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# LY393558, a 5-hydroxytryptamine reuptake inhibitor and 5-$HT_{1B/1D}$ receptor antagonist: effects on extracellular levels of 5-hydroxytryptamine in the guinea pig and rat

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**Abstract**

The stimulation of terminal 5-$HT_{1B/1D}$ autoreceptors limits the effects of selective serotonin reuptake inhibitors on extracellular levels of 5-hydroxytryptamine (5-HT, serotonin) in vivo. Microdialysis studies show that acute oral administration of LY393558—a 5-HT reuptake inhibitor and antagonist at both the human 5-$HT_{1B}$ and 5-$HT_{1D}$ receptor—in the dose range 1–20 mg/kg, increases extracellular levels of 5-HT in both the guinea pig hypothalamus and rat frontal cortex. In both species, the levels of 5-HT that were attained were higher than following an acute, maximally effective dose of fluoxetine (20 mg/kg orally), reaching approximately 1500% in the guinea pig hypothalamus and 700% in the rat frontal cortex. In both species, the response to LY393558 (10 mg/kg p.o.) was impulse dependent, being absent in the presence of tetrodotoxin delivered at 1 μM via the microdialysis probe. The sensitivity to tetrodotoxin contrasted with the effects seen with DL-fenfluramine. Studies in rats showed that the microdialysate 5-HT concentration achieved in the frontal cortex after an acute challenge with LY393558 (5 mg/kg p.o.) was significantly greater than following a chronic regime of fluoxetine treatment (10 mg/kg/day orally for 21 days). Moreover, in rats chronically treated with LY393558 (5 mg/kg/day orally for 21 days), the mean basal concentration, 24 h after the final pretreatment dose, was of the same magnitude as that following chronic fluoxetine. However, in contrast to the response seen in fluoxetine-pretreated animals, a challenge dose of LY393558 still elicited a further increase in extracellular 5-HT in LY393558-pretreated animals. LY393558 is a potent 5-HT reuptake inhibitor and 5-$HT_{1B/1D}$ receptor antagonist. Microdialysis studies show that acute oral administration increases extracellular levels of 5-HT, by an impulse-dependent mechanism, above those produced by a maximally effective dose of fluoxetine, and in rats to levels only achieved following chronic fluoxetine treatment. Its neurochemical profile in vivo suggests that it may be a more effective antidepressant with the potential for producing an earlier onset of clinical activity than selective serotonin reuptake inhibitors. 

*Keywords:* 5-$HT_{1B/1D}$ receptor; 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; 5-HT (5-hydroxytryptamine, serotonin) release; Microdialysis; Antidepressant

## 1. Introduction

Major depression is a common illness affecting more than 5% of the population. It is well established that a significant component of the symptomatology of depression can be attributed to a reduction in serotonergic function. Enhancing serotonin (5-hydroxytryptamine, 5-HT) function by selective serotonin reuptake inhibitors has improved the treatment of this disorder, producing therapeutic benefits and having a better safety and patient compliance profile compared to the tricyclic antidepressants (Murphy et al., 1995).

Although selective serotonin reuptake inhibitors offer a significant advance in the treatment of major depression, there are limitations to their effectiveness. Some reports suggest that up to 30% of patients fail to show an adequate response (Thase and Rush, 1995), and in those that do respond, therapeutic improvement is not immediate, but requires treatment for 2–4 weeks (Montgomery, 1995).

Selective serotonin reuptake inhibitors increase 5-HT transmission by blocking reuptake of released 5-HT. However, activation of somatodendritic 5-$HT_{1A}$ (Hjorth, 1993; Gartside et al., 1995) and terminal 5-$HT_{1B/1D}$ autoreceptors (Rollema et al., 1996) counteracts, or diminishes the effectiveness of their acute treatment. One of the hypotheses proposed to explain the delayed onset of the antidepressant action of selective serotonin reuptake inhibitors is the time required for autoreceptor desensitisation (Good-

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win, 1996; Leonard, 1996). Therefore, one approach for more efficient and fast acting antidepressant drugs would be to pharmacologically mimic 5-HT autoreceptor desensitisation by the concomitant blockade of terminal autoreceptors with selective antagonists (Glennon and Westkaemper, 1993; Briley and Moret, 1993; Moret and Briley, 2000). This combination would prevent feedback inhibition of terminal 5-HT release, leading to an increased effectiveness of the selective serotonin reuptake inhibitor on synaptic levels of 5-HT. Preclinical evidence favouring such a strategy has been obtained from the combined systemic administration of sertraline (a selective serotonin reuptake inhibitor) with GR127935, a nonselective 5-$HT_{1B/1D}$ receptor antagonist (Skingle et al., 1993). In these experiments, the combination resulted in a more pronounced and long-lasting increase in extracellular levels of 5-HT in the guinea pig hypothalamus, indicative of the synergistic effect of this pharmacological intervention (Rollema et al., 1996). Therefore, a drug combining 5-HT reuptake inhibition with antagonism of 5-$HT_{1B/1D}$ presynaptic autoreceptors would be expected, on acute administration, to elevate synaptic 5-HT (by an impulse-dependent mechanism) to levels that are only achieved following chronic administration of a selective serotonin reuptake inhibitor alone. Increasing synaptic levels of 5-HT to this extent following acute administration may produce a more rapid therapeutic effect.

