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LY367265, an inhibitor of the 5-hydroxytryptamine transporter and 5-hydroxytryptamine$_{2A}$ receptor antagonist: a comparison with the antidepressant, nefazodone

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Abstract

The potential antidepressant, LY367265 (1-[2-[4-(6-fluoro-1*H*-indol-3-yl)-3,6-dihydro-1(2*H*)-pyridinyl]ethyl]-5,6-dihydro-1*H*,4*H*-[1,2,5]thiadiazolo[4.3.2-ij]quinoline-2,2,-dioxide) has been shown to have a higher affinity for the 5-hydroxytryptamine (5-HT) transporter (K_i = 2.3 nM) and 5-HT$_{2A}$ (K_i = 0.81 nM) receptor than the clinically effective antidepressant, nefazodone. It is a potent inhibitor of [^{3}H]5-HT uptake into rat cortical synaptosomes (IC_{50} = 3.1 nM) and shows selectivity over that for [^{3}H]noradrenaline (IC_{50} > 1000 nM). It potentiates potassium-induced [^{3}H]5-HT outflow from prelabelled guinea pig cortical slices both in the presence (EC_{50} = 950 nM) and absence (EC_{50} = 250 nM) of a saturating concentration of the 5-HT transport inhibitor, paroxetine, indicating a low level of activity at the 5-HT$_{1B/1D}$ autoreceptor. These studies indicate that LY367265 is a putative antidepressant which, because of its 5-HT$_{2A}$ receptor antagonist activity, has the potential to produce less sleep disturbance and sexual dysfunction than selective serotonin uptake inhibitors.

Keywords: 5-HT transporter; 5-HT$_{2A}$ receptor; Nefazodone

1. Introduction

The introduction of selective serotonin reuptake inhibitors has led to a marked improvement in the treatment of depressive disorders by producing therapeutic benefit without the serious adverse side-effects associated with the tricyclic antidepressants (Montgomery, 1995). These compounds, however, are not without their own limitations. It has been reported (Thase and Rush, 1995) that up to 30% of patients fail to respond to first-line selective serotonin uptake inhibitor treatment, although this failure rate can be reduced when switching between treatments occurs. In those patients that do respond, side effects such as sexual dysfunction, sleep disturbances, nausea, anxiety and reduced appetite have been reported (Montgomery, 1995; Davis et al., 1997). Also, like other antidepressants, selective serotonin uptake inhibitors take 2 to 3 weeks before producing a clear therapeutic improvement (Montgomery, 1995).

It has been suggested that the lack of clinical efficacy of short-term administration of selective serotonin uptake inhibitors is due to the increase in synaptic concentrations of 5-hydroxytryptamine (5-HT), leading to stimulation of terminal presynaptic 5-HT$_{1B/1D}$ (Rollema et al., 1996; Marcoli et al., 1999; Middlemiss et al., 1999) or somatodendritic 5-HT$_{1A}$ autoreceptors (Casanovas et al., 1997). This results in an inhibition of 5-HT release and limits their ability to elevate synaptic concentrations of 5-HT. Therapeutic improvement is achieved only after these autoreceptors down-regulate. In support of this hypothesis, Perez et al. (1997) have reported that the 5-HT$_{1A}$ receptor antagonist, pindolol, increases the effectiveness of fluoxetine therapy.

Recently nefazodone has been introduced for the treatment of depression. This compound has a mechanism of

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action distinct from other currently available agents in that it has been reported to potently inhibit post-synaptic 5-HT_{2A} receptors and moderately inhibit serotonin and noradrenaline uptake (Eison et al., 1990). When administered to patients with major depression, it has been shown to have efficacy equivalent to that of selective serotonin uptake inhibitors (Baldwin et al., 1996; Feiger et al., 1996; Rioux et al., 1996) but to produce fewer sexual, gastrointestinal and sleep disturbances. This reduced incidence of side effects has been attributed to its 5-HT_{2A} receptor antagonist activity (Davis et al., 1997). In support of this claim, a number of studies have implicated 5-HT_2 receptors in sexual function and sleep disorders. For instance, stimulation of these receptors disrupts sexual function, whilst 5-HT_2 receptor antagonists can improve sexual behaviour in animals (Foreman et al., 1992). In addition, mirtazapine, an antidepressant with potent 5-HT_2 receptor antagonist activity, did not produce sexual dysfunction in patients who had discontinued previous selective serotonin uptake inhibitor treatment because of this side effect (Koutouvidis et al., 1999). It has been shown also to have a beneficial effect in the one-third of untreated depressed patients with sexual difficulties (Boyarsky et al., 1999).

