7.58 ± 0.20	7.61 ± 0.13	115 ± 3

E_{max} = % basal binding. Data are means ± s.e.mean; n = 3–4 independent experiments for each determination.

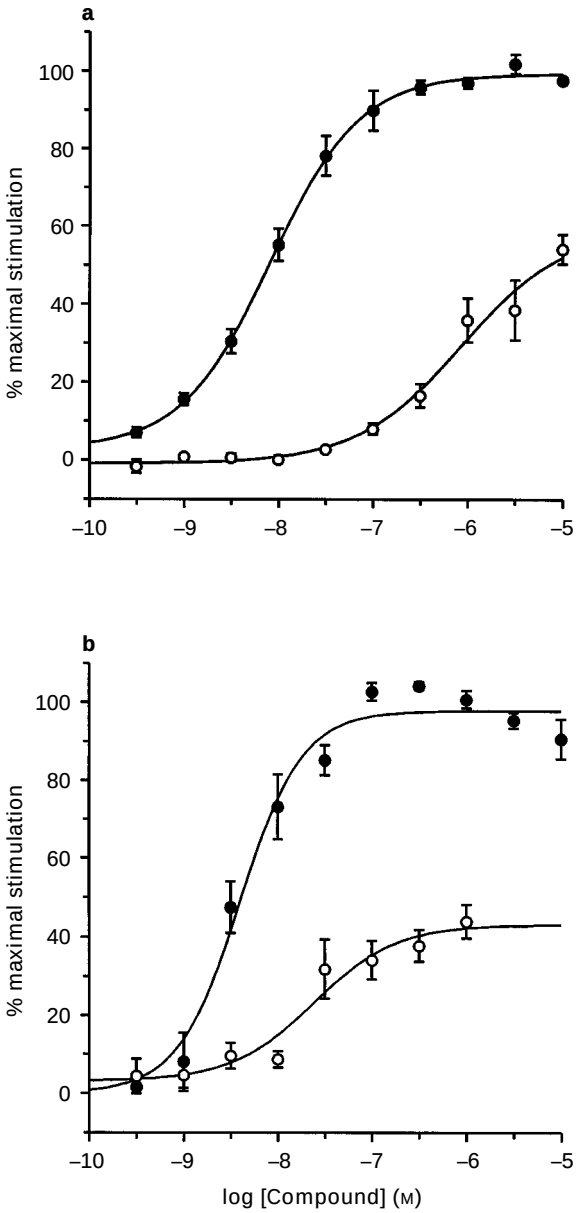

Figure 4 The effects of 5-HT and (+)-WAY 100135 on [^{35}S]-GTPγS binding to h5-HT$_{1B}$ (a) and h5-HT$_{1D}$ (b) receptors stably expressed in CHO cells. Stimulation of [^{35}S]-GTPγS binding by 5-hydroxytryptamine (●) and (+)-WAY 100135 (○) is expressed as a percentage of maximum with respect to 5-HT. Points are means and vertical lines show s.e.mean ($n=4$).

Under 'pseudo single pulse' conditions, (+)-WAY 100135 decreased 5-HT efflux, by 32%. This reduction was seen within 20–30 min, a time scale typical of the effects of an agonist in our stimulated brain slice paradigm (Davidson & Stamford, 1995). Furthermore, CP 94253, the 5-HT$_{1B}$ receptor agonist and 5-carboxamidotryptamine, the non-selective 5-HT$_1$ receptor agonist, also decreased 5-HT efflux (to 51 and 48% respectively on stimulations of 20 pulses at 100 Hz–data not shown) over a comparable timescale.

Although WAY 100135 has been shown to block the effects of 8-OH-DPAT on dorsal raphe cell firing (Mundey *et al.*, 1994), in some models WAY 100135 has been shown to have agonist activity at 5-HT$_{1A}$ receptors (Escandon *et al.*, 1994), an effect also manifested by the (+)-isomer (Assie & Koek, 1996). However, the agonist effect of (+)-WAY 100135 in our experiments cannot be mediated via 5-HT$_{1A}$ receptors, since, as stated above, the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT had no effect on 5-HT efflux at a concentration (1 μM) previously shown to inhibit 5-HT efflux in the dorsal raphe nucleus (Davidson & Stamford, 1995) and well above the concentration needed to inhibit raphe cell firing (Rigdon & Wang, 1991). Furthermore, the effect of (+)-WAY 100135 could be blocked by the selective 5-HT$_{1B/D}$ receptor antagonist, GR 127935 (Skingle *et al.*, 1995), suggesting that this agonist action is mediated via a 5-HT$_{1B}$ or 5-HT$_{1D}$ receptor. The modest decline in 5-HT efflux observed after GR 127935 may be an indication of a partial agonist action of GR 127935 itself at 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Pauwels & Palmier, 1995; Watson *et al.*, 1996).

Under circumstances where there was appreciable 'tone' at the autoreceptor, (+)-WAY 100135 acted as an antagonist. Such conditions were readily achieved by blockade of the 5-HT transporter with the SSRI paroxetine. Paroxetine (100 nM) increased 5-HT efflux and re-uptake half-life when given alone. In combination with (+)-WAY 100135, the effect of paroxetine on efflux was potentiated to a similar degree as previously seen with methiothepin (Davidson & Stamford, 1994). The absence of effect of (+)-WAY 100135 on 5-HT re-uptake means that the increased effect of paroxetine was not due to an enhancement of 5-HT uptake blockade.

