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European Journal of Pharmacology 493 (2004) 85–93

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The role of the 5-HT_{1D} receptor as a presynaptic autoreceptor in the guinea pig

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Received 6 November 2003; received in revised form 15 April 2004; accepted 20 April 2004

Abstract

The present study investigated the role of the 5-hydroxytryptamine (5-HT, serotonin)$_{1D}$ receptor as a presynaptic autoreceptor in the guinea pig. In keeping with the literature, the 5-HT_{1B} selective antagonist, 1′-methyl-5-[[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro [furo[2,3-f]indole-3,4′-piperidine]oxalate (SB224289) potentiated [^{3}H]5-HT outflow from pre-labelled slices of guinea pig cerebral cortex confirming its role as a presynaptic autoreceptor in this species. In addition, the 5-HT_{1D} receptor-preferring antagonists, 1-[2-[4-(6-fluoro-1*H*-indol-3-yl)-3,6-dihydro-2*H*-pyridin-1-yl]-ethyl]-3-pyridin-4-yl-methyl-tetrahydro-pyrimidin-2-one (LY367642), (*R*)-1-[2-(4-(6-fluoro-1*H*-indol-3-yl-)-3,6-dihydro-1(2*H*)-pyridinyl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-6-carboxamide (LY456219), (*S*)-1-[2-(4-(6-fluoro-1*H*-indol-3-yl-)-3,6-dihydro-1(2*H*)-pyridinyl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-6-carboxamide (LY456220) and 1-[2-[4-(4-fluoro-benzoyl)-piperidin-1-yl]-ethyl]-3,3-dimethyl-1,2-dihydro-indol-2-one (LY310762), potentiated [^{3}H]5-HT outflow from this preparation with potencies (EC_{50} values = 31–140 nM) in the same range as their affinities for the guinea pig 5-HT_{1D} receptor (K_i values = 100–333 nM). The selective 5-HT_{1D} receptor agonist, *R*-2-(4-fluoro-phenyl)-2-[1-[3-(5-[1,2,4]triazol-4-yl-1*H*-indol-3-yl)-propyl]-piperidin-4-ylamino]-ethanol dioxylate (L-772,405), inhibited [^{3}H]5-HT outflow. In microdialysis studies, administration of either SB224289 or LY310762 at 10 mg/kg by the intraperitoneal (i.p.) route, potentiated the increase in extracellular 5-HT concentration produced by a maximally effective dose of the selective serotonin re-uptake inhibitor, fluoxetine (at 20 mg/kg i.p.). In addition, the 5-HT_{1D} receptor-preferring antagonist and 5-HT transporter inhibitor, LY367642 (at 10 mg/kg i.p.), elevated extracellular 5-HT concentrations to a greater extent than a maximally effective dose of fluoxetine. It is concluded that the 5-HT_{1D} receptor, like the 5-HT_{1B} receptor, may be a presynaptic autoreceptor in the guinea pig.

Keywords: 5-HT autoreceptor; 5-HT_{1B} receptor; 5-HT_{1D} receptor; Neurotransmitter release; Selective serotonin re-uptake inhibitor; Antidepressant

1. Introduction

The 5-HT_{1B} receptor has been shown to be a presynaptic autoreceptor controlling transmitter release in the rat (Engel et al., 1986), guinea pig (Selkirk et al., 1998; Bühlen et al., 1996) and human (Fink et al., 1995; Marcoli et al., 1999; Middlemiss et al., 1999). The role of the 5-HT_{1D} receptor is, however, less well defined. In the raphé nucleus of the guinea pig, the mRNA for the 5-HT_{1D} receptor is localised in cell bodies containing mRNA for the serotonin transporter (Bonaventure et al., 1998a,b). These results indicate that the 5-HT_{1D} receptor may function as an autoreceptor regulating serotonin neurotransmission in this species. In human post-mortem brain tissue, 5-HT_{1D} mRNA was found in the pyramidal cell layer of the hippocampal CA3 field (Pasqualetti et al., 1996). These authors, however, restricted their investigations to the prefrontal, parietal and occipital cortex, hippocampus, caudate nucleus and insula-claustrum-putamen. In a more recent study, Bidmon et al. (2001) demonstrated 5-HT_{1B} and 5-HT_{1D} mRNA within subdivisions of the dorsal raphé nucleus and concluded that both receptors may serve as autoreceptors in man. Attempts to show the presence of 5-HT_{1D} receptors in mammalian brain

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doi:10.1016/j.ejphar.2004.04.029

have been less successful. Bonaventure et al. (1998b) used autoradiography to study the binding of the 5-$HT_{1B/1D}$ receptor ligand, [^{3}H]alniditan, to guinea pig brain slices in the presence and absence of the 5-HT_{1D} receptor antagonist, ketanserin. They were unable to detect any evidence of binding to 5-HT_{1D} receptors in any of the brain areas studied. More recently, Varnäs et al. (2001) used whole hemisphere autoradiography to study the distribution of 5-HT_{1B} and 5-HT_{1D} receptors in the human brain with *N*-[4-[^{3}H]methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide ([^{3}H]GR 125743). Although the ligand was suitable for examining the distribution of 5-HT_{1B} receptors, the selectivity of ketanserin and SB 224289 precluded definitive observation of 5-HT_{1D} receptors.

