



# RS-127445: a selective, high affinity, orally bioavailable 5-HT<sub>2B</sub> receptor antagonist

\*<sup>1</sup>Douglas W. Bonhaus, <sup>1</sup>Lee A. Flippin, <sup>1</sup>Robert J. Greenhouse, <sup>1</sup>Saul Jaime, <sup>1</sup>Cindy Rocha, <sup>1</sup>Mark Dawson, <sup>1</sup>Kristine Van Natta, <sup>1</sup>L.K. Chang, <sup>1</sup>Tess Pulido-Rios, <sup>1</sup>Andrea Webber, <sup>1,2</sup>Edward Leung, <sup>1</sup>Richard M. Eglén & <sup>1</sup>Graeme R. Martin

<sup>1</sup>Departments of Molecular Pharmacology, Medicinal Chemistry and Metabolism Bioanalysis and Pharmacokinetics, Center for Biological Research, Neurobiology Unit, Roche Bioscience, Palo Alto, California, CA 94304, U.S.A.

**1** Efforts to define precisely the role of 5-HT<sub>2B</sub> receptors in normal and disease processes have been hindered by the absence of selective antagonists. To address this deficiency, we developed a series of naphthylpyrimidines as potentially useful 5-HT<sub>2B</sub> receptor antagonists.

**2** RS-127445 (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine) was found to have nanomolar affinity for the 5-HT<sub>2B</sub> receptor ( $pK_i = 9.5 \pm 0.1$ ) and 1,000 fold selectivity for this receptor as compared to numerous other receptor and ion channel binding sites.

**3** In cells expressing human recombinant 5-HT<sub>2B</sub> receptors, RS-127445 potently antagonized 5-HT-evoked formation of inositol phosphates ( $pK_B = 9.5 \pm 0.1$ ) and 5-HT-evoked increases in intracellular calcium ( $pIC_{50} = 10.4 \pm 0.1$ ). RS-127445 also blocked 5-HT-evoked contraction of rat isolated stomach fundus ( $pA_2 = 9.5 \pm 1.1$ ) and ( $\pm$ )- $\alpha$ -methyl-5-HT-mediated relaxation of the rat jugular vein ( $pA_2 = 9.9 \pm 0.3$ ). RS-127445 had no detectable intrinsic activity in these assays.

**4** In rats, the fraction of RS-127445 that was bioavailable *via* the oral or intraperitoneal routes was 14 and 60% respectively. Intraperitoneal administration of RS-127445 (5 mg kg<sup>-1</sup>) produced plasma concentrations predicted to fully saturate accessible 5-HT<sub>2B</sub> receptors for at least 4 h.

**5** In conclusion, RS-127445 is a selective, high affinity 5-HT<sub>2B</sub> receptor antagonist suitable for use *in vivo*. The therapeutic potential of this molecule is being further evaluated.

**Keywords:** 5-HT; serotonin; 5-HT<sub>2B</sub> receptor; RS-127445

**Abbreviations:** RS-127445, (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine); SB 204741, (N-(1-methyl-1H-indol-5-yl)N'-(benzyl)-2,3,4,9-tetrahydro-1H- $\beta$ -carboline); U46619, (9,11-dideoxymethyl-5-isothiazolyl) urea); LY-23728, (1-(3,4-dimethoxy-3- $\alpha$ , 11  $\alpha$ -methano-epoxy-PGF<sub>2</sub> $\alpha$ ); maximum plasma concentration, ( $T_{max}$ ); maximum plasma concentration, ( $C_{max}$ ); The plasma half-life, ( $T_{1/2}$ ); Elimination rate constant, ( $\beta$ ); The area under the concentration-time curve from 0–8 hours, ( $AUC_{0-8}$ ); HEK, (human embryonic kidney); CHO, (Chinese hamster ovary)

## Introduction

The 5-HT<sub>2B</sub> receptor is a G-protein coupled receptor that was first identified in rat stomach fundus (Clineschmidt *et al.*, 1985; Baez *et al.*, 1990). Initially termed a 5-HT<sub>1</sub>-like or 5-HT<sub>2F</sub> receptor (Kursar *et al.*, 1992), its subsequent cloning and functional expression revealed structural and transduction properties similar to 5-HT<sub>1C</sub> and 5-HT<sub>2</sub> receptors (Foguet *et al.*, 1992; Kursar *et al.*, 1994). This led to a reorganization of the 5-HT<sub>2</sub> receptor family with this receptor being designated as the 5-HT<sub>2B</sub> receptor (Hoyer *et al.*, 1994; Baxter *et al.*, 1995; Hoyer & Martin, 1997).

5-HT<sub>2B</sub> receptors are widely expressed in peripheral tissues of various species. 5-HT<sub>2B</sub> receptor mRNA or protein immunoreactivity has been found throughout the gastrointestinal tract including smooth muscle of the stomach fundus, oesophagus, small intestine and colon. The receptor has also been found in the placenta, uterus, lung and prostate. 5-HT<sub>2B</sub> receptors are present in many vascular beds and have been localized to both vascular smooth muscle and vascular endothelial cells (Schmuck *et al.*, 1994; Kursar *et al.*, 1994; Bonhaus *et al.*, 1995; Ullmer *et al.*, 1995; 1996; Choi & Maroteaux, 1996).