The involvement of 5-HT_2 receptor inhibition in the reduced incidence of sleep disturbance observed with nefazodone is supported by the observation that the 5-HT_2 receptor antagonists, ritanserin and seganserin, produce an increase in slow wave sleep (SWS) and enhance slow wave activity (SWA) in humans and animals (Idzikowski et al., 1986; Borbély et al., 1988; Dijk et al., 1989). This effect is probably due to inhibition of the 5-HT_{2A} receptor as Landolt et al. (1999) have shown that the selective 5-HT_{2A} receptor antagonist, {*trans*, 4-[(3*Z*)3-(2-dimethylaminoethyl)oxyimino-3(2-flurophenyl)propen-1-yl]phenol hemifumarate} (SR 46349B), produced an increase in both SWS and SWA in healthy male volunteers with no influence on the amount and latency to rapid eye movement sleep. These effects are similar to those produced by sleep deprivation.

We report here the in vitro pharmacology of 1-[2-[4-(6-fluoro-1*H*-indol-3-yl)-3,6-dihydro-1(2*H*)-pyridinyl]ethyl]-5,6-dihydro-1*H*,4*H*-[1,2,5]thiadiazolo[4.3.2-ij]quinoline-2,2,-dioxide (LY367265) (Fig. 1), a potent antagonist of both the 5-HT_{2A} receptor and the 5-HT transporter.

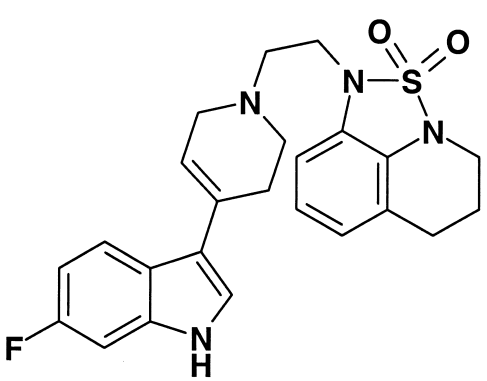

Fig. 1. Chemical structure of LY367265.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade. Radiolabelled 5-HT (5-hydroxy[G-^{3}H]tryptamine creatinine sulphate), 8-OH-DPAT (8-Hydroxy-[^{3}H]DPAT), *N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)-benzamide) GR125743 ([*N*-methyl-^{3}H]GR125743) were ob-tained from Amersham International and 2,5-dimethoxy-4-iodoamphetamine (DOI) ([^{125}I]-(+/−)DOI) from NEN Life Science Products. Imipramine, ketanserin, ritanserin, clozapine and methysergide were obtained from Sigma. Indalpine, nefazodone, amesergide and *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 (GR127935) were synthesised at the Lilly Research Centre and paroxetine was a gift from SmithKline Beecham. Depending on their aqueous solubility, test compounds were dissolved at 10 mM in deionised water or dimethyl sulphoxide (DMSO) and diluted to their final concentrations in the appropriate buffer. The maximum concentration of DMSO present in any of the assays was shown not to affect the results.

2.2. Binding to the 5-HT transporter in rat cortex

Male Lister Hooded rats (300–400 g) were killed by cervical dislocation and their brains rapidly removed. Cortical tissue was homogenised in 40 vol. assay buffer (50 mM Tris–HCl buffer pH 7.4), centrifuged at 40,000 × *g* for 10 min at 4°C and the membranes washed and then resuspended in 40 vol. assay buffer. Following incubation at 37°C for 20 min to remove endogenous 5-HT, and two further centrifugations as previously described, the membranes were suspended in 20 vol. assay buffer containing 150 mM NaCl and 5 mM KCl and the protein concentration estimated (Lowry et al., 1951). Membranes prepared in this manner could be stored at −70°C for up to 1 week.

Competition studies were performed by incubating 150 μg protein in 1.0 ml of assay buffer containing 150 mM NaCl, 5 mM KCl, 0.2 nM [^{3}H]citalopram and appropriate concentrations of the competing ligand. Non-specific binding was defined using 10 mM fluoxetine. Samples were incubated at 37°C for 90 min, filtered through GF/B filters pre-soaked in assay buffer containing 0.1% (w/v) polyethylenimine. The filters were washed five times with assay buffer, dried, and the bound tritium determined by liquid scintillation spectrometry. The results were analysed using an automatic spline-fitting program and K_i values

determined from the IC_{50} data using the Cheng–Prusoff equation (Cheng and Prusoff, 1973) and a K_d value for [^{3}H]citalopram binding of 2.5 nM (determined by Scatchard analysis. Data not shown).