Demonstration of 5-HT_{1D} receptor function has also been equivocal. The 5-HT_{1D} receptor-selective antagonist, 3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol (BRL15572; Price et al., 1966) did not affect 5-HT release from guinea pig cortical slices (Schlicker et al., 1997) nor from synaptosomes prepared from human cortical biopsy material (Marcoli et al., 1999). These latter authors, however, demonstrated that BRL15572 blocked the 5-HT-induced inhibition of potassium-stimulated glutamate release from the same preparation and concluded that the 5-HT_{1D} receptor was not a presynaptic autoreceptor in the human cortex but was a heteroreceptor on glutamatergic terminals where it inhibited glutamate release. There is, however, evidence that the 5-HT_{1D} receptor can operate as an autoreceptor controlling 5-HT release. Davidson and Stamford (1995) have shown that the 5-$HT_{1B/D}$ receptor agonist, sumatriptan, inhibited electrically stimulated 5-HT release from the rat dorsal raphé nucleus and that this effect was not reversed by the 5-HT_{1B} receptor-selective antagonist, isamoltane. More recently, several authors have confirmed the involvement of the 5-HT_{1D} receptor in controlling the release of 5-HT within the rat dorsal raphé nucleus (Davidson and Stamford, 2000; Hopwood and Stamford, 2001; Roberts and Price, 2001). In slices of the rat ventral lateral geniculate nucleus, Davidson and Stamford (1996) demonstrated that isamoltane antagonised the inhibition of electrically stimulated 5-HT release produced by the 5-HT_{1B} receptor agonist, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one (CP 93129). It did not, however, reverse the inhibition produced by sumatriptan, an agonist with a higher affinity for the 5-HT_{1D} than 5-HT_{1B} receptor. The non-selective 5-$HT_{1B/1D}$ receptor antagonist, *N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxyamide (GR127935), inhibited the effects of both agonists. They concluded that both the 5-HT_{1B} and 5-HT_{1D} receptors function as autoreceptors in the lateral geniculate nucleus. The role of the 5-HT_{1D} receptor in controlling 5-HT release in the raphé nucleus is strengthened by the observation that the 5-$HT_{1B/1D}$ agonist sumatriptan but not the selective 5-HT_{1B} receptor agonist, CP 93129, negatively regulates electrically stimulated 5-HT release from dorsal raphé nucleus slices from wild type and 5-HT_{1B} receptor knock-out mice (Piñeyro et al., 1995a). In in vivo studies, Piñeyro et al. (1995b) have shown that (±) mianserin, which has a 10 000 fold higher affinity for the 5-HT_{1D} than for the 5-HT_{1B} receptor, inhibited the ability of 1-[3-trifluoromethyl]-phenylpiperazine, a mixed 5-HT_1 receptor agonist, to decrease 5-HT release in the dorsal raphé nucleus of the anaesthetised rat. This observation coupled with a lack of inhibition of 5-HT release from mesencephalic slices, in vitro, by the 5-HT_{1B} receptor agonist, led them to conclude that the 5-HT_{1D} and not the 5-HT_{1B} receptor was involved in controlling 5-HT release in the rat raphé nucleus.

It has been proposed that the clinical efficacy of short-term administration of a selective serotonin re-uptake inhibitor is limited by the resulting stimulation of presynaptic 5-HT autoreceptors leading to decreased 5-HT release (Rollema et al., 1996; Marcoli et al., 1999; Middlemiss et al., 1999). Moreover, therapeutic improvement is achieved only after these receptors down-regulate and higher synaptic concentrations of 5-HT are achieved. The fact that the ability of an acute dose of a selective serotonin re-uptake inhibitor to elevate extracellular concentrations of 5-HT is limited by presynaptic autoreceptor stimulation was clearly shown by Rollema et al. (1996). They demonstrated, using microdialysis techniques, that the co-administration of the selective serotonin re-uptake inhibitor, sertraline, and the 5-$HT_{1B/1D}$ receptor antagonist, GR127935, led to a greater elevation in the concentration of 5-HT in the dialysate than could be achieved by the acute administration of the selective serotonin re-uptake inhibitor alone.