Since activation of 5-HT<sub>2B</sub> receptors has been shown to contract stomach and intestinal smooth muscle (Vane, 1959; Baxter *et al.*, 1994; Borman & Burleigh, 1995), it is likely that a primary function of these receptors is to mediate contractile responses to 5-HT in those smooth muscle tissues in which it is expressed. However, recent findings suggest that 5-HT<sub>2B</sub> receptors in vascular endothelium may mediate a nitric oxide-dependent relaxation (Glusa & Richter, 1993; Bodelsson *et al.*, 1993; Ellis *et al.*, 1995). Thus while the predominant function of peripheral 5-HT<sub>2B</sub> receptors in most smooth muscle tissues may be to mediate contraction, their role in vascular tissues may be more complex, depending on the specific vascular bed examined and the pathological state of the tissue (Watts *et al.*, 1996).

Efforts to obtain a more complete understanding of peripheral 5-HT<sub>2B</sub> receptors in normal and pathological states have been hampered by the absence of suitable high affinity, selective ligands. Most antagonists commonly used to characterize the function of 5-HT<sub>2</sub> receptors, such as mianserin, ritanserin, mesulergine and methysergide, fail to discriminate between 5-HT<sub>2</sub> receptor subtypes. Recently, several 5-HT<sub>2B</sub>/5-HT<sub>2C</sub> selective antagonists, with low affinity for the 5-HT<sub>2A</sub> receptor, have been described (Forbes *et al.*, 1995; Nozulak *et al.*, 1995; Kennett *et al.*, 1996). However, most of these ligands fail to adequately discriminate between the 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptor subtypes (Baxter, 1996).

\* Author for correspondence at: Roche Bioscience, 3401 Hillview Avenue, Building R2-101, Palo Alto, CA 94303, USA E-mail: doug.bonhaus@roche.com <sup>2</sup>Current address: Medco Research Inc. P.O.Box 13886, Research Triangle Park, NC 27709, USA

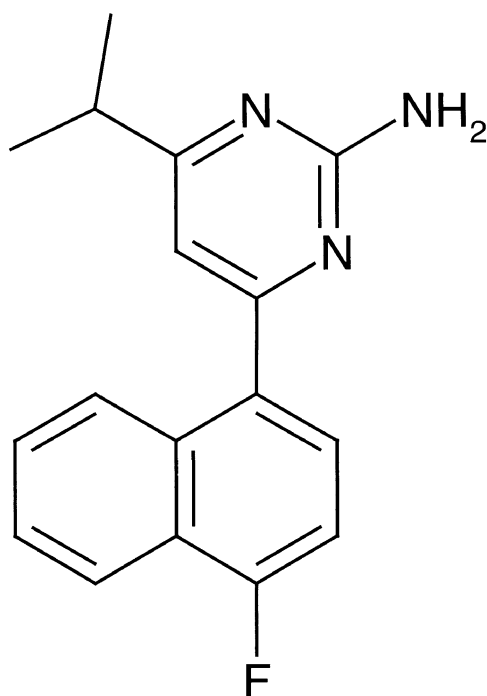
Recently several 5-HT<sub>2B</sub> selective ligands have emerged. These include SB 204741 (N-(1-methyl-1H-indol-5-yl)N'-(3-methyl-5-isothiazoyl) urea) which has 100 fold selectivity for the human recombinant 5-HT<sub>2B</sub> receptor, albeit with relatively low affinity (Bonhaus *et al.*, 1995; Baxter, 1996) and a series of compounds, based on a  $\beta$ -carboline structure, (Audia *et al.*, 1996). These ligands (e.g., LY-23728: 1-(3,4-dimethoxybenzyl)-2,3,4,9-tetrahydro-1H- $\beta$ -carboline) are selective for the 5-HT<sub>2B</sub> receptor as compared to the 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors and have relatively high affinity, but their overall receptor specificity has not been reported nor their suitability for *in vivo* studies.

To further define the function of 5-HT<sub>2B</sub> receptors, and ultimately to test the clinical utility of 5-HT<sub>2B</sub> antagonists in various disease states, we initiated a programme aimed at developing selective, high affinity 5-HT<sub>2B</sub> receptor antagonists suitable for *in vivo* applications. These efforts yielded a series of naphthylpyrimidines having high affinity and selectivity for the 5-HT<sub>2B</sub> receptor. The *in vitro* pharmacology and *in vivo* pharmacokinetic properties of one of these compounds, RS-127445(2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine) is described in Figure 1.

## Methods

### Cell culture

HEK-293 cells stably expressing human recombinant 5-HT<sub>2B</sub> receptors were maintained in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and 250  $\mu\text{g ml}^{-1}$  G-418. CHO-K1 cells stably expressing the human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> or 5-HT<sub>2C</sub> receptors were maintained in Ham's F-12 supplemented with 10% newborn calf serum and 300  $\mu\text{g ml}^{-1}$  G-418. All cells were cultured in a humidified atmosphere at 37°C.