In this paper, we report the in vivo activity of 1-[2-[4-(6-fluoro-1*H*-indol-3-yl)-3,6-dihydro-1(2*H*)-pyridinyl]ethyl]-3-isopropyl-6-(methylsulphonyl)-3,4-dihydro-1*H*-2,1,3-benzothiadiazine-2,2-dioxide (LY393558), a compound that possesses dual pharmacological activity as a selective serotonin reuptake inhibitor and an antagonist at the 5-$HT_{1B}$ and 5-$HT_{1D}$ receptor (Pullar et al., in press). In an in vitro assay to measure autoreceptor activity, LY393558 has been shown to augment $K^+$-evoked release of [$^3$H]5-HT from guinea pig cortical slices (in the presence of a saturating concentration of paroxetine), indicative of autoreceptor antagonist activity. In this paper, we describe the effects of LY393558, in vivo, on extracellular levels of 5-HT in both the guinea pig and rat following both acute and chronic administration. We compare the response obtained with LY393558 with that produced by a maximally effective dose of fluoxetine, and provide preclinical data suggesting that a compound possessing dual activity as a selective serotonin reuptake inhibitor and terminal autoreceptor antagonist may have a shorter onset of clinical activity.

## 2. Materials and methods

### 2.1. Animals

Dunkin Hartley guinea pigs (female; 350–400 g, Harlan UK) or Lister Hooded rats (male; 290–320 g, Harlan UK) used in these experiments were housed on a 12/12-h dark/light cycle; food and water were available ad libitum.

### 2.2. Dialysis probe construction

Concentric microdialysis probes were constructed using 23-gauge stainless steel tubing (thin wall; Coopers Needle Works, UK), glass capillary tubing (Scientific Glass Engineering, UK) and cellulose acetate dialysis membrane (Hospal, UK; 200 μm O.D.). The shaft of the microdialysis probe consisted of stainless steel tubing (16 mm) with a small hole drilled midway along its length. Glass capillary tubing (3 cm; 140 μm O.D., 40 μm I.D.) was inserted into a piece of dialysis tubing (approximately 8 mm in length) which had been sealed with epoxy glue at one end. The dialysis tubing containing the 'inlet' glass capillary was then inserted into one end of the metal tubing and sealed using epoxy glue leaving 5 mm of dialysis tubing exposed. An 'outlet' glass capillary (170 μm O.D., 110 μm I.D.) was then inserted through the hole midway along the shaft of the steel tubing, and sealed with epoxy glue. The 'outlet' glass capillary was then strengthened using a sheath consisting of an 8-mm length of stainless steel tubing. The whole arrangement was then fixed together with epoxy glue. The length of "active" membrane was adjusted using epoxy glue to suit the brain area into which it was to be implanted (e.g. 2 mm for hypothalamus and 3.5 mm for frontal cortex). In vitro recoveries of 5-HT and 5-hydroxyindoleacetic acid through these probes (from a 100-nM solution) were approximately 7.8% and 13.6%, respectively, at 1 μl/min.

### 2.3. Brain microdialysis surgery

Guinea pigs or rats 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 using the following co-ordinates: guinea pig hypothalamus (from interaural line and dura surface in a flat head position), caudal +9.0 mm, lateral +1.5 mm, vertical −10.2 mm (Rapisarda and Bacchelli, 1977); rat frontal cortex (from bregma and dura in a flat head position): caudal +3.2 mm, lateral 3.5 mm, vertical −4.5 mm (Paxinos and Watson, 1986). The probes were implanted while being perfused with artificial cerebrospinal fluid (aCSF) at 5 μl/min, containing (in mM): NaCl (120), KCl (5), $CaCl_2$ (1.25), $MgCl_2$ (0.8), $Na_2HPO_4$ (1.4), $NaH_2PO_4$ (0.25) 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 24–48 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 aCSF 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 oral route (p.o.) or by subcutaneous (s.c.) or intraperitoneal (i.p.) injection.

### 2.4. Histology

Verification of probe placements was conducted either macroscopically or in 30-μm coronal sections stained with Cresyl violet.

### 2.5. High-performance liquid chromatography (HPLC) details

The HPLC system consisted of a Rheos 4000 pump (Flux Instruments), an on-line degasser, a $75 \times 2.1$-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 30 °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 ethylenediaminetetraacetic 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.

### 2.6. Data analysis

Data from experiments were converted from peak areas using a calibration curve and reported as nanomolar. The three samples before 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 MANOVA 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).

### 2.7. Materials

All chemicals for HPLC were Analar or HPLC grade. Domitor®, Antisedan® and Vetergesic® were obtained from Vetdrug. 5-HT creatinine sulphate, 5-hydroxyindoleacetic acid, DL-fenfluramine and tetrodotoxin were purchased from Sigma (Poole, UK). *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) and LY393558 (as free base or tartrate salt) were synthesised at Lilly Research Centre. All drugs for acute systemic administration were dissolved in 50% β-cyclodextrin (BCD) or water, while for chronic administration, the vehicle consisted of water for fluoxetine, or 1% carboxymethylcellulose (CMC) for LY393558. For oral administration, drugs were administered in a volume of 2 ml/kg (plus 2 ml water in the guinea pig). The doses used for all drugs were calculated as free base weight.

## 3. Results

### 3.1. Effect of fluoxetine on extracellular levels of 5-HT in the guinea pig hypothalamus

Oral administration of fluoxetine (10 and 20 mg/kg) increased extracellular levels of 5-HT in the guinea pig hypothalamus—reaching 250–300% of their pre-injection control (Fig. 1A). The increase represented a maximal response to fluoxetine as there was no significant difference between the two doses tested [$F(1,6) = 0$, $P$ = NS]. Following a different route of administration, fluoxetine administered using the intraperitoneal route (at 10–40 mg/kg), also increased extracellular 5-HT equally [effect of dose: $F(2,9) = 2.01$, $P$ = NS; Fig. 1B], and the maximal response obtained was comparable to that seen after oral administration.