2.3. Binding to human 5-HT_{2A} receptors

Hm2.3 cells stably transfected with the human 5-HT_{2A} receptor were harvested by centrifugation at $2200 \times g$ and the cell pellet frozen at -70°C until required.

Cell pellets were homogenized in an equal volume of phosphate-buffered saline and, after centrifugation at $40{,}000 \times g$ for 10 min at 4°C, re-homogenized in 10 vol. (original cell pellet volume) of assay buffer (50 mM Tris–HCl containing 0.5 mM EDTA pH 7.4). After centrifugation at $1000 \times g$ for 10 min, the supernatant was centrifuged at $40{,}000 \times g$ for 20 min at 4°C, the resulting pellet resuspended in assay buffer (1 ml per 300 million cells; 3–4 mg protein/ml) and stored in liquid nitrogen for up to 4 weeks.

Competition studies were performed in 0.25 ml of assay buffer containing 50 μg protein, 0.4 nM [^{3}H]ketanserin and appropriate concentrations of the competing ligand. Non-specific binding was defined using 10 mM methysergide. Samples were incubated at 37°C for 30 min followed by filtration through GF/B filters pre-soaked in 50 mM Tris–HCl containing 0.1% (w/v) polyethylenimine; pH 7.4. The filters were washed with 50 mM Tris–HCl pH 7.4 at 4°C, dried and the bound tritium determined by liquid scintillation spectrometry. The results were analysed using an automatic spline-fitting program and K_i data determined from the IC_{50} values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973) and a K_d value for [^{3}H]ketanserin binding of 0.5 nM (determined by Scatchard analysis. Data not shown).

2.4. Receptor selectivity

Membranes from LM (tk-) cells stably transfected with either the human 5-HT_{1B} or 5-HT_{1D} receptors were produced using the method described for the human 5-HT_{2A} binding assay and were stored in liquid nitrogen for up to 4 weeks.

Competition studies were performed in 0.25 ml buffer (50 mM Tris–HCl containing 0.5 mM EDTA, 10 mM $MgSO_4$, 5.7 mM ascorbic acid, 16 μM pargyline, pH 7.75) containing 150 μg membrane protein, 2 nM [^{3}H]GR125743 and appropriate concentrations of the competing ligand. Non-specific binding was defined using 100 μM 5-HT. Samples were incubated at 37°C for 30 min followed by filtration through GF/B filters pre-soaked in 50 mM Tris–HCl containing 0.1% (w/v) polyethylenimine; pH 7.4. The filters were washed with 50 mM Tris–HCl pH 7.4 at 4°C, dried and the bound tritium determined by liquid scintillation spectrometry. The results were analysed using an automatic spline-fitting program and K_i data determined from the IC_{50} values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973) and a K_d value for [^{3}H]GR125743 binding of 2.0 nM for each receptor (determined by Scatchard analysis. Data not shown).

Standard receptor-binding assay methods were used to evaluate the ability of LY367265 to interact with 5-HT_{1A} (Wong et al., 1991), 5-HT_{2B} and 5-HT_{2C} (Wainscott et al., 1996) receptors. The source of the receptor and the ligand used are shown in Table 3.

2.5. [^{3}H]5-HT and [^{3}H]noradrenaline uptake into rat cortical synaptosomes

Male Lister–Hooded rats (180–250 g) were killed by asphyxiation with carbon dioxide and cervical dislocation followed by decapitation. The cerebral cortex was homogenised in 20 vol. ice-cold sucrose (0.32 M), centrifuged at $2000 \times g$ for 10 min at 4°C and the supernatant recentrifuged for 20 min at $12{,}500 \times g$. The pellet was resuspended in ice-cold 0.32 M sucrose, layered onto 0.8 M sucrose and centrifuged at $12{,}500 \times g$ for 30 min. The pellet was resuspended in 8 vol. (original wet weight) ice-cold assay buffer pH 7.4 (10 mM HEPES, 133 mM NaCl, 4.85 mM KCl, 1.2 mM KH_2PO_4, 1.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 11.1 mM glucose, 10 mM pargyline).