**Figure 1** Structure of RS-127445 (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine).

### Radioligand binding

CHO-K1 cells expressing human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> or 5-HT<sub>2C</sub> receptors were harvested using 2 mM EDTA in phosphate buffered saline. Cell membranes were prepared by four cycles of homogenization (Brinkman P10 disrupter) and centrifugation (48,000  $\times g$  for 15 min). As previously described, each assay was established so as to achieve steady state conditions and to optimize specific binding (Bonhaus *et al.*, 1995). For the 5-HT<sub>2A</sub> receptor, membranes from  $1 \times 10^6$  cells were incubated with 0.2 nM [<sup>3</sup>H]-ketanserin at 32°C for 60 min. Nonspecific binding was determined using 10  $\mu\text{M}$  methysergide. For the 5-HT<sub>2B</sub> receptor, membranes from  $1.5 \times 10^6$  cells were incubated with 0.2 nM [<sup>3</sup>H]-5-HT at 4°C for 120 min. Nonspecific binding was determined using 10  $\mu\text{M}$  5-HT. For the 5-HT<sub>2C</sub> receptor, membranes from  $3 \times 10^5$  cells were incubated with 0.5 nM [<sup>3</sup>H]-mesulergine at 32°C for 60 min. Nonspecific binding was determined using 10  $\mu\text{M}$  methysergide. Assays were terminated by vacuum filtration through glass fibre filters (GF/B) which had been pretreated with 0.1% polyethylenimine. Total and bound radioactivity was determined by liquid scintillation counting. Greater than 90% specific binding was achieved in each of these assays.

The selectivity of RS-127445 for 5-HT<sub>2B</sub> receptors was further examined by testing the compound for affinity at over 100 additional ion channel or receptor binding sites. These studies were conducted by Cerep (Celle L'Evescault France) using standard protocols.

### Inhibition of 5-HT evoked formation of inositol phosphates

HEK-293 cells expressing the human 5-HT<sub>2B</sub> receptor were incubated with [<sup>3</sup>H]-myoinositol (1.67  $\mu\text{Ci ml}^{-1}$ ) in 162 cm<sup>2</sup> flasks overnight at 37°C in an inositol free Ham's F12 medium containing 10% dialyzed foetal bovine serum. The cells were harvested, washed five times with phosphate buffered saline and resuspended in inositol free Ham's F12 media at density of approximately  $3 \times 10^6$  cells ml<sup>-1</sup>. RS-127445 (10  $\mu\text{M}$ ) was initially dissolved in 10% (v v<sup>-1</sup>) DMSO with 90% inositol free Ham's F12 medium. Subsequent dilutions were made with inositol free Ham's F12 medium. 5-HT was dissolved in inositol free Ham's F12 medium containing 100 mM LiCl and 1 mM ascorbate. RS-127445, vehicle or other antagonists were pre-incubated with 240  $\mu\text{l}$  of cell suspension at 37°C for 20 min. The reactions were initiated by addition of 5-HT. Sixty minutes later, the reactions were terminated by adding 50  $\mu\text{l}$  of ice-cold 20% perchloric acid, chilled in an ice-water bath for 10 min and then neutralized with 160  $\mu\text{l}$  of 1 N KOH. Each sample was diluted with 2 ml of 50 mM Tris-HCl, pH 7.4 at room temperature. The aqueous portion (2.2 ml) was transferred onto Dowex AG1X8 columns (1 ml, 1:1, w v<sup>-1</sup>) which had been washed with 5 ml of distilled water. The columns were then washed with 18 ml of distilled water and the inositol phosphates were eluted with 3 ml of 1 N HCl. The eluted radioactivity was determined by liquid scintillation spectroscopy using a Packard 1900CA analyzer.

### Inhibition of 5-HT evoked increases in intracellular calcium

HEK-293 cells expressing human 5-HT<sub>2B</sub> receptors were seeded in 96-well poly-D-lysine coated plates at a density of 50,000 cells well<sup>-1</sup> in Dulbecco's modified Eagle's medium containing 10% (v v<sup>-1</sup>) dialyzed foetal bovine serum and

250  $\mu\text{g ml}^{-1}$  G-418. After an overnight incubation, the cells were washed three times with Hank's buffered saline solution containing 2 mM  $\text{CaCl}_2$ , 10 mM HEPES and 2.5 mM probenecid. The cells were incubated with 2  $\mu\text{M}$  Fluo-3 AM in Hank's buffered saline solution for 60 min at 37°C. The Fluo-3 loaded cells were then washed four times with Hank's buffered saline solution.

Antagonists were added at least 40 min prior to the addition of agonist. Agonist induced changes in intracellular calcium concentrations were determined at room temperature using a Fluorometric Imaging Plate Reader (Molecular Devices Corporation, Sunnyvale, CA, U.S.A.).

#### *Inhibition of 5-HT evoked contraction of the isolated rat stomach fundus*

Functional responses in rat isolated stomach were measured as described by Baxter *et al.* (1994). Male Sprague-Dawley rats were euthanized by  $\text{CO}_2$  asphyxiation. Longitudinal stomach fundus muscle, devoid of the mucosal layer, was mounted in the longitudinal plane under 1 g tension in a 10 ml organ bath containing Tyrode's physiological salt solution (pH 7.4 at 37°C). Antagonists were equilibrated with the tissue for at least 1 h.

Concentration-response curves to 5-HT were then generated in the presence of 0.1 mM pargyline, 30  $\mu\text{M}$  cocaine and 30  $\mu\text{M}$  corticosterone.