When given alone, paroxetine presumably increases the amount of 5-HT in the synapse by blocking re-uptake sites. This increased synaptic and perisynaptic concentration of 5-HT then results in a tonic activation of the terminal 5-HT autoreceptors. Under these circumstances, an antagonist such as methiothepin blocks this counteracting tonic activation and unmasks the true effect of the uptake blocker on 5-HT efflux (Davidson & Stamford, 1995). The simplest explanation for the potentiation of paroxetine exhibited by (+)-WAY 100135 is that, whereas the drug is an agonist in circumstances of low intrinsic tone at the autoreceptor, it can act as an antagonist at the vLGN 5-HT autoreceptor under these conditions of high activation.

Interestingly, when examined on long stimulus trains, where one might expect an antagonist to have an effect, (+)-WAY 100135 appeared inactive, although both methiothepin and GR 127935 showed clear 5-HT$_{1B/D}$ receptor antagonism. This suggests that (+)-WAY 100135 only shows antagonist properties when the concentration of the endogenous ligand is high and that its antagonist effects are not readily seen when the concentration of endogenous ligand at the autoreceptor is low. On these long-train stimulations (20 pulses, 20 Hz) the peak extracellular concentration of 5-HT measured at the carbon fibre microelectrode was 15 ± 4 nM (mean$\pm$s.e.mean, $n=4$). However, with paroxetine present, the 5-HT concentration measured on pseudo single pulse stimulations was nearly 4 times greater (56 ± 16 nM). On long stimulations, the agonist and antagonist properties of (+)-WAY 100135 may cancel each other out. In other words (+)-WAY 100135 may behave as a partial agonist whose net effect is determined by the degree of endogenous 'tone' at the receptor.

In order to shed further light on the responses observed in the vLGN slices, we examined the activity of (+)-WAY 100135 at stably expressed human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. (+)-WAY 100135 has only modest affinity for cloned h5-HT$_{1B}$ receptors, similar to that observed for 5-HT$_{1B}$ receptors in native tissues (Fletcher *et al.*, 1993), but showed clear activity at 5-HT$_{1D}$ receptors. Due to the relatively low levels of expression of 5-HT$_{1D}$ receptors in native tissues (in virtually all CNS tissue 5-HT$_{1B}$ receptors predominate), and a lack of selective 5-HT$_{1D}$ receptor ligands, it is difficult to confirm the affinity and degree of partial agonism which would be displayed in native tissue. However, these data are consistent with the effects of (+)-WAY 100135 on vLGN 5-HT efflux. Strikingly, the vLGN has a relatively high density of 5-HT$_{1D}$ receptors compared with many other brain regions (Bruinvels *et al.*, 1993).

It is tempting therefore to speculate that the inhibition (or potentiation) of 5-HT efflux in the vLGN is preferentially mediated via 5-HT$_{1D}$ rather than 5-HT$_{1B}$ receptors. However, such a conclusion cannot be justified by the voltammetric data. In those experiments, (+)-WAY 100135 was used at a concentration of 1 μM. Although this concentration is forty fold

greater than the EC_{50} at h5-HT$_{1D}$ receptors, it is also still nearly twice the EC_{20} at the h5-HT$_{1B}$ receptor and thus, in a mixed autoreceptor population consisting mainly of 5-HT$_{1B}$ receptors, a significant action at 5-HT$_{1B}$ receptors cannot be excluded. It is also important to remember that there are significant pharmacological differences between human and rat 5-HT$_{1B}$ receptors (Hartig *et al*., 1996). Caution must therefore be used when extrapolating from data obtained at recombinant h5-HT$_{1B}$ receptors to native r5-HT$_{1B}$ autoreceptors.

In conclusion, we have shown that (+)-WAY 100135 can show both agonist and antagonist properties at 5-HT$_{1B/D}$ autroreceptors in a 5-HT terminal area, the vLGN. These effects are dependent on the conditions under which the drug is tested, in particular the stimulation protocol used. In circumstances where there is little endogenous tone at the autoreceptor, a compound such as (+)-WAY 100135 with low intrinsic activity will act as an agonist. On the other hand, when the receptor is activated by 5-HT, the compound may antagonize endogenous autoinhibition. Our studies on recombinant human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors further support the hypothesis that (+)-WAY 100135 acts as a partial agonist at one or other of these receptors. Although (+)-WAY 100135 had a higher potency at h5-HT$_{1D}$ than h5-HT$_{1B}$ receptors, one must be cautious in concluding that (+)-WAY 100135 is acting solely via 5-HT$_{1D}$ receptors in the rat vLGN, since there are recognised pharmacological differences between human and rat 5-HT$_{1B}$ receptors (Hartig *et al*., 1996). Although one might tentatively suggest that the effects we have observed are consistent with actions mediated via a 5-HT$_{1D}$ autoreceptor, an action at native rat 5-HT$_{1B}$ receptors cannot be excluded.

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