Test compounds (10 mM) in DMSO were diluted with assay buffer and tested over a 5 log unit concentration range in duplicate. An aliquot (25 μl) of either the diluted test compound, assay buffer (total uptake) or 100 μM paroxetine (5-HT non-specific uptake) or 100 μM nomifensine (noradrenaline non-specific uptake) were incubated at 37°C for 15 min. After the addition of the synaptosome preparation (50 μl) the incubation was continued for a further 15 min. Following the addition of 175 μl [^{3}H]5-HT (71.4 nM) or [^{3}H]noradrenaline (71.4 nM) to each well, the incubation continued for a third period of 15 min. The uptake of the [^{3}H]5-HT or [^{3}H]noradrenaline into the synaptosomes was stopped by filtration through GF/B filters presoaked in 0.9% (w/v) NaCl containing 0.1% (w/v) polyethylenimine. The filters were dried and the radioactivity determined by scintillation spectroscopy. The results were analysed using an automatic spline-fitting program and IC_{50} values determined.

2.6. GTPγ[^{35}S] binding to human 5-HT_{1B} and 5-HT_{1D} receptors

2.6.1. Membrane preparation

LM (tk-) or AV12 cells stably tranfected with human 5-HT_{1B} or 5-HT_{1D} receptors, respectively, were washed with and then homogenised in Tris/EDTA buffer (20 mM Tris–HCl, 5 mM EDTA pH 7.4). After centrifugation at $1500 \times g$ for 10 min at 4°C, the supernatant was recentrifuged at $40{,}000 \times g$ for 18 min at 4°C and the resultant pellet washed with Tris/EDTA buffer and finally resus-

pended in assay buffer (100 mM NaCl, 10 mM $MgCl_2$, 1.0 mM EDTA and 20 mM HEPES, pH 7.4). The protein concentration was measured (Lowry et al., 1951) adjusted with assay buffer to 400 μg/ml for the 5-HT_{1B} containing membranes and 600 μg/ml for the 5-HT_{1D} before storage at −70°C for up to 6 weeks.

2.6.2. 5-HT_{1B} GTPγ[^{35}S] assay

All reagents were diluted in assay buffer containing 167 μg/ml dithiothreitol. Membranes from the LM (tk-) cells (10 μg), in a final volume of 250 μl, were pre-incubated for 30 min at 30°C with GDP (10 μM) and 5-HT (0 to 10 μM) in the presence and absence of 300 nM test compound. Following the addition of GTPγ[^{35}S] (0.2 nM) and 1.5 mg wheatgerm agglutinin treated polyvinyltoluene Scintillation Proximity Assay beads, the incubation was continued for a further 1 h before centrifugation at 650 × *g* for 10 min at 4°C. The radioactivity in close proximity to the beads was assessed by scintillation spectroscopy. K_b values for the test compounds were calculated from the dextral shift of the 5-HT dose–response curve.

2.6.3. 5-HT_{1D} GTPγ[^{35}S] assay

This assay was essentially the same as that for 5-HT_{1B} GTPγ[^{35}S] with the following exceptions. Each incubation contained 15 μg membrane protein from the 5-HT_{1D} expressing AV12 cells in 250 ml of assay buffer containing no dithiothreitol.

2.7. [^{3}H]5-HT outflow from guinea pig cortical slices

The assay was essentially similar to that described previously (Harvey et al., 1996). Male guinea pigs (350–400 g) were killed by asphyxiation with carbon dioxide and their brains rapidly removed. Cortical slices (350 μm × 350 μm) were prepared, washed once in basal buffer (10 mM HEPES, 133 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 11.1 mM glucose, 10 μM pargyline pH 7.4) and incubated in basal buffer at 25 mg wet weight per ml with [^{3}H]5-HT (50 nM) for 30 min at 37°C. The slices were washed three times in basal buffer and transferred to baskets (10 mm i.d. polypropylene tubes with 150 mm nylon mesh bases) at approximately 5 mg wet weight per basket. The baskets were used to transfer the tissue between the washing and release buffers.