#### *Inhibition of ( $\pm$ ) $\alpha$ -methyl-5-HT evoked relaxation of the rat jugular vein*

Sprague-Dawley rats were euthanized as described above. Right and left external jugular veins were dissected, cleaned of connective tissues and cut into ring segments approximately 5 mm long. Tungsten hooks (0.125 mm diameter) were inserted through the lumen of the vein and connected to tension transducers. Tissues were kept in 10 ml organ baths containing Krebs's solution supplemented with cocaine (30  $\mu\text{M}$ ), corticosterone (30  $\mu\text{M}$ ), ketanserin (0.3  $\mu\text{M}$ ) and indomethacin (3  $\mu\text{M}$ ) at 37°C at a resting tension of 0.5 g. Prior to the initiation of any studies, monamine oxidases were inactivated by a 30 min pre exposure of the tissue to pargyline (0.1 mM). The veins were then exposed to 0.1  $\mu\text{M}$  U46619 (9,11-dideoxy-9  $\alpha$ , 11  $\alpha$ -methano-epoxy-PGF<sub>2</sub> $\alpha$ ; a thromboxane A<sub>2</sub> mimetic) until a stable contraction was attained. Acetylcholine (0.1  $\mu\text{M}$ ) was used to verify the integrity of the endothelium and to determine the maximum amount of nitric oxide-dependent relaxation that was achievable. After washout of the acetylcholine and recontraction with U46619, cumulative concentration-response curves to ( $\pm$ )- $\alpha$ -methyl-5-HT were constructed. When maximum relaxation was reached, the baths were rinsed, and the tissues were maintained undisturbed for 2 h. Antagonists were then added to the bath and allowed to equilibrate with the tissue for at least 1 h before a second concentration-response curve to ( $\pm$ )- $\alpha$ -methyl-5-HT was generated.

#### *Pharmacokinetics of RS-127445 in rats*

Male Sprague-Dawley rats (200 g) were allowed to acclimatize for at least 2 days. The animals were fasted overnight prior to dosing and for 4 h following dosing. Water was allowed *ad libitum*.

To compare the plasma kinetics of RS-127445 following different routes of administration, 90 rats were distributed into three treatment groups of 30 rats each. A single dose of RS-

127445 (5 mg  $\text{kg}^{-1}$ ) dissolved (2.5 mg  $\text{ml}^{-1}$ ) in ethanol:propylene glycol:water (10:50:40, v v v<sup>-1</sup>), was administered to each rat. At 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 h after dosing, the rats were anaesthetized and blood samples were collected by cardiac puncture.

The relationship between the dose of RS-127445 and the achieved plasma concentration was also examined. Male Sprague-Dawley rats were distributed into three treatment groups. The rats within each group (40) received 1.0, 3.0 or 10.0 mg of RS-127445 by the intraperitoneal route. A blood sample was then collected at 0.5, 1.0, 2.0, 3.0 or 4.0 h following dosing. No more than one blood sample was taken from any one rat. Plasma was stored frozen at -20°C until analysed.

An internal standard was added to aliquots of plasma (0.5 ml) and adjusted to pH 7.2 with phosphate buffer. RS-127445 and the internal standard were extracted into ethyl acetate:hexane (1:9, v v<sup>-1</sup>). The organic extract was evaporated to dryness under a stream of nitrogen at 38°C and dissolved in methanol:phosphate buffer (pH 3.0). An aliquot of the dissolved extract was subjected to reverse-phase HPLC on a 150  $\times$  4.6 mm, 5  $\mu\text{m}$  C18 column using a mobile phase of 40% acetonitrile in 50 mM sodium phosphate buffer containing 20 mM 1-heptane sulfonic acid (pH 3.0) at a flow rate of 1 ml  $\text{min}^{-1}$ . RS-127225 was detected at a wavelength of 305 nm. The lower limit of quantification of the assay was 4 ng  $\text{ml}^{-1}$ .

#### *Methods of data analysis*

For competition binding curves IC<sub>50</sub> values were obtained using non-linear iterative curve fitting routines and a logistic, four parameter (variable Hill slope) equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / 1 + 10^{(\text{LogIC}_{50} - X)\text{Hill Slope}}$$

(Prism-GraphPad Software Inc., San Diego CA, U.S.A.). When Hill slopes were found to be not different than 1.0, estimates of affinity ( $K_i$  values) were obtained using the Cheng-Prusoff equation (Cheng & Prusoff, 1973). The data are presented as a negative log of the  $K_i$  value (p $K_i$ ).

For the inositol phosphate studies, EC<sub>50</sub> values for 5-HT concentration-response curves were generated. Estimates of affinity (p $K_B$  values) were then obtained using Schild regression analysis of the ratios of EC<sub>50</sub> values determined in the presence of at least three different concentrations of antagonist. Regression lines with slopes not significantly different from 1.0 were constrained to 1.0.

For the calcium influx studies, IC<sub>50</sub> values for antagonist inhibition of 5-HT evoked responses were generated. Since the endpoint of this assay is achieved within seconds of adding the agonist there is no opportunity for the antagonist to re-equilibrate to new steady-state levels of binding. Thus, no attempt was made to generate affinity estimates on the basis of shifts in agonist-response curves.

For the functional studies using rat isolated stomach fundus or jugular vein, agonist concentration-response curves were generated and Schild regression analysis was conducted using at least three different concentrations of antagonist. However, as RS-127445 produced a decrease in the maximum response to 5-HT or ( $\pm$ )- $\alpha$ -methyl-5-HT, results from these studies are presented as pA<sub>2</sub> rather than p $K_B$  values.

Pharmacokinetic calculations were conducted using standard analytical methods. When concentrations of RS-127445 were below the quantification limit of the assay, the values were set to zero. The peak time to maximum plasma

concentration ( $T_{max}$ ) and the maximum plasma concentration ( $C_{max}$ ) values were estimated by inspection of the plots of the log plasma concentration versus time. The area under the concentration-time curve from 0–8 h ( $AUC_{0-8}$ ) was calculated using the linear trapezoidal rule. The plasma half-life ( $T_{1/2}$ ) was determined using the relationship  $0.693/\beta$ , where  $\beta$  is the elimination rate constant calculated by linear regression analysis of the terminal phase of the log plasma concentration vs time curve. Bioavailability was calculated by dividing the  $AUC_{0-8}$  obtained following oral or intraperitoneal dosing by the  $AUC_{0-8}$  obtained with intravenous administration of RS-127445.