### 3.2. Effect of GR127935, a nonselective 5-$HT_{1B/1D}$ receptor antagonist, on the 5-HT response to fluoxetine in the guinea pig hypothalamus

To explore the extent to which stimulation of 5-$HT_{1B/1D}$ receptors limit the extracellular response to 5-HT reuptake inhibition, the nonselective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (Skingle et al., 1993) was administered after a systemic challenge with fluoxetine, when extracellular levels of 5-HT had reached a maximum. The capacity to cause a further enhancement in extracellular levels of 5-HT was then compared to animals that received fluoxetine followed by the vehicle control. In these experiments, fluoxetine administered at 10 mg/kg i.p. increased levels of 5-HT to approximately 200% (Fig. 2). Analysis of the response to fluoxetine between the two groups of animals (i.e. those that were to receive a subsequent administration of GR127935 or vehicle) revealed no significant difference between the two response profiles up to 3 h after its administration [$F(1,13) = 0$, $P$ = NS]. At 3 h, animals then received GR127935 at 0.3 mg/kg s.c., or vehicle. In animals that received the mixed 5-$HT_{1B/1D}$ receptor antag-

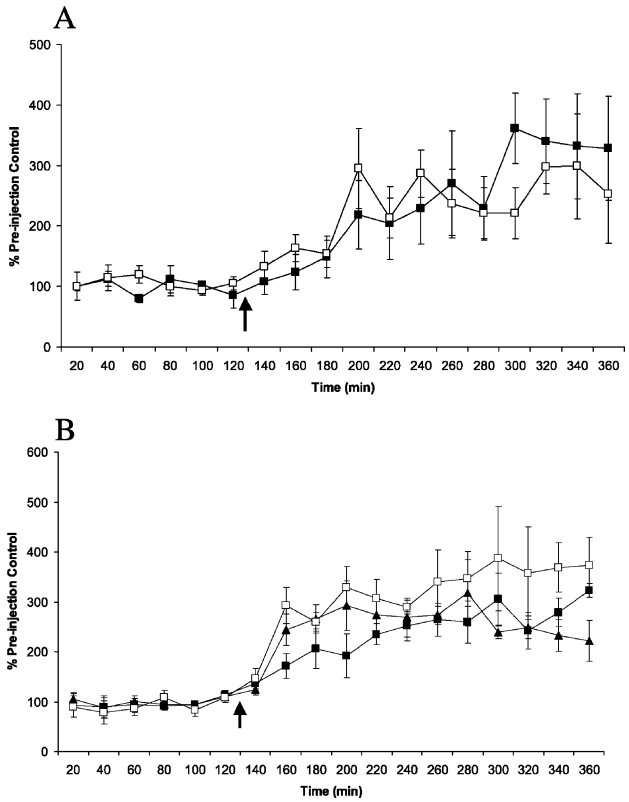


Fig. 1. (A and B) The effect of fluoxetine on extracellular levels of 5-HT in the guinea pig hypothalamus following oral (A) and intraperitoneal (B) administration. Fluoxetine was administered (at arrow) orally at 10 mg/kg (closed squares, $n = 4$) and 20 mg/kg (open squares, $n = 4$), and at 10 mg/kg (closed squares, $n = 4$), 20 mg/kg (closed triangles, $n = 4$) and 40 mg/kg (open squares, $n = 4$) by the intraperitoneal route. The data are expressed as a percentage of a pre-injection control, and represent the mean ± S.E.M.

onist, a further significant increase in extracellular levels of 5-HT occurred—reaching 350–400% [versus vehicle administration: $F(1,13) = 17.0$, $P = 0.0012$; Fig. 2]. At this dose, GR127935 when administered alone had previously been shown to be unable to significantly alter levels of 5-HT (data not shown).

### 3.3. Comparison between a maximally effective dose of fluoxetine and LY393558 on extracellular levels of 5-HT in the guinea pig hypothalamus

Oral administration of LY393558 produced a dose-dependent and significant increase in extracellular 5-HT [$F(5,24) = 33.0$, $P < 0.0001$; Fig. 3]. At the lowest dose tested (1 mg/kg), extracellular levels of 5-HT reached 200–250% relative to the pre-injection control [versus vehicle control: $F(1,24) = 9.6$, $P = 0.0048$], while after the highest dose of LY393558 (20 mg/kg), levels of 5-HT reached approximately 1500% [versus vehicle control: $F(1,24) = 135.8$, $P < 0.0001$]. The increase obtained with both 10 and 20 mg/kg was significantly greater than that produced by a maximally effective dose of fluoxetine [fluoxetine at 20 mg/kg versus LY393558 at 10 mg/kg: $F(1,24) = 9.9$, $P = 0.0044$; versus LY393558 20 mg/kg: $F(1,24) = 36.5$, $P < 0.0001$].

### 3.4. Comparison between fluoxetine and paroxetine on levels of 5-HT in the guinea pig hypothalamus

A comparison was made between the effect of a maximally effective dose of fluoxetine and that of a high dose of paroxetine. This was conducted to address the possibility that the potent 5-HT reuptake inhibitor activity of LY393558 may contribute towards the extent of the extracellular 5-HT response—that is, producing a response that exceeds the maximal response obtained by fluoxetine. Paroxetine has been reported to have a higher affinity than fluoxetine for the 5-HT transporter and to be functionally a more potent inhibitor of synaptosomal 5-HT uptake (Pullar