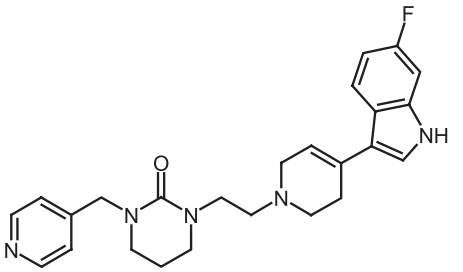
In order to determine whether the 5-HT_{1D} receptor operates as an autoreceptor in the guinea pig, we have studied the effects of the selective 5-HT_{1D} receptor agonist, L-772,405 (Russell et al., 1999), four 5-HT_{1D} receptor antagonists, LY310762, LY456219, LY456220 and LY367642 (see Fig. 1 for structures), which have a higher affinity for the guinea pig 5-HT_{1D} receptor than for the 5-HT_{1B} receptor, and the 5-HT_{1B} receptor selective antagonist, SB224289 (Selkirk et al., 1997), on 5-HT release both in vitro and in vivo.

2. Methods

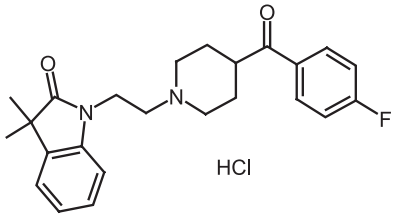
2.1. 5-HT_{1B} and 5-HT_{1D} receptor binding

The method used for measuring the affinity of compounds for the guinea pig 5-HT_{1B} and 5-HT_{1D} receptors was essentially as described for the human receptors by Pullar et al. (2000). Briefly, membranes from L-M (tk-) cells stably transfected with either the guinea pig 5-HT_{1B} or 5-HT_{1D} receptors were stored in liquid nitrogen for up to 4 weeks.

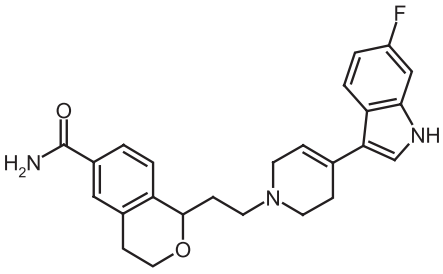
Competition studies were performed in 0.25 ml buffer (50 mM Tris–HCl containing 0.5 mM EDTA, 10 mM $MgSO_4$, 5.7 mM ascorbic acid, 16 μM pargyline. pH 7.75) containing 150 μg membrane protein, 2 nM [^{3}H]GR125743 and appro-



LY 367642



LY 310762



Isomer 1 = LY 456219

Isomer 2 = LY 456220

Fig. 1. Chemical structure of LY310762, LY367642, LY456219, and LY456220.

priate concentrations of the competing ligand. Non-specific binding was defined using 100 μM 5-HT. 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).

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 mg protein in 1.0 ml of assay buffer containing 150 μM NaCl, 5 mM KCl, 0.2 nM [^{3}H]citalopram and appropriate concentrations of the competing ligand. Non-specific binding was defined using 10 μM 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. [^{3}H]-5-HT outflow from guinea pig cortical slices

The assay was essentially similar to that described previously (Pullar et al., 2000). Male Dunkin Hartley Guinea Pigs (350–400 g, Harlan, UK) were killed by asphyxiation with CO_2 and their brains rapidly removed. Cortical slices (350 × 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/ml wet weight 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 μm 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 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). All 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 propan-2-ol (0.7 ml). The tritium label 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 tritium label 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 minus that of basal release. The percentage increase in release produced by the com-

pound 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.

2.4. Guinea pig microdialysis

2.4.1. Animals

All procedures complied with the UK Animals (Scientific Procedures) Act 1986. Dunkin Hartley guinea pigs (female; 350–400 g, Harlan) used in these experiments were housed on a 12:12 h dark/light cycle; food and water were available ad libitum.

2.4.2. Surgery and microdialysis

Guinea pigs were firstly sedated with Domitor® (medetomidine hydrochloride 1 mg/ml; 0.15 ml s.c.) and anaesthetised with isoflurane (3%) delivered with oxygen (2 l/min). On attaining surgical anaesthesia, the animals were positioned in a stereotaxic frame and anaesthesia was maintained on 1–2% isoflurane with oxygen (2 l/min). Body temperature was maintained at 36–37 °C using a heated pad.

Microdialysis probes were implanted into the hypothalmus using the following coordinates (from interaural line and dura surface in a flat head position): AP +9.0 mm, LM +1.5 mm, DV −10.2 mm (Rapisarda and Bacchelli, 1977). The probes were implanted whilst being perfused at 5 μl/min with artificial CSF containing 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 0.8 mM $MgCl_2$, 1.4 mM Na_2HPO_4, 0.25 mM NaH_2PO_4 at pH 7.4. Probes were secured with skull screws and dental cement, and the wound sutured. Perfusion was then stopped and the probes sealed. Animals were then administered Antisedan® (altipamezole hydrochloride 5 mg/ml; 0.15 ml intramuscularly) and Vetergesic® (buprenorphine; 0.08 ml s.c.). Animals were allowed 48 h to recover from surgery.