## Materials

[<sup>3</sup>H]-Mesulergine (75 Ci mmol<sup>-1</sup>, 99% pure), [<sup>3</sup>H]-myoinositol (18.6 Ci mmol<sup>-1</sup>, 99% pure and [<sup>3</sup>H]-5-HT (91 Ci mmol<sup>-1</sup>, 99% pure) were obtained from Amersham International (Cardiff, Wales, U.K.). [<sup>3</sup>H]-Ketanserin (62 Ci mmol<sup>-1</sup>, 99% pure) was obtained from Dupont-NEN Life Sciences (Boston, MA, U.S.A.). Ritanserin, mianserin, yohimbine and other ligands were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or Mallinckrodt Baker, Inc (Paris, KY, U.S.A.). LY23728 (1-(3,4-dimethoxy-benzyl)-2,3,4,9-tetrahydro-1H- $\beta$ -carboline) and RS-127445 were synthesized in the Department of Chemistry, Neurobiology Unit, Roche Bioscience. The fluorescent probe Fluo-3 AM was obtained from Molecular Probes (Eugene, OR, U.S.A.). Analytical solvents were HPLC grade and were purchased from Burdick and Jackson (Muskegon, MI, U.S.A.). The HPLC column used to isolate RS-127445 was a Higgins Analytical HAI-sil C18 column (Higgins Analytical, Inc., Mountain View, CA, U.S.A.). Hank's Balanced Salt Solution, Dulbecco's Modified Eagle Medium and other reagents for tissue culture were obtained from Gibco BRL (Grand Island, NY, U.S.A.).

## Results

### Radioligand binding

RS-127445 potently displaced [<sup>3</sup>H]-5-HT from human recombinant 5-HT<sub>2B</sub> receptors expressed in CHO-K1 cells. The affinity ( $pK_i$  value) of RS-127445 for the 5-HT<sub>2B</sub> receptor was  $9.5 \pm 0.1$  ( $n=9$ ). RS-127445 was selective for the 5-HT<sub>2B</sub> receptor, having approximately 1000 fold lower affinity for

the human recombinant 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>5</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors, a 5-HT<sub>1A</sub> receptor in rat brain membranes, a 5-HT<sub>1B/D</sub> receptor in bovine caudate, and a monoamine uptake site in rabbit platelets (Table 1).

RS-127445 (10  $\mu$ M tested in duplicate) had no appreciable affinity (less than 50% inhibition of specific binding) at the following binding sites: adenosine A<sub>1</sub> and A<sub>2A</sub> receptors; adrenergic  $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  receptors; adenosine, choline, dopamine and serotonin uptake sites; angiotensin AT<sub>1</sub> receptors; Atrial Natriuretic Factor receptor; bombesin and; bradykinin B<sub>1</sub> and B<sub>2</sub> receptors; Calcitonin Gene Related Peptide receptor; the benzothiazepine, dihydropyridine and phenylalkylamine binding sites on L-type Ca<sup>2+</sup> channels; a conotoxin (GVIA) binding site on N-type Ca<sup>2+</sup> channels; cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors; the cholecystokinin CCK<sub>A</sub> and CCK<sub>B</sub> receptors; dopamine D<sub>1</sub>, D<sub>2L</sub>, D<sub>2S</sub>, D<sub>4.2</sub>, D<sub>4.4</sub>, D<sub>4.7</sub> and D<sub>5</sub> receptors; endothelin ET<sub>A</sub> and ET<sub>B</sub> receptors; an EGF binding site; an estrogen receptor; the GABA, benzodiazepine and picrotoxin binding sites on the GABA receptor; a peripheral benzodiazepine binding site; the galanin and glucocorticoid receptors; the AMPA and kainate glutamate receptors; the NMDA, glycine and phencyclidine binding sites on the NMDA receptor; the strychnine-sensitive glycine receptor; histamine H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptors; the inositol trisphosphate binding site; an insulin receptor; interleukin 1 $\alpha$ , 2, 6 and 8 receptors; the leukotriene B<sub>4</sub> and D<sub>4</sub> receptors; muscarinic M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> receptors; neurokinin NK<sub>1</sub> receptors; neuropeptide Y<sub>1</sub> and Y<sub>2</sub> receptors; a neurotensin receptor, a central nicotinic acetylcholine receptor,  $\delta$ ,  $\kappa$  and  $\mu$  opiate receptors; a phorbol ester binding site; a platelet activating factor receptor; the platelet derived growth factor receptor; the K<sub>A</sub>, K<sub>ATP</sub>, K<sub>V</sub> and SK<sub>Ca</sub> potassium channels; the progesterone and purinergic P<sub>2X</sub> receptors; sigma  $\sigma_1$  and  $\sigma_2$  binding sites; the batrachotoxin binding site on sodium channels; the somatostatin, testosterone and thromboxane A<sub>2</sub> receptors and, binding sites on the TRH, TGF- $\beta$ , TNF- $\alpha$ , VIP<sub>1</sub> and vasopressin V<sub>1</sub> receptors.