In order to obtain a stable baseline release, the slices were incubated for 11 min in basal buffer (0.5 ml), transferred for 4 min to a second tube containing basal buffer (0.5 ml) and then, for a further 4 min, to basal buffer (0.5 ml) or to a buffer in which NaCl had been substituted with KCl, on an equimolar basis, to give a KCl concentration of 30 mM (release sample). In experiments in which the 5-HT transporter had been blocked, all the buffers used in the 11-min and the two 4-min incubations contained 1-μM paroxetine. Following the incubations, the tissue was digested with soluene-350 (0.7 ml) and the baskets rinsed with 0.7-ml propan-2-ol. The tritium in the tissue samples and in the buffers from the three incubation periods was estimated by liquid scintillation spectroscopy. The compounds being tested were present throughout the three incubation periods and were each tested in six replicates. The basal release was measured in four replicates and the control release in eight replicates. The 30 mM potassium-induced [^{3}H]5-HT outflow was greater than 90% calcium dependent regardless of whether 1 μM paroxetine was present in the incubation medium.

The tritium in the release sample was expressed as the percentage of the total tritium in the tissue at the time the sample was collected (% fractional release). Stimulated release was calculated as the % fractional release produced by the high potassium buffer (16.63 ± 0.98 and 9.18 ± 0.61 in the presence and absence of 1 μM paroxetine, respectively) minus that produced by the basal buffer (4.08 ± 0.25 and 2.98 ± 0.25 in the presence and absence of 1 μM paroxetine, respectively) over the same time interval. The percentage increase in release produced by the compound was calculated as the increase over the control stimulated release, where the control release is 100%. For individual experiments the mean of the replicate data was calculated. The results are the means and standard errors of at least three separate experiments.

3. Results

3.1. Inhibition of the 5-HT and noradrenaline transporters

As can be seen from Table 1, LY367265, like the selective serotonin uptake inhibitors fluoxetine, indalpine and paroxetine, has a higher affinity for the rat 5-HT transporter than nefazodone or imipramine. It is also a

Table 1
Receptor binding affinities (K_i, nM) for the 5-HT transporter and inhibition of [^{3}H]5-HT and [^{3}H]noradrenaline uptake (IC_{50}, nM) into rat brain synaptosomes

Compound	[^{3}H]Citalopram binding K_i (nM)	[^{3}H]5-HT uptake into synaptosomes IC_{50} (nM)	[^{3}H]Noradrenaline uptake into synaptosomes IC_{50} (nM)
LY 367265	2.3 ± 0.3	3.1 ± 1.6	> 1000
Fluoxetine	6.2 ± 1.0	35 ± 5	> 1000
Paroxetine	0.093 ± 0.008	0.7 ± 0.2	180 ± 18
Indalpine	2.0 ± 0.1	6.5 ± 1.3	NT
Nefazodone	120 ± 20	290 ± 60	300 ± 44
Imipramine	51 ± 0.4	36 ± 9	140 ± 20

Data expressed as mean ± S.E.M. of at least three independent experiments.
NT = Not Tested.

Table 2
Affinity (K_i, nM) of LY367265 and comparators for the human 5-HT_{2A} receptor

Compounds	K_i (nM)
LY367265	0.81 ± 0.07
Ketanserin	0.87 ± 0.13
Ritanserin	0.57 ± 0.14
Clozapine	4.7 ± 0.18
Amesergide	25 ± 3.9
Nefazodone	5.8 ± 0.22

Data expressed as mean ± S.E.M. of at least three independent experiments.

potent inhibitor of [^{3}H]5-HT uptake into rat brain synaptosomes.

Unlike nefazodone and imipramine, fluoxetine and paroxetine as well as LY367265 are much more effective at inhibiting the 5-HT transporter than that for noradrenaline (Table 1).

3.2. Receptor profile

LY367265 has a similar affinity for the human 5-HT_{2A} receptor expressed in AV12 cells to that of the potent 5-HT_{2A} receptor antagonists ketanserin and ritanserin and has six to seven times higher affinity than the antipsychotic, clozapine, or the antidepressant, nefazodone (Table 2). The 5-HT_2 receptor antagonist, amesergide, has a 30-times lower affinity for the 5-HT_{2A} receptor than LY367265.

LY367265 has been assessed for its ability to interact with other serotinergic receptors. As can be seen from Table 3, it has at least a 28 times lower affinity for these receptors than for the human 5-HT_{2A} receptor (Table 3).

3.3. GTPγ[^{35}S] binding to human 5-HT_{1B} and 5-HT_{1D} receptors

GR127935 gave a K_b at the 5-HT_{1B} receptor of 0.19 ± 0.06 nM and at the 5-HT_{1D} receptor of 0.10 ± 0.02 nM whilst LY367265, like nefazodone, was inactive at both receptors (Table 4).