On the day of test, animals were connected with a tether and harness to a liquid swivel, and the probes perfused with artificial CSF at a rate of 1 μl/min. After 30 min, samples were collected every 20 min. Baseline samples were collected for 2 h before drugs were administered systemically by the intraperitoneal (i.p.) route.

Verification of probe placements was conducted either macroscopically or histologically from 30 μm coronal sections stained with cresyl violet.

2.4.3. High-performance liquid chromatography

The high-performance liquid chromatography (HPLC) system consisted of a Rheos 4000 pump (Flux Instruments), an on-line degasser, a 75 × 2.1 mm column (C_{18} 5 μ, Higgins Analytical) and a Triathlon autosampler (Presearch). Detection of 5-HT was accomplished with an Antec electrochemical detector (Presearch) with the glassy-carbon electrode maintained at +0.80 V versus an Ag/AgCl reference electrode. Chromatographic separation and electrochemical detection were performed at 35 °C. The mobile phase consisted of a 150 mM phosphate buffer (NaH_2PO_4), containing 2% isopropanol, 0.74 mM L-octane sulphonic acid, 0.1 mM ethylenediaminetetra-acetic acid, at pH 2.90; the flow rate was 0.4 ml/min. Peaks were displayed, integrated and stored using a Millenium-32 data acquisition system (Waters).

These HPLC conditions enabled the resolution of 5-HT from the dopamine metabolite 3-methoxytyramine. The limit of detection was approximately 0.1 nM.

2.4.4. Data analysis

Data from experiments were converted from peak areas using a calibration curve and reported as nM. The three samples prior to drug or vehicle administration were averaged to yield a pre-injection control value (equivalent to 100%). All samples were expressed as a percentage of this control value.

Differences in response to drug administration were analysed by analysis of variance (ANOVA) with repeated measures following log-transformation (natural logarithm) of percentage data. Significance was taken at the 5% level (JMP v3.2.6, SAS Institute, USA).

2.5. Materials

All reagents were of analytical grade. Radiolabelled 5-HT (5-hydroxy[G-^{3}H]-tryptamine creatinine sulphate), citalopram ([*N*-methyl-^{3}H] citalopram) and GR125743 ([*N*-methyl-^{3}H]-GR125743) were obtained from Amersham Pharmacia Biotechnology and pargyline from Sigma. 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro [furo[2,3-f]indole-3,4′-piperidine]oxalate (SB224289), *R*-2-(4-fluoro-phenyl)-2-[1-[3-(5-[1,2,4]triazol-4-yl-1*H*-indol-3-yl)-propyl]-piperidin-4-ylamino]-ethanol dioxylate (L-772,405), 1-[2-[4-(6-fluoro-1*H*-indol-3-yl)-3,6-dihydro-2*H*-pyridin-1-yl]-ethyl]-3-pyridin-4-ylmethyl-tetrahydro-pyrimidin-2-one (LY367642), (*R*)-1-[2-(4-(6-fluoro-1*H*-indol-3-yl-)-3,6-dihydro-1(2*H*)-pyridinyl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-6-carboxamide (LY456219), (*S*)-1-[2-(4-(6-fluoro-1*H*-indol-3-yl-)-3,6-dihydro-1(2*H*)-pyridinyl)ethyl]-3,4-dihydro-1*H*-2-benzopyran-6-carboxamide (LY456220) and 1-[2-[4-(4-fluoro-benzoyl)-piperidin-1-yl]-ethyl]-3,3-dimethyl-1,2-dihydro-indol-2-one (LY310762) were provided by the Eli Lilly Research Laboratories and paroxetine was a gift from Glaxo SmithKline.

3. Results

3.1. Receptor profile

LY367642, LY456220, LY456219 and LY310762 had a higher affinity for the guinea pig 5-HT_{1D} receptor than for

the 5-HT_{1B} receptor (Table 1). In agreement with the literature, SB224289 was selective for the 5-HT_{1B} (Selkirk et al., 1997, 1998) and L-772,405 for the 5-HT_{1D} receptor (Russell et al., 1999). In addition, LY367642, LY455219 and LY456220 had a high affinity for the rat brain 5-HT transporter (Table 1).