### Effect of RS-127445 on 5-HT evoked increases in the formation of inositol phosphates

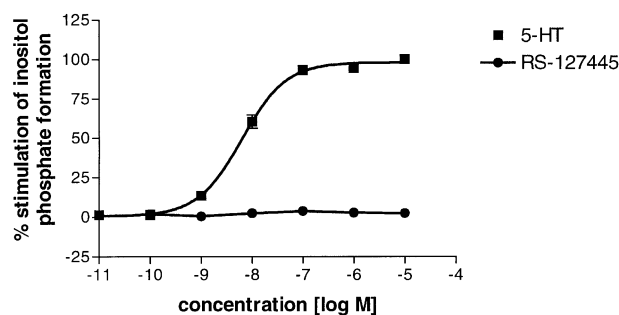
5-HT concentration-dependently increased the rate of formation of inositol phosphates in HEK-293 cells expressing the human recombinant 5-HT<sub>2B</sub> receptor ( $pEC_{50}$  value of  $8.2 \pm 0.1$ ). By contrast, RS-127445, at concentrations up to 100  $\mu$ M, had no stimulatory effect on inositol phosphate production (Figure 2).

RS-127445 potently inhibited the 5-HT evoked increase in the formation of inositol phosphates in cells expressing 5-HT<sub>2B</sub>

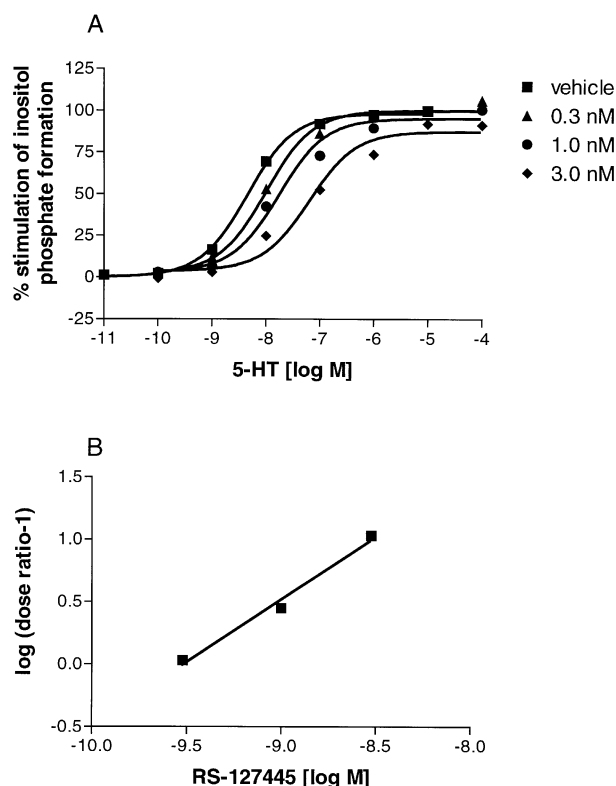
**Table 1** Affinity of RS-127445 at selected receptors

Receptor	$pK_i$	Hill slope
5-HT <sub>1A</sub>	$5.5 \pm 0.1$	$0.92 \pm 0.03$
5-HT <sub>1B/D</sub>	< 6.0	not determined
5-HT <sub>2A</sub>	$6.3 \pm 0.2$	$1.05 \pm 0.07$
5-HT <sub>2B</sub>	$9.5 \pm 0.1$	$1.16 \pm 0.07$
5-HT <sub>2C</sub>	$6.4 \pm 0.1$	$0.96 \pm 0.12$
5-HT <sub>3</sub>	< 6.0	not determined
5-HT <sub>5</sub>	< 6.0	not determined
5-HT <sub>6</sub>	< 6.0	not determined
Monoamine uptake	$5.7 \pm 0.1$	$1.10 \pm 0.9$

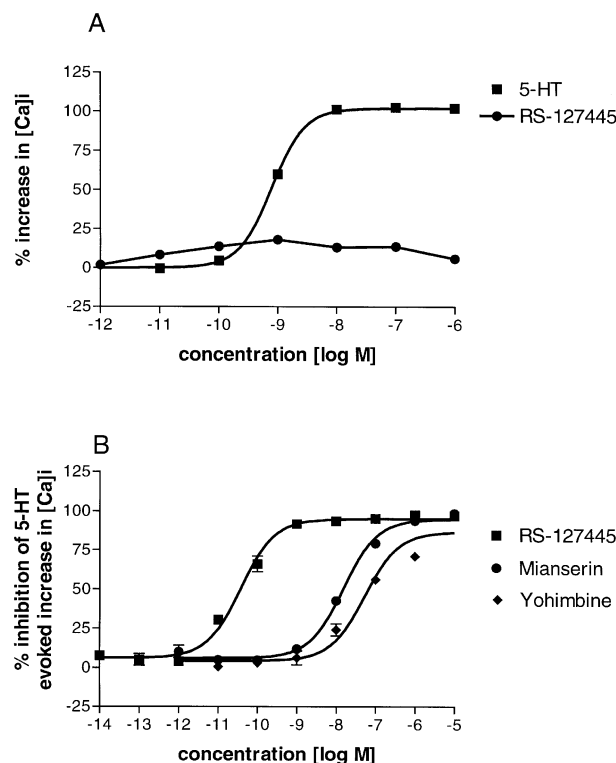
Values are the means  $\pm$  s.e.mean for at least three separate determinations. The data pertaining to the 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors were obtained as described in the Methods section. The data pertaining to the 5-HT<sub>1A</sub>, 5-HT<sub>1B/D</sub>, 5-HT<sub>3</sub>, 5-HT<sub>5</sub> and 5-HT<sub>6</sub> receptors and the monoamine uptake site were obtained from Cerep using standard protocols as described (Cerep, 1997 pharmacological model Catalogue).