Table 3
The receptor type, species, tissue used in the 5-HT receptor binding assays and the affinity (K_i, nM) of LY367265 for these receptors

Receptor	Species	Tissue	Radioligand	K_i (nM)
5-HT_{1A}	Rat	Cerebral cortex	[^{3}H]8-OH-DPAT	490 ± 80
5-HT_{1B}	Human	Transfected LM (tk-)	[^{3}H]GR125743	490 ± 44
5-HT_{1D}	Human	Transfected L M (tk-)	[^{3}H]GR125743	81 ± 10
5-HT_{2A}	Human	Transfected Hm2.3	[^{3}H]ketanserin	0.81 ± 0.07
5-HT_{2B}	Human	Transfected AV12	[^{3}H]5-HT	23 ± 6
5-HT_{2C}	Human	Transfected AV12	[^{125}I]DOI	32 ± 2

Data expressed as mean ± S.E.M. of at least three independent experiments.

Table 4
K_b value (nM) for the inhibition of 5-HT-stimulated [^{35}S]-GTPγS binding to cells stably transfected with human 5-HT_{1B} or 5-HT_{1D} receptors

Compound	5-HT_{1B} receptor K_b (nM)	5-HT_{1D} receptor K_b (nM)
LY367265	> 1000	> 1000
GR127935	0.19 ± 0.06	0.10 ± 0.02
Nefazodone	> 1000	> 1000

Data expressed as mean ± S.E.M. of at least three independent experiments.

3.4. [^{3}H]5-HT outflow from guinea pig cortical slices

Paroxetine and LY367265 produced an increase in the potassium-induced [^{3}H]5-HT outflow from guinea pig cortical slices with their relative potencies (EC_{50} = 50 nM and 250 nM, respectively) in keeping with their ability to inhibit the 5-HT transporter (Fig. 2). Fluoxetine was less active and did not reach a maximum potentiation of [^{3}H]5-HT outflow at the highest concentration tested. If it is assumed that fluoxetine is able to produce a maximum potentiation similar to that of paroxetine and LY367265, an EC_{50} value of about 2.5 μM is obtained, giving a relative potency similar to that obtained for the inhibition of [^{3}H]5-HT uptake into synaptosomes (Table 1). Nefazodone produced only a very small increase of 23% at 10 μM. Concentrations of test compound greater than 10 μM could not be used in this assay as they produced an increase in the basal, non-stimulated, outflow of [^{3}H]5-HT. The potentiation produced by LY 367265 was due not only to its inhibition of the 5-HT transporter as it was still able to induce an increase of [^{3}H]5-HT outflow (EC_{50} = 950

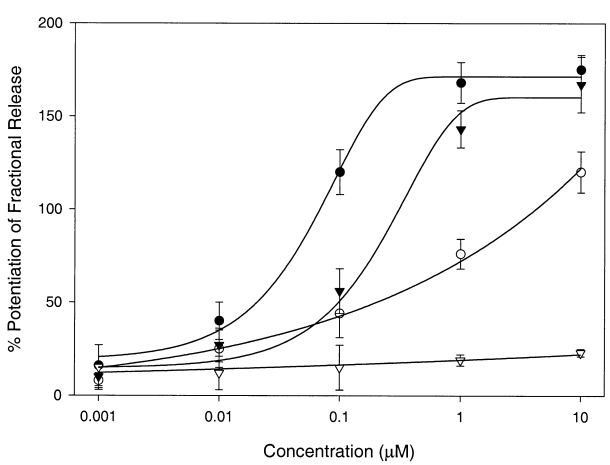

Fig. 2. Potentiation of potassium-induced [^{3}H]5-HT outflow from guinea pig cortical slices. The potentiation in each experiment is expressed as the percentage increase in fractional release above that obtained in the absence of compound. Paroxetine (●), LY367265 (▼), fluoxetine (○) and nefazodone (▽) were tested in log concentration increments from 1 nM to 10 μM and each data point is the mean of, at least, five experiments. All curves are fitted with a four-parameter logistic equation.