3.2. [^{3}H]-5-HT outflow from guinea pig cortical slices

The four 5-HT_{1D} receptor antagonists, LY310762, LY456219, LY456220 and LY367642, as well as the 5-HT_{1B} receptor antagonist, SB224289, potentiated the potassium-induced [^{3}H]5-HT outflow from guinea pig cortical slices with EC_{50} values in the range 30–140 nM (Fig. 2, Table 2). The maximum potentiation of the potassium-induced outflow which was obtained with any of the antagonists was about 40%. In the case of LY367642, LY456219 and LY456220 this potentiation of outflow was not due to their interaction with the 5-HT transporter as the assay was carried out in the presence of a maximally effective concentration (1 μM) of the selective serotonin re-uptake inhibitor, paroxetine (Pullar et al., 2000). Under these conditions the 5-HT transport inhibitor fluoxetine, at a concentration of 1 μM, produced only a 3% potentiation of [^{3}H]5-HT outflow (data not shown).

The selective 5-HT_{1D} receptor agonist, L-772,405, decreased the potassium-induced outflow of [^{3}H]5-HT (Fig. 2) with an IC_{50} value of 240 ± 24 nM (Table 2).

3.3. Effect of 5-HT_{1B} and 5-HT_{1D} receptor antagonists on basal and fluoxetine-evoked levels of 5-HT in the guinea pig hypothalamus, in vivo

Administration of the selective 5-HT_{1B} receptor antagonist, SB224289 (10 mg/kg i.p.) 2 h after fluoxetine (20 mg/kg, i.p) produced a further significant enhancement in extracellular levels of 5-HT in the guinea pig hypothalamus compared to animals receiving fluoxetine and then vehicle (Fig. 3; fluoxetine + vehicle versus fluoxetine + SB224289: $F[df\ 1,9] = 10.42$, $p < 0.0001$). In these experiments, there was no significant difference in the extent of the fluoxetine response between the two treatment groups prior to the second drug administration ($F[df\ 1,9] = 0.93$, $p = 0.36$). In SB224289-treated animals the levels of 5-HT after fluoxetine were increased from 261 ± 46% to a maximum of 596 ± 89%, while after vehicle the levels remained unchanged at approximately 200%, compared to the pre-injection control. In an additional group of animals, SB 224289 administered alone, also produced a significant increase in basal levels of 5-HT compared to vehicle controls ($F[df\ 1,9] = 7.79$, $p = 0.021$), reaching a maximum of 202 ± 25% (Fig. 3).

Systemic administration of LY310762 (10 mg/kg i.p.) also produced a further significant enhancement in the 5-HT response to fluoxetine (20 mg/kg i.p.) when compared to animals receiving a control vehicle injection (Fig. 4; fluoxetine + vehicle versus fluoxetine + LY310762: $F[df\ 1,12] = 8.72$, $p = 0.0121$). In these experiments the fluoxetine response also failed to differ significantly between the two treatment groups prior to the second drug administration ($F[df\ 1,12] = 0.39$, $p = 0.54$). In fluoxetine

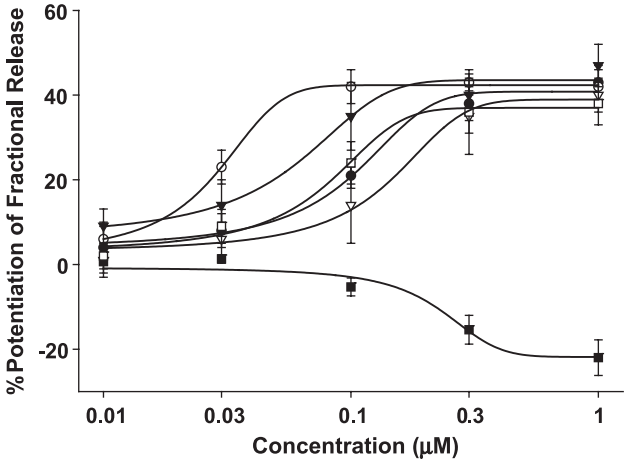


Fig. 2. Potentiation of potassium-induced [^{3}H]5-HT outflow from guinea pig cortical slices in the presence of 1.0 μM paroxetine. The potentiation in each experiment is expressed as the % increase in fractional release above that obtained in the absence of compound. SB224289 (●), LY310762 (○), LY367642 (□), LY456219 (▼), LY456220 (▽) and L-772,405 (■) were tested in log concentration increments and the points are the mean values and the vertical bars represent the S.E.M. of at least five independent experiments. All curves are fitted with a four-parameter logistic equation.