**Figure 2** Effect of 5-HT and RS-127445 on the formation of [<sup>3</sup>H]-inositol phosphates in HEK-293 cells expressing the human 5-HT<sub>2B</sub> receptor. Each data point is the means  $\pm$  s.e.mean of three determinations (error bars are not shown when smaller than the symbol).



**Figure 3** (A) 5-HT mediated stimulation of inositol phosphate formation in the presence of ascending concentrations of RS-127445. Each data point is the means  $\pm$  s.e. mean of 12 different determinations. (B) Schild regression of the data in A.



**Figure 4** (A) Effects of 5-HT and RS-127445 on intracellular calcium concentrations in HEK-293 cells expressing the human 5-HT<sub>2B</sub> receptor. Each data point is the means  $\pm$  s.e. mean of four (RS-127445) or eleven (5-HT) different determinations. (B) Inhibition of 5-HT (10 nM) evoked increases in intracellular calcium concentrations by RS-127445, mianserin and yohimbine. Each data point is the means  $\pm$  s.e. mean of seven to eleven different determinations.

receptors, producing parallel rightward shifts in the concentration response curves of 5-HT (Figure 3A). Schild regression analysis generated a  $pK_B$  value of  $9.5 \pm 0.1$  with the slope of the Schild regression being not different from 1.0 ( $0.95 \pm 0.23$ ) (Figure 3B).

#### *Effect of RS-127445 on 5-HT evoked increases in intracellular calcium concentration*

5-HT concentration-dependently increased intracellular calcium concentrations in HEK-293 cells expressing 5-HT<sub>2B</sub> receptors ( $pEC_{50}$  value of  $9.2 \pm 0.1$ ) while RS-127445, at concentrations up to  $100 \mu M$ , had no stimulatory effect on calcium influx (Figure 4A).

RS-127445 potentially blocked the 5-HT (10 nM) evoked increases in intracellular calcium concentrations in the HEK-293 cells expressing the 5-HT<sub>2B</sub> receptor ( $pIC_{50}$  value of  $10.4 \pm 0.1$ ) (Figure 4B). The rank-order of potencies of a series of 5-HT<sub>2</sub> receptor antagonists in inhibiting 5-HT evoked inositol phosphate formation and 5-HT mediated increases in intracellular calcium concentrations were similar (Table 2).

#### *Effect of RS-127445 on 5-HT evoked contraction of the rat stomach fundus*

5-HT concentration-dependently contracted the isolated rat stomach fundus with an  $pEC_{50}$  value of  $8.3 \pm 0.2$ . By contrast, RS-127445, at concentrations from  $0.1$ – $10 \mu M$ , had no contractile effect on the isolated fundus.

RS-127445 potentially blocked the 5-HT evoked contraction of the isolated fundus. The concentration-response curves to 5-HT were shifted to the right in a concentration-dependent manner. There was also a progressive decrease in the maximum response (Figure 5A). The intercept value on the Schild regression line corresponded to a  $pA_2$  value of  $9.5 \pm 1.1$ . The slope of the regression was statistically significantly less than unity ( $0.58 \pm 0.09$ ) (Figure 5B).

To test whether slow dissociation kinetics may have accounted for the insurmountable nature of the antagonism produced by RS-127445, tissues were exposed to RS-127445 (10 nM) and then washed for varying periods of time. Extensive washing of the isolated stomach fundus resulted in a time-dependent reduction in the block produced by RS-127445. However, even after 60 min of washout, the magnitude of the remaining effect corresponded to 50% of the original concentration of RS-127445 (Figure 6).

#### *Effect of RS-127445 on ( $\pm$ ) $\alpha$ -methyl-5-HT evoked relaxation of the rat jugular vein*

The 5-HT<sub>2B</sub> receptor agonist ( $\pm$ ) $\alpha$ -methyl-5-HT, in a concentration-dependent manner, relaxed rat isolated jugular

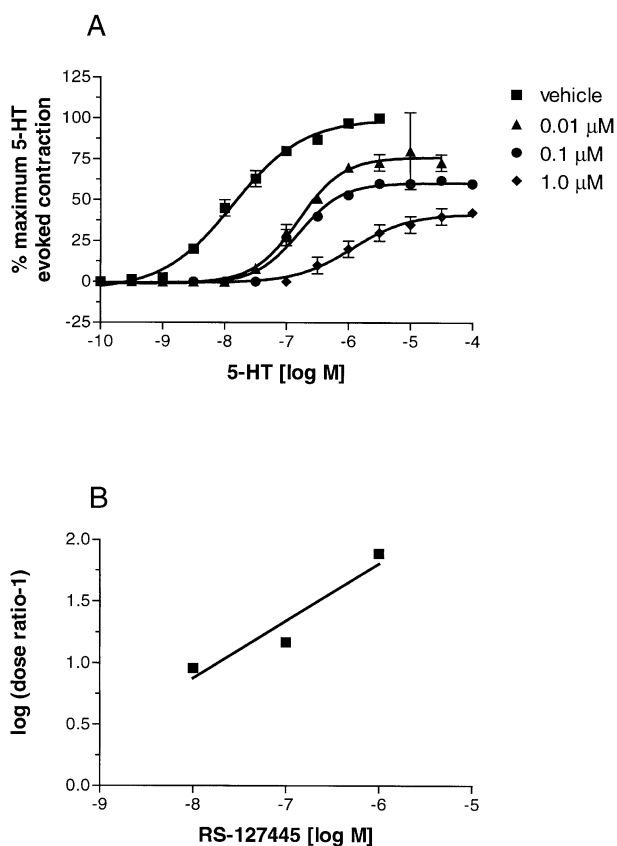
**Table 2** Affinity of selected ligands at human recombinant 5-HT<sub>2B</sub> receptors