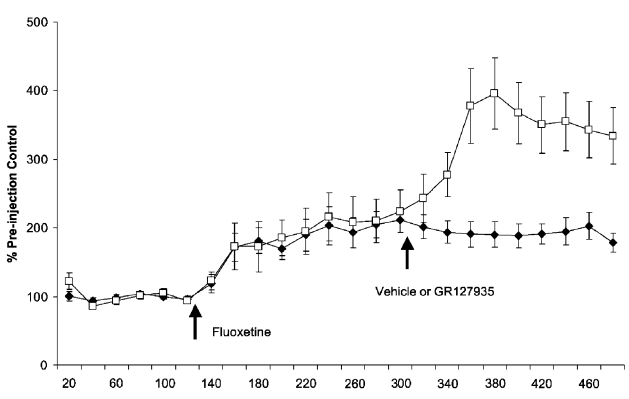


Fig. 2. The effect of GR127935 (0.3 mg/kg s.c.) on the 5-HT response to fluoxetine (10 mg/kg i.p.) in the guinea pig hypothalamus. The data are expressed as a percentage of a pre-injection control, and represent the mean ± S.E.M. Fluoxetine was administered (at arrow) 2 h after onset of recording followed by GR127935 or vehicle (at arrow) after a further 3 h (closed triangles: fluoxetine + vehicle, $n = 8$; open squares: fluoxetine + GR127935, $n = 7$).

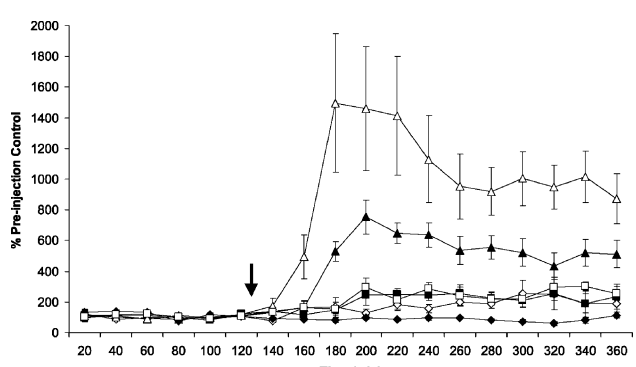


Fig. 3. Effect of LY393558 (in a dose range 1–20 mg/kg) administered orally on extracellular levels of 5-HT in the guinea pig hypothalamus. The data are expressed as a percentage of a pre-injection control, and represent the mean ± S.E.M. (Vehicle: closed diamonds, $n = 6$; fluoxetine 20 mg/kg: open squares, $n = 4$; LY393558 1 mg/kg: open diamonds, $n = 5$; 5 mg/kg: closed squares, $n = 5$; 10 mg/kg: closed triangles, $n = 5$; 20 mg/kg: open triangles, $n = 5$; drugs administered at arrow.)

et al., 2000). Administration of a high dose of paroxetine (10 mg/kg p.o.) produced increases in extracellular levels of 5-HT in the guinea pig hypothalamus that failed to differ significantly from the highest dose of fluoxetine [paroxetine versus fluoxetine at 20 mg/kg: $F(1, 20) = 1.44$, $P$ = NS; Fig. 4]. The increase obtained with paroxetine (between 150% and 200%), which is also a more potent 5-HT uptake inhibitor than LY393558 (Pullar et al., in press), was still less than that obtained previously with LY393558 (Fig. 3).

### 3.5. Effect of tetrodotoxin on levels of 5-HT evoked by DL-fenfluramine and LY393558 in the guinea pig hypothalamus

To explore the neuronal specificity/selectivity of the response evoked by a high dose of LY393558, experiments were conducted to determine whether the response was impulse dependent. To address this issue, the effect of LY393558 was determined in the absence and presence of the sodium channel blocker tetrodotoxin, and compared to the tetrodotoxin dependency of the response evoked by DL-fenfluramine—a nonspecific impulse-independent releaser of 5-HT.

#### 3.5.1. DL-Fenfluramine-evoked response

Perfusion of tetrodotoxin through the microdialysis probe at 1 μM produced a significant decrease in extracellular levels of 5-HT [$F(1,5) = 104.0$, $P = 0.0002$]. In the continued presence of tetrodotoxin, the subsequent administration of DL-fenfluramine (20 mg/kg i.p.) still elevated levels of 5-HT to the same extent as that achieved in the absence of tetrodotoxin [fenfluramine response in absence and presence of tetrodotoxin: $F(1,5) = 2.77$, $P$ = NS; Fig. 5A].

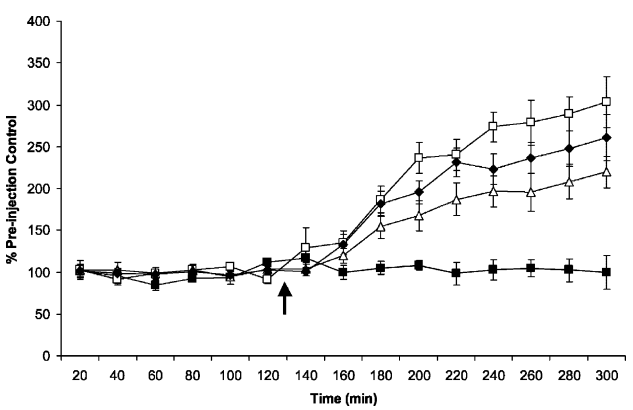


Fig. 4. Comparison between fluoxetine (10 and 20 mg/kg p.o.) and paroxetine (10 mg/kg p.o.) on extracellular levels of 5-HT in the guinea pig hypothalamus. The data are expressed as a percentage of a pre-injection control, and represent the mean ± S.E.M. (Vehicle: closed squares, $n = 5$; fluoxetine 10 mg/kg p.o.: open triangles, $n = 6$; fluoxetine 20 mg/kg p.o.: closed diamonds, $n = 7$; paroxetine 10 mg/kg p.o.: open squares, $n = 7$; drugs administered at arrow.)