Table 1
Affinity (K_i, nM) of selected compounds for the guinea pig 5-HT_{1B} and 5-HT_{1D} receptors and for the rat serotonin transporter

Compound	Guinea Pig 5-HT_{1B} Receptor K_i (nM)	Guinea Pig 5-HT_{1D} Receptor K_i (nM)	Rat 5-HT Transporter K_i (nM)
SB224289	96 ± 4	>1000	>1000
LY367642	(44 ± 6%)[a]	100 ± 27	0.39 ± 0.08
LY456219	(35 ± 6%)[a]	113 ± 36	0.17 ± 0.01
LY456220	(21 ± 5%)[a]	333 ± 25	0.31 ± 0.01
LY310762	(32 ± 7%)[a]	249 ± 32	>1000
L-772,405	318 ± 23	29 ± 2	>1000

Data expressed as mean ± S.E.M. of at least three independent experiments.
[a] Percent inhibition of binding at 1000 nM.

Table 2
EC_{50} values (nM) for the potentiation of potassium-stimulated [^{3}H]5-HT release from guinea pig cortical slices in the presence of 1 μM paroxetine

Compounds	EC_{50} (nM)
SB224289	110 ± 10
LY367642	90 ± 15
LY456219	72 ± 14
LY456220	140 ± 12
LY310762	31 ± 6
L-772,405	240 ± 24[a]

Data expressed as mean ± S.E.M. of at least five independent experiments.
[a] IC_{50} value.

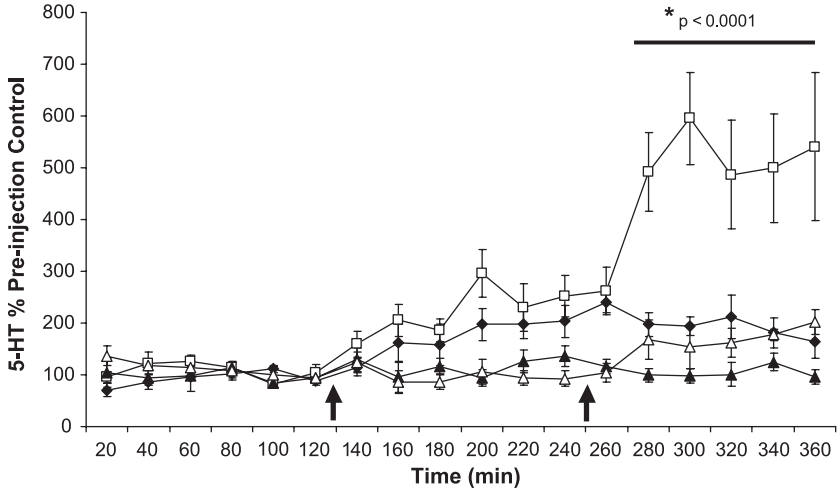


Fig. 3. The effect of SB224289 (10 mg/kg i.p.) on the 5-HT response to fluoxetine (20 mg/kg i.p.) in the guinea pig hypothalamus. The data are expressed as a percentage of a pre-injection control, and represents the mean ± S.E.M. Fluoxetine or vehicle were administered (at arrow) 2 h after onset of recording, followed by SB224289 or vehicle (at arrow) after a further 2 h. Fluoxetine + vehicle, $n = 4$ (♦); fluoxetine + SB224289, $n = 7$ (□); vehicle + vehicle, $n = 6$ (▲); vehicle + SB224289, $n = 5$ (▽). Bar represents the statistical comparison between fluoxetine + vehicle versus fluoxetine + SB224289.

treated animals, levels of 5-HT increased from 312 ± 43% to a maximum of 683 ± 191% after LY310762; in control animals, levels of 5-HT remained unchanged (250%). LY310762 administered alone also significantly increased basal levels of 5-HT above vehicle controls ($F[df\ 1,11] = 9.78$, $p = 0.0096$), reaching a maximum of 258 ± 72% compared to the pre-injection control (Fig. 4).

LY367642, a compound that is both a 5-HT uptake inhibitor and antagonist at the 5-HT_{1D} receptor, administered at 10 mg/kg s.c. produced a significant increase in extracellular levels of 5-HT (Fig. 5). Moreover, the levels of 5-HT attained with the single molecule, reaching a maximum of 501 ± 79%, were significantly greater than that achieved with fluoxetine (Fig. 5; 20 mg/kg; maximal increase, 241 ± 20%; LY367642 versus fluoxetine: $F[df\ 1,8] = 10.88$, $p = 0.0109$). The response to fluoxetine represents a maximal response that can be attained in this brain area by inhibition of 5-HT reuptake alone (Mitchell et al., 2001).