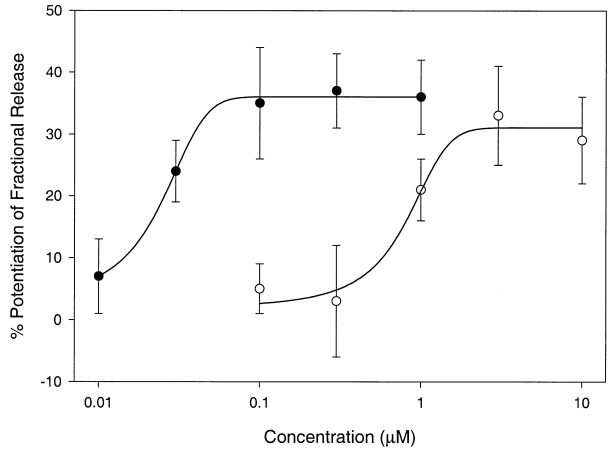

Fig. 3. Potentiation of potassium-induced [^{3}H]5-HT outflow from guinea pig cortical slices in the presence of 1.0 μM paroxetine. The potentiation in each experiment is expressed as the percentage increase in fractional release above that obtained in the absence of compound. GR127935 (●) and LY367265 (○) were tested in log concentration increments and each data point is the mean of, at least, five experiments. All curves are fitted with a four-parameter logistic equation.

nM) when the 5-HT transporter was inhibited by a maximally effective concentration (1 μM) of paroxetine (Fig. 3). Under the same conditions, fluoxetine, at 1 μM, was inactive (3% potentiation. Results not shown). This potentiation of [^{3}H]5-HT outflow when the 5-HT transporter is fully blocked, may be due to inhibition of the presynaptic 5-HT$_{1B}$ or 5-HT$_{1D}$ autoreceptor since the mixed 5-HT$_{1B/1D}$ receptor antagonist GR127935 has a similar, although more potent, effect (EC_{50} = 25 nM);(Fig. 3).

4. Discussion

The potential antidepressant LY367265 is a potent, inhibitor of the 5-HT transporter with an IC_{50} value for the uptake of [^{3}H]5HT into rat brain cortical synaptosomes about one tenth that of fluoxetine. Like fluoxetine, it has a high degree of selectivity for this transporter over that for noradrenaline (> 300 times). As would be expected from an inhibitor of the 5-HT reuptake, LY367265 potentiates the potassium-stimulated outflow of [^{3}H]5-HT from prelabelled guinea pig cortical slices with a potency, relative to fluoxetine, in keeping with their activities on synaptosomes. Unlike in the case of fluoxetine, however, this potentiation of [^{3}H]5-HT outflow by LY367265 is not fully inhibited by a maximally effective concentration of the selective serotonin uptake inhibitor, paroxetine. Under the same conditions, the 5-HT$_{1B/1D}$ receptor antagonist, GR127935, in common with selective 5-HT$_{1B}$ or 5-HT$_{1D}$ receptor antagonists (data not shown), potentiates the potassium-stimulated outflow of 5-HT from guinea pig cortical slices. This effect has been ascribed to inhibition of inhibitory 5-HT autoreceptors (Bühlen et al., 1996). LY367265 has a moderate affinity for the 5HT$_{1D}$ receptor ($K_i = 81 \pm 10$ nM) and a lower affinity for the 5HT$_{1B}$ receptor ($K_i = 490 \pm 44$ nM). Although this affinity would indicate a very low level of activity at these receptors, there is evidence (Matzen et al., 2000) that, with compounds structurally related to LY367265, the affinity in binding assays does not reflect function. For this reason we evaluated the ability of LY367265 to inhibit 5-HT-stimulated GTPγS binding to human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor-expressing cell lines. At a concentration of 300 nM, it failed to show any antagonist activity on either cell line. At 1 μM, however, it has been shown (M.L. Cohen, personal communication) that LY367265 produces a parallel dextral shift of the concentration response curve to the 5-HT$_{1B/1D}$ receptor agonist, sumatriptan, in the rabbit saphenous vein. This suggests that it is a competitive antagonist of the 5-HT$_{1D\text{-like}}$ receptor mediating contractile responses in this tissue (Cohen and Schenck, 1999). This activity may be responsible for the potentiation of 5-HT release obtained with LY367265. Although, for the reasons set out previously, a compound possessing presynaptic 5-HT$_{1D}$ or 5-HT$_{1B}$ receptor antagonism as well as 5-HT transporter inhibition may produce an earlier onset of antidepressant efficacy, it is unlikely that the low level of 5-HT$_{1D}$ or 5-HT$_{1B}$ receptor antagonism in this compound will be of functional significance at clinically effective antidepressant doses.