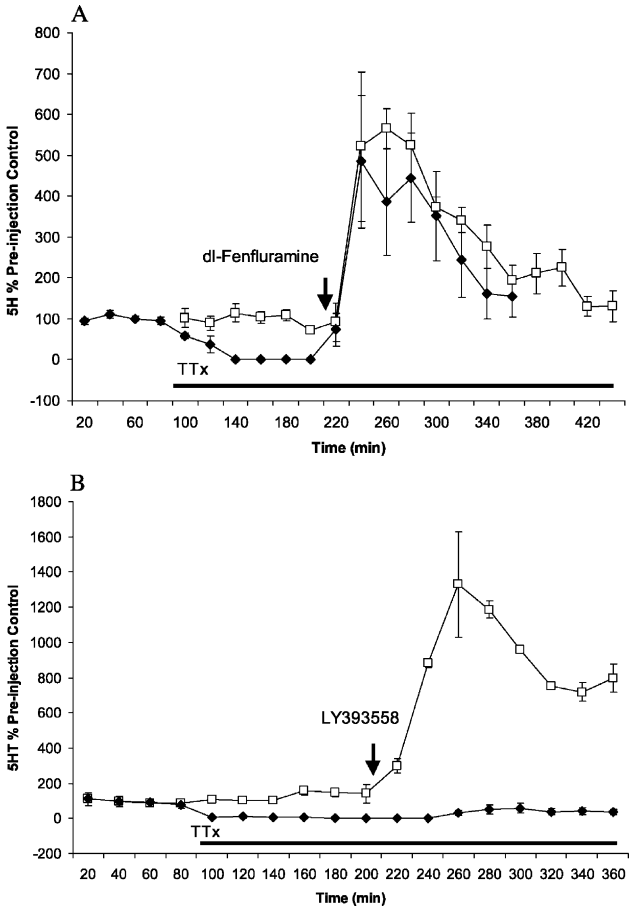


Fig. 5. (A and B) The effect of tetrodotoxin (TTx) on the extracellular 5-HT response to DL-fenfluramine (A) and LY393558 (B). The data are expressed as a percentage of a pre-injection control, and represent the mean ± S.E.M. Control response to DL-fenfluramine or LY393558: open squares, $n = 4$; TTx + DL-fenfluramine or LY393558: closed diamonds, $n = 3$. TTx was perfused in the hypothalamus, by inclusion in the aCSF, during the 100-min sample collection, and continued for duration of the experiment (as depicted by line). Drugs were administered (at arrow) 2 h after onset of TTx administration.

#### 3.5.2. LY393558-evoked response

Basal levels of 5-HT were significantly [$F(1,5) = 95.2$, $P = 0.0002$] reduced by perfusion with tetrodotoxin (1 μM). However, in the presence of tetrodotoxin, the response to a subsequent administration of LY393558 at 20 mg/kg p.o. was completely abolished [5-HT response in presence and absence of tetrodotoxin: $F(1,5) = 47.2$, $P$ = 0.001; Fig. 5B].

### 3.6. Effect of GR127935 on the 5-HT response to fluoxetine in the rat frontal cortex

Species differences have been reported in the pharmacology of the 5-$HT_{1B}$ receptor (Hartig et al., 1992), while the pharmacology of the (cloned) 5-$HT_{1D}$ receptor is conserved among various mammalian species, such as human, guinea pig and rat (Wurch et al., 1997). To explore

potential species differences in the activity of LY393558, experiments were also conducted in the rat. Initially, however, the nonselective antagonist GR127935 was utilised to explore the contribution made by terminal 5-$HT_{1B/1D}$ receptors in limiting the extracellular response to fluoxetine. In these experiments, a significantly greater increase in the extracellular 5-HT response to fluoxetine (10 mg/kg i.p.) was obtained in animals pretreated with GR127935 [0.3 mg/kg s.c.; versus vehicle controls: $F(1,6) = 17.56$, $P = 0.0057$; Fig. 6].

### *3.7. Effect of a maximally effective dose of fluoxetine and LY393558 on extracellular levels of 5-HT in the rat frontal cortex*

The maximal response that can be obtained by fluoxetine in the rat frontal cortex was determined following oral administration at 10, 20 and 40 mg/kg. Fluoxetine increased levels of 5-HT in the rat frontal cortex, reaching between 200% and 350% of their pre-injection control (Fig. 7). Overall, there was no significant difference between the responses obtained with each dose [$F(2,10) = 0.91$, $P$ = NS].

Administration of LY393558 at doses of 1–20 mg/kg p.o. significantly increased extracellular levels of 5-HT (Fig. 8) above vehicle controls [$F(5,26) = 52.2$, $P < 0.0001$]. A significant increase was obtained with the lowest dose tested [1 mg/kg; versus vehicle: $F(1,26) = 11.6$, $P = 0.0022$], and a maximal increase was observed with 5 mg/kg [$F(1,26) = 132.0$, $P < 0.0001$], with levels of 5-HT reaching approximately 600–700% of the pre-injection control. Administration of LY393558 at 10 and 20 mg/kg also significantly increased levels of 5-HT [10 mg/kg: $F(1,26) = 153.2$, $P < 0.0001$; 20 mg/kg: $F(1,26) = 103.3$, $P < 0.0001$], but neither response was significantly greater than that obtained with 5 mg/kg [versus 10 mg/kg: $F(1,26) = 1.1$, $P$ = NS; versus 20 mg/kg: $F(1,26) = 0$, $P$ = NS]. The response obtained with LY393558 (at 1–20 mg/kg) was long lasting, with levels of 5-HT being elevated above vehicle controls for the duration of the recording (4 h).