4. Discussion

The 5-HT_{1D} receptor antagonists used in this study (LY367642, LY456219, LY456220 and LY310762) had a higher affinity for the guinea pig 5-HT_{1D} receptor than for

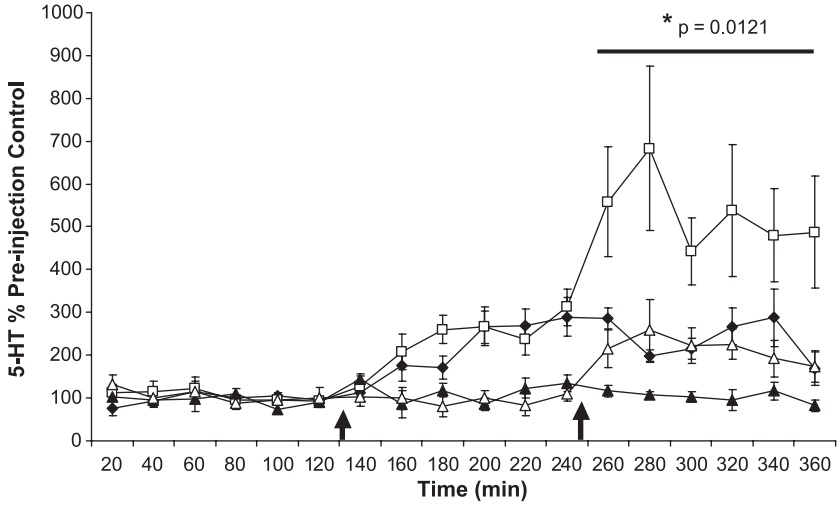


Fig. 4. The effect of LY310762 (10 mg/kg i.p.) on the 5-HT response to fluoxetine (20 mg/kg i.p.) in the guinea pig hypothalamus. The data are expressed as a percentage of a pre-injection control, and represents the mean ± S.E.M. Fluoxetine or vehicle were administered (at arrow) 2 h after onset of recording followed by LY310762 or vehicle (at arrow) after a further 2 h. Fluoxetine + vehicle, $n = 7$ (♦); fluoxetine + LY310762, $n = 7$ (□); vehicle + vehicle, $n = 7$ (▲); vehicle + LY310762, $n = 6$ (△). Bar represents the statistical comparison between fluoxetine + vehicle versus fluoxetine + LY310762.

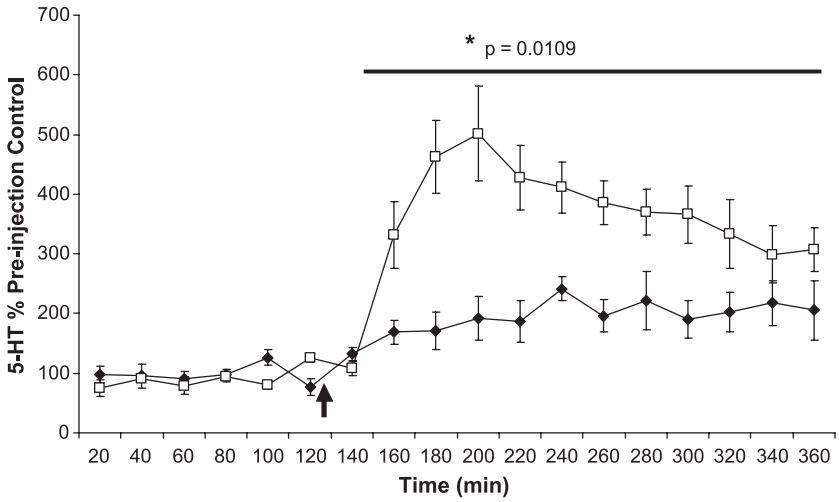


Fig. 5. The effect of LY367642 (10 mg/kg i.p.) on extracellular levels of 5-HT in the guinea pig hypothalamus: comparison with a maximal dose of fluoxetine (20 mg/kg i.p.). The data are expressed as a percentage of a pre-injection control, and represents the mean ± S.E.M. LY367642 or fluoxetine administered (at arrow) two hours after onset of recording. Fluoxetine, $n=5$ (♦); LY367642, $n=5$(□). Bar represents the statistical comparison between fluoxetine and LY367642.

the 5-HT_{1B} receptor. Assuming single site occupancy, the selectivity for the 5-HT_{1D} receptor was between 8 and 14 fold. The 5-HT_{1D} receptor agonist, L-772,405, had an 11 fold selectivity for the 5-HT_{1D} receptor.