In addition to potent inhibition of the 5-HT transporter, LY367265 has a high affinity for the human 5-HT$_{2A}$ receptor and has been shown by its ability to inhibit DOI-induced hyperthermia in guinea pigs to be an antagonist at this receptor (N.A. Moore, personal communication). This activity, however, does not explain the observed potentiation of [^{3}H]5-HT outflow from prelabelled guinea pig cortical slices in the presence of paroxetine as we have found that the 5-HT$_{2A}$ receptor antagonist, MDL100907, is inactive in this paradigm (data not shown).

Selective serotonin uptake inhibitors have been reported to produce undesirable side effects, the most prominent of which are sexual dysfunction and sleep disturbances (Montgomery 1995; Davis et al., 1997). These have been attributed to stimulation of the 5-HT$_{2A}$ receptor. This is supported by the observation that 5-HT$_2$ receptor antagonists improve sexual behaviour in rats (Foreman et al., 1992) and reverse the inhibition of sexual behaviour produced by the 5-HT$_2$ receptor agonist DOI (Klint and Larsson, 1995; Watson and Gorzalka, 1991). In rats, the dose-related increase in wakefulness and decrease in deep slow wave sleep, produced by the 5-HT$_2$ receptor agonist, (−)-2,5-dimethoxy-4-methyl-amphetamine (DOM), are reversed by pretreatment with the 5-HT$_2$ receptor antagonists, ritanserin and cinanserin (Dugovic et al., 1989). It has also been shown (Landolt et al., 1999) that the selective 5-HT$_{2A}$ receptor antagonist, SR49349B, increases slow-wave sleep when given to human subjects 3 h before bed time.

In addition to a reduced incidence of side-effects, the presence of 5-HT_{2A} receptor antagonist activity may contribute towards an antidepressant activity. For instance, it has been shown (Hrdina et al,. 1993) that the number of 5-HT_2 binding sites is significantly increased in the prefrontal cortex and amygdala from suicide depressives and a similar finding has been reported for 5-HT_2 receptors in platelets (Hrdina et al 1995). Although others have failed to observe differences in the number of 5-HT_2 binding sites in depressed patients (Attar-Levy et al., 1999; Lowther et al., 1994; Meyer et al., 1999), there seems to be general agreement that antidepressant treatment reduces 5-HT_2 receptor binding (Attar-Levy et al., 1999; Deakin, 1988; Yatham et al., 1999). Clinical experience also supports the view that 5-HT_2 antagonism may produce an antidepressant effect in that the selective 5-HT_2 receptor antagonist, ritanserin, has been shown to have some antidepressant efficacy in a number of studies (Murphy et al., 1995; Stefanski and Goldberg, 1997).

Nefazodone, is a potent 5-HT_{2A} receptor antagonist with moderate inhibition of 5-HT and noradrenaline uptake (Davis et al., 1997). It is an effective antidepressant (Victor and Vieweg, 1998) and has been reported to improve depression-related insomnia (Fontaine et al., 1994; Rickels et al., 1994) and to lead to a lower incidence of sleep disturbances than selective serotonin uptake inhibitors (Rush et al., 1998). In a multicentre trial, Feiger et al. (1996) showed that, unlike the selective serotonin uptake inhibitor sertraline, nefazodone did not adversely effect sexual function. This compound has only a low affinity for the 5-HT transporter ($K_i = 120 \pm 20$ nM: K_i for fluoxetine $= 6.2 \pm 1.0$ nM) and this is reflected in the high clinically effective dose for the treatment of major depression of 300–500 mg/day (Victor and Vieweg, 1998). Its affinity for the 5-HT_{2A} receptor ($K_i = 5.8 \pm 0.22$ nM) is more than 20 times that for the 5-HT transporter. It is likely, therefore, that the dose required to produce a maximum effect at 5-HT_{2A} receptors will be well below the therapeutic dose for antidepressant activity. With LY367265, the affinity for the 5-HT_{2A} receptor is less than three times that for the transporter. At any single dose, therefore, receptor occupancy of the 5-HT_{2A} receptor and the 5-HT transporter will be more nearly equal than with nefazodone.

It is concluded that LY367265 should be an effective antidepressant producing fewer sleep disturbances and less sexual dysfunction than selective serotonin uptake inhibitors. As it has a higher affinity for the 5-HT transporter than does nefazodone, the clinical dose would be predicted to be smaller.

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