At 5, 10 and 20 mg/kg of LY393558, the response elicited by LY393558 was significantly greater than that produced by a maximally effective (20 mg/kg) oral dose of fluoxetine [fluoxetine versus LY393558 at 5 mg/kg: $F(1,26) = 32.8$, $P < 0.0001$; versus LY393558 at 10 mg/kg: $F(1,26) = 43.8$, $P < 0.0001$; versus LY393558 at 20 mg/kg: $F(1,26) = 25.6$, $P < 0.0001$].

As seen previously in the guinea pig hypothalamus, in the rat frontal cortex, perfusion of tetrodotoxin at 1 μM through the probe significantly attenuated the response to a systemic challenge with LY393558 [10 mg/kg p.o.;

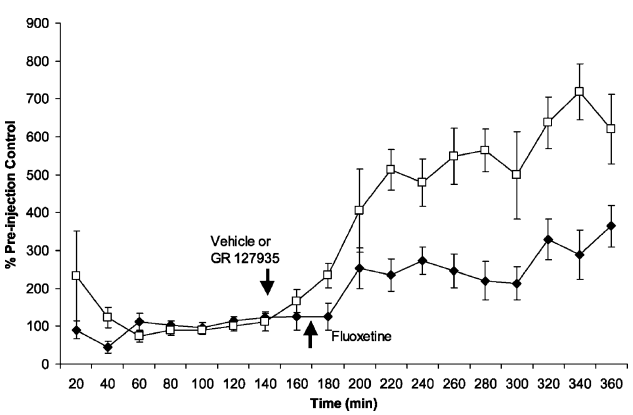


Fig. 6. The effect of GR127935 (0.3 mg/kg s.c.) on the 5-HT response to fluoxetine (10 mg/kg i.p.) in the rat frontal cortex. The data are expressed as a percentage of a pre-injection control, and represent the mean ± S.E.M. Fluoxetine was administered 30 min after GR127935 or vehicle (drugs administered at arrows). (Vehicle + fluoxetine: closed triangles, $n = 4$; GR127935 + fluoxetine: open squares, $n = 4$.)

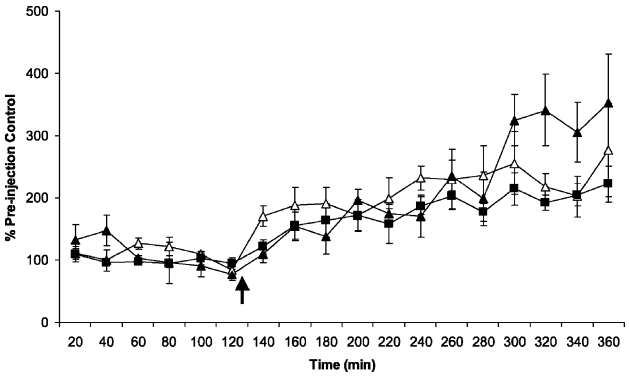


Fig. 7. Effect of fluoxetine administered orally in the dose range 10–40 mg/kg on extracellular levels of 5-HT in the rat frontal cortex. The data are expressed as a percentage of a pre-injection control, and represent the mean ± SEM. Fluoxetine at 10 mg/kg: open triangles, $n = 4$; 20 mg/kg: closed triangles, $n = 4$; 40 mg/kg: closed squares, $n = 5$; drug administered at arrow.

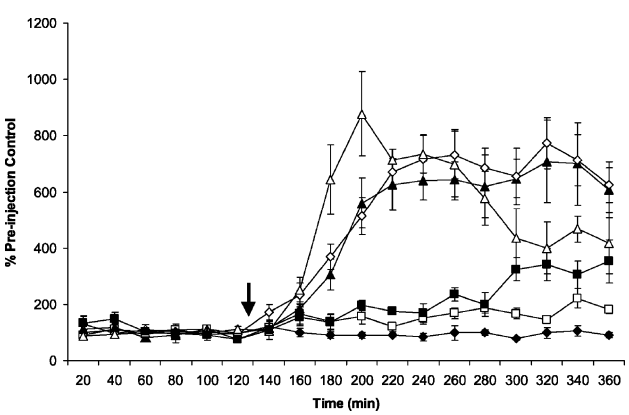


Fig. 8. Shows the effect of LY393558 (in a dose range 1–20 mg/kg) administered orally on extracellular levels of 5-HT in the rat frontal cortex. The data are expressed as a percentage of a pre-injection control, and represent the mean ± SEM. Vehicle: closed diamonds, $n = 4$; fluoxetine 20 mg/kg: closed squares, $n = 4$; LY393558 at 1 mg/kg: open squares, $n = 6$; 5 mg/kg: closed triangles, $n = 7$; 10 mg/kg: open diamonds, $n = 7$; 20 mg/kg: open triangles, $n = 4$; drugs administered at arrow.

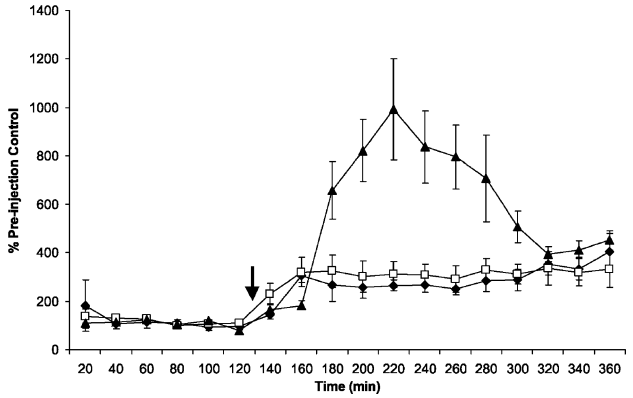


Fig. 9. Comparsion between the extracellular response caused by paroxetine, fluoxetine and LY393558 in the rat frontal cortex. The data are expressed as a percentage of a pre-injection control, and represent the mean ± SEM. Paroxetine (10 mg/kg i.p.): open squares, $n = 6$; fluoxetine (20 mg/kg i.p.): closed triangles, $n = 6$; LY393558 (5 mg/kg i.p.): closed triangles, $n = 4$; drugs administered at arrow.