In keeping with earlier findings (Pullar et al., 2001) the 5-HT_{1B} antagonist SB224289, potentiated potassium-stimulated [^{3}H]5-HT from guinea pig cortical slices. This was found also to be the case with the 5-HT_{1D} receptor-preferring antagonists LY310762, LY456219, LY456220 and LY367642. The increase in potassium-induced outflow of [^{3}H]5-HT resulting from the presence of 1 μM paroxetine (>150%, Pullar et al., 2000) will increase the tone of the presynaptic autoreceptor enabling antagonist activity to be more easily detected. Pullar et al. (2000) have shown that the 5-$HT_{1B/1D}$ receptor antagonist GR127935 potentiated potassium-induced outflow of [^{3}H]5-HT from guinea pig cortical slices and Middlemiss et al. (1999) have demonstrated that the 5-HT_{1B} receptor antagonist, SB236057, reversed 5-HT-induced suppression of electrically evoked [^{3}H]5-HT release from the same tissue. These activities were ascribed to the inhibition of presynaptic autoreceptors. The increase in [^{3}H]5-HT outflow produced by SB224289 in the present study helps to confirm the role of the 5-HT_{1B} receptor as a presynaptic autoreceptor in this species. In addition, the ability of the 5-HT_{1D} receptor preferring antagonists, LY456219, LY456220 and LY367642, to potentiate the potassium-stimulated outflow of [^{3}H]5-HT with EC_{50} values similar to their K_i's for binding to the guinea pig 5-HT_{1D} receptor, suggests that the 5-HT_{1D} receptor may have a similar presynaptic autoreceptor role in the guinea pig. LY310762, however, is more effective at potentiating [^{3}H]5-HT release from guinea pig cortical slices ($EC_{50}=31\pm6$ nM) than would be expected from its affinity for the guinea pig 5-HT_{1D} receptor ($K_i=249\pm32$ nM). The reason for this discrepancy is unknown. Notably, LY310762, unlike the other 5-HT_{1D} preferring antagonists used in the present study, is not a serotonin transport inhibitor. However, as the in vitro release experiments are carried out in the presence of a maximally effective concentration of the selective serotonin re-uptake inhibitor paroxetine, the absence of this activity should not influence the observed EC_{50} value. The possibility that the 5-HT_{1D} receptor functions as a presynaptic autoreceptor in the guinea pig is strengthened by the observation that the 5-HT_{1D} agonist, L-772,405, inhibits potassium-stimulated [^{3}H]5-HT outflow from guinea pig cortical slices. The IC_{50} obtained in this assay with L-772,405 (240 ± 24 nM) is higher than the K_i of this compound for the 5-HT_{1D} receptor (29 ± 2 nM). This may be due to the presence of a saturating concentration of paroxetine leading to a high tone at the receptor and thus reducing the sensitivity of the assay to agonists.

Rollema et al. (1996), using microdialysis techniques, have shown that, in the guinea pig, the co-administration of a selective serotonin reuptake inhibitor, sertraline, and the mixed 5-$HT_{1B/1D}$ receptor antagonist, GR127935, produced a greater elevation in the concentration of 5-HT in the dialysate of the hypothalamus than could be achieved by the acute administration of the selective serotonin reuptake inhibitor alone. They argued that this was because the ability of an acute dose of a selective serotonin reuptake inhibitor to elevate the extracellular concentration of 5-HT was limited by stimulation of the 5-$HT_{1B/1D}$ presynaptic autoreceptor. A similar finding was reported for the guinea pig hippocampus (Pullar et al., 1996). The increase in 5-HT concentrations in the guinea pig hypothalamus has been confirmed by Mitchell et al. (2001) using the selective serotonin re-uptake inhibitor, fluoxetine. In addition, these authors have shown that LY393558, a compound which blocks both the 5-HT reuptake site and the 5-$HT_{1B/1D}$

receptor, was able to elevate the extracellular concentration of 5-HT in the rat frontal cortex and guinea pig hypothalamus to a greater extent than a maximally effective dose of the selective serotonin re-uptake inhibitor, fluoxetine. In the present study, both the 5-HT_{1B} and the 5-HT_{1D} receptor antagonists (SB224289 and LY310762 respectively) have been shown to potentiate the effects of a maximally effective dose of fluoxetine. In addition, the 5-HT_{1D} receptor antagonist with potent 5-HT transporter inhibitory activity, LY367642, elevated extracellular 5-HT concentrations to a greater extent than the acute administration of a maximally effective dose of fluoxetine. As in the case of the in vitro 5-HT release data, this points towards a role for both the 5-HT_{1B} and 5-HT_{1D} receptors as presynaptic autoreceptors in this species.

It has been suggested (Rollema et al., 1996; Marcoli et al., 1999; Middlemiss et al., 1999) that the clinical efficacy of short-term selective serotonin re-uptake inhibitor administration is limited by the resulting stimulation of terminal presynaptic autoreceptors leading to a decrease in 5-HT release. As the 5-HT_{1D} receptor, as well as the 5-HT_{1B} receptor, may be a presynaptic autoreceptor, the administration of a selective serotonin re-uptake inhibitor along with a 5-HT_{1D} receptor antagonist should lead to early onset of antidepressant activity provided the 5-HT_{1D} receptor functions as an autoreceptor in man.

In conclusion, we have shown that the 5-HT_{1D} receptor may have a similar presynaptic autoreceptor role to the 5-HT_{1B} receptor in the cortex and hypothalamus of the guinea pig.

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