$F(1,6) = 208.6,\ P < 0.0001$], but failed to alter the response to systemic DL-fenfluramine [10 mg/kg i.p.; $F(1,4) = 0,\ P =$ NS]. Figures not shown.

### 3.8. A comparison between the 5-HT response to LY393558 and that produced by either paroxetine or fluoxetine

In the guinea pig hypothalamus, the response to LY393558 far exceeded that evoked by a maximally effective dose of fluoxetine, as well as that of the more potent 5-HT reuptake inhibitor, paroxetine. In the rat frontal cortex, both fluoxetine (20 mg/kg i.p.) and paroxetine (10 mg/kg i.p.) increased extracellular levels of 5-HT to the same extent [300–350%; fluoxetine versus paroxetine: $F(1,13) = 0.11,\ P =$ NS; Fig. 9]. The response evoked by LY393558 (5 mg/kg i.p.), on the other hand, reached approximately 800% of the pre-injection control, far exceeding the response evoked by either reuptake inhibitor [versus fluoxetine: $F(1,13) = 7.72,\ P = 0.016$; versus paroxetine: $F(1,13) = 6.12,\ P = 0.028$].

### 3.9. Effect of chronic (21-day) treatment with fluoxetine and LY393558 on basal levels of 5-HT and the response to a subsequent challenge in the rat frontal cortex

A drug combining 5-HT reuptake inhibition and 5-$HT_{1B/1D}$ receptor antagonism should produce levels of 5-HT after an acute administration that can be attained after a period of chronic treatment with a reuptake inhibitor alone. Chronic studies were therefore undertaken with fluoxetine and LY393558 to determine (i) how 5-HT levels achieved after acute LY393558 administration compare to those after chronic fluoxetine treatment, and (ii) whether the response to LY393558 is altered after chronic treatment.

Comparison of the concentration of 5-HT in the dialysate samples from rats chronically pretreated with fluoxetine (10 mg/kg p.o./day) or LY393558 (5 mg/kg p.o./day), 24 h after the last pretreatment, revealed a significantly higher concentration of 5-HT in each drug-treated group when compared to their respective vehicle controls [$F(7,62) = 7.85,\ P < 0.0001$; basal fluoxetine versus water: $F(1,62) = 9.07,\ P = 0.0038$; basal LY393558 versus CMC: $F(1,62) = 5.09,\ P = 0.0276$; Fig. 10]. There was no significant difference between the two drug treatment groups. Fig. 10 shows the concentration of 5-HT in the dialysate samples for the basal, and challenge periods (i.e. 2 h before drug challenge and 2 h post-challenge).

In vehicle (CMC)-treated animals, LY393558 evoked a significant elevation in the dialysate concentration of 5-HT [$F(1,62) = 27.9,\ P < 0.0001$]. The concentration of 5-HT following this acute administration of LY393558 was significantly higher than that reached following an acute fluoxetine challenge [$F(1,62) = 18.76,\ P < 0.0001$] and significantly higher than the basal concentration reached 24 h after chronic treatment with fluoxetine [$F(1,62) = 5.29,\ P = 0.0248$].

Although basal concentrations of 5-HT were higher in LY393558-pretreated animals, a subsequent administration of the drug still produced a significant increase in the concentration of 5-HT [$F(1,62) = 5.55,\ P = 0.0216$]. In these animals, the concentration of 5-HT did not differ significantly from that achieved following an acute response to LY393558 in vehicle-treated animals. Moreover,

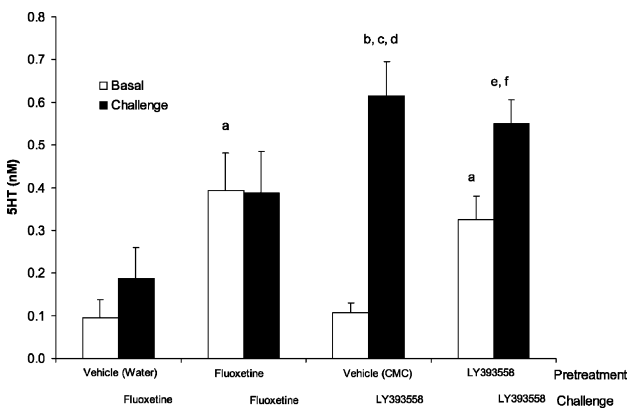


Fig. 10. The effect of chronic treatment with LY393558 (5 mg/kg p.o./day) or fluoxetine (10 mg/kg p.o.) in the rat on the basal concentrations of 5-HT in the frontal cortex 24 h after the last dose, and after a subsequent drug challenge. The basal dialysate concentration in vehicle (water or CMC) or drug-treated groups (Pretreatment) represents the mean dialysate concentration of 5-HT 2 h before drug challenge. The response to drug administration (Challenge) represents the mean dialysate concentration over a 2-h period beginning 2 h after administration of either LY393558 or fluoxetine given at their pretreatment dose. The data represent the mean ± S.E.M., $n = 8–9$ per group. (Significant comparisons, $P < 0.05$: a = basal concentration of 5-HT in chronic drug versus vehicle-pretreated groups; b = acute drug response in vehicle-pretreated animals; c = acute LY393558 response versus fluoxetine response in vehicle-pretreated animals; d = acute LY393558 response in vehicle-pretreated animals versus basal levels in chronic fluoxetine-pretreated group; e = LY393558 response versus basal levels in LY393558-pretreated animals; f = LY393558 response in LY393558-pretreated animals versus acute fluoxetine response in vehicle-pretreated animals.)

the response to LY393558 in animals pretreated with the drug was still significantly higher than that achieved following acute fluoxetine treatment [$F(1,62) = 13.56$, $P < 0.0001$].

## 4. Discussion

LY393558 is a potent inhibitor of 5-HT reuptake and an antagonist at both the 5-$HT_{1B}$ and 5-$HT_{1D}$ receptor (Pullar et al., in press). In vitro release studies have shown LY393558 to potentiate 5-HT release from guinea pig cortical slices following potassium stimulation (Pullar et al., in press). These studies were conducted in the presence of a maximally effective concentration of paroxetine, and the increase in 5-HT release has been ascribed to an inhibition of presynaptic autoreceptors.

We now report the effects of LY393558 (1–20 mg/kg p.o.) on extracellular levels of 5-HT in vivo, using microdialysis. These studies show that acute oral administration increases extracellular levels of 5-HT in both the guinea pig hypothalamus and rat frontal cortex by an impulse-dependent mechanism, and in both species, the levels reached are higher than following an acute, maximally effective dose of fluoxetine or paroxetine. Chronic studies in rats show that the microdialysate 5-HT concentration in the frontal cortex achieved after an acute challenge with LY393558 (5 mg/kg p.o.) was significantly greater than after a chronic regime of fluoxetine treatment (24 h after 10 mg/kg/day for 21 days). Furthermore, in rats treated chronically with LY393558 for 21 days (5 mg/kg p.o./day), the mean basal concentration 24 h after the last pretreatment was of the same magnitude as that obtained in animals receiving chronic fluoxetine. However, unlike that seen in fluoxetine-pretreated animals, a subsequent challenge with drug in LY393558-treated animals still elicited a further increase in extracellular 5-HT.

Presynaptic autoreceptors limit the ability of selective serotonin reuptake inhibitors to elevate synaptic concentrations of 5-HT, as reported previously (Rollema et al., 1996; Roberts et al., 1997), but also shown here with the 5-$HT_{1B/1D}$ antagonist GR127935 in both the guinea pig hypothalamus and rat frontal cortex. Such an effect may have consequences for the therapeutic activity of selective serotonin reuptake inhibitors in the treatment of depression. It is well known that one major drawback in the treatment of depression with selective serotonin reuptake inhibitors is the time for onset of clinical activity—therapeutic improvement is not immediate, but requires treatment for 2–4 weeks (Montgomery, 1995). One hypothesis proposed to explain this therapeutic delay is the time for autoreceptors to down-regulate and for higher synaptic concentrations to be achieved (Goodwin, 1996; Leonard, 1996). Preclinically, there is evidence for autoreceptor desensitisation following chronic 5-HT reuptake inhibition. For instance, chronic pretreatment of rats with citalopram has been reported to reduce the efficacy of the 5-HT receptor agonist lysergic acid diethylamine (LSD) to inhibit electrically evoked release from hypothalamic slices in vitro (Moret and Briley, 1990), and chronic paroxetine, to reduce the effect of CP-93,129 to inhibit electrically evoked release from dorsal raphé slices (Davidson and Stamford, 2000). In vivo microdialysis studies have shown that, the nonselective 5-$HT_{1B}$ receptor antagonist methiothepin, has a greater maximal effect on 5-HT outflow from rats treated acutely with citalopram than those treated chronically (Moret and Briley, 1996), while chronic administration of the tricyclic antidepressant clomipramine, which has a high affinity for the 5-HT transporter, also reduces the sensitivity of presynaptic 5-$HT_{1B}$ receptors, as measured by the response to the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (Newman et al., 2000).

It follows that one way to circumvent the time delay for autoreceptor desensitisation is to pharmacologically antagonise autoreceptor function (Matzen et al., 2000; Moret and Briley, 2000). An agent combining 5-HT reuptake inhibition with inhibition of 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors, would be expected on acute administration, to elevate synaptic 5-HT to a concentration that can be achieved by the chronic administration of a selective serotonin reuptake inhibitor. In so doing, such a drug may have a shorter onset of antidepressant activity. Following acute administration of LY393558, the increase in extracellular levels of 5-HT far exceeded those evoked by a maximally effective acute dose of fluoxetine, where activation of terminal (and somatodendritic) autoreceptors limit the response. As indicated above, chronic administration of a selective serotonin reuptake inhibitor desensitises autoreceptor processes, and as shown in these studies in rats, chronic treatment with fluoxetine produced basal concentrations of 5-HT in the microdialysate fraction that exceeded those evoked by acute fluoxetine treatment. In agreement with the proposed mechanism, the level of 5-HT evoked by an acute LY393558 administration was equivalent to that obtained after chronic fluoxetine. Moreover, chronic treatment with LY393558 did not alter the magnitude of the response to a subsequent drug challenge, and the extracellular concentration of 5-HT reached was still higher than that produced by acute fluoxetine, and as high as that achieved after chronic fluoxetine treatment.

In conclusion, LY393558 is a potent inhibitor of the 5-HT transporter and an antagonist of 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors. In vitro release studies have shown the compound to be a potent inhibitor at the terminal autoreceptor (Pullar et al., in press). In vivo, LY393558 increases extracellular levels of 5-HT above those evoked by a maximally effective acute dose of fluoxetine, and to levels that are normally only seen after chronic fluoxetine treatment. Its neurochemical profile in vivo suggests that it may be a more effective antidepressant with the potential for producing an earlier onset of clinical activity than selective serotonin reuptake inhibitors.

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