Compound	Binding (pK <sub>i</sub> )	Inositol phosphate (pK <sub>B</sub> )	Intracellular calcium (pIC <sub>50</sub> )
RS-127445-190	$9.5 \pm 0.1$	$9.5 \pm 0.1$	$10.4 \pm 0.1$
LY23728	$9.0 \pm 0.1$	$9.5 \pm 0.2$	$8.8 \pm 0.02$
Ritanserin	$9.0 \pm 0.1$	$8.6 \pm 0.2$	$7.4 \pm 0.1$
Mianserin	$8.8 \pm 0.1$	$7.4 \pm 0.3$	$7.8 \pm 0.1$
Yohimbine	$7.5 \pm 0.03$	$7.7 \pm 0.1$	$7.3 \pm 0.1$

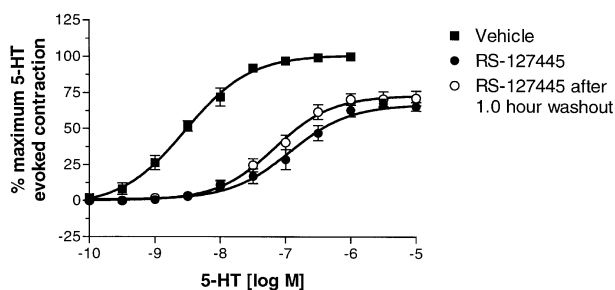
Values are the means  $\pm$  s.e. mean for at least three separate determinations.

veins which had been pre-contracted with the U44619. RS-127445, at concentrations up to 10  $\mu$ M had no relaxant effect on this preparation but blocked the ( $\pm$ )- $\alpha$ -methyl-5-HT mediated response.

As was found in the studies with the isolated stomach fundus, RS-127445 produced rightward shifts in the agonist-mediated concentration-response curves with progressive decreases in the maximum response attained by ( $\pm$ )- $\alpha$ -methyl-5-HT (Figure 7A). The intercept of the Schild regression line corresponded to a pA<sub>2</sub> value of  $9.95 \pm 0.07$  with a slope not statistically different than 1.0 ( $1.18 \pm 0.08$ ) (Figure 7B).



**Figure 5** (A) Inhibition of 5-HT-evoked contraction of the rat isolated stomach fundus by ascending concentrations of RS-127445. Each data point is the means  $\pm$  s.e. mean of at least three different determinations. (B) Schild regression analysis of the data in A.



**Figure 6** Effect of 1 h of washout on the inhibition of 5-HT-evoked contraction of the rat isolated stomach fundus produced by 0.1  $\mu$ M RS-127445. Each data point is the means  $\pm$  s.e. mean of at least three different determinations. Similar findings were obtained using 1.0  $\mu$ M RS-127445.

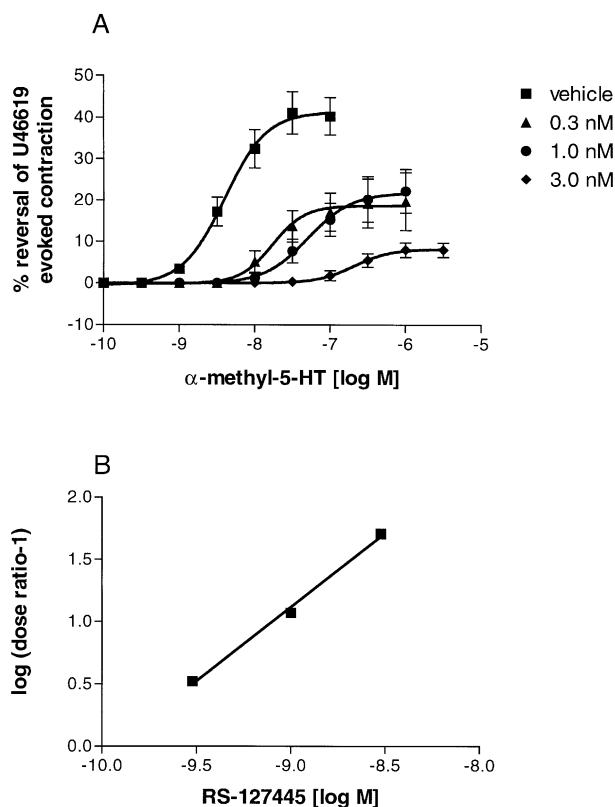
### In vivo studies with RS-127445

RS-127445 (5 mg kg<sup>-1</sup>) was administered to rats by oral, intraperitoneal and intravenous routes. Peak plasma concentrations were rapidly achieved with the highest concentrations being found at the first time-point measured following intravenous and intraperitoneal administration (0.08 h) and by 0.25 h following dosing by the oral route of administration. RS-127445 was cleared from plasma with an estimated terminal elimination half-life of approximately 1.7 h. The bioavailability of RS-127445, when administered by the oral and intraperitoneal routes was approximately 14 and 62% of that obtained by intravenous administration (Figure 8A). To test whether plasma levels were proportional to the dose administered, RS-127445 was given by the intraperitoneal route at doses of 1, 3 and 10 mg kg<sup>-1</sup>. Increasing the dose of RS-127445 resulted in proportional increases in its concentration in the plasma (Figure 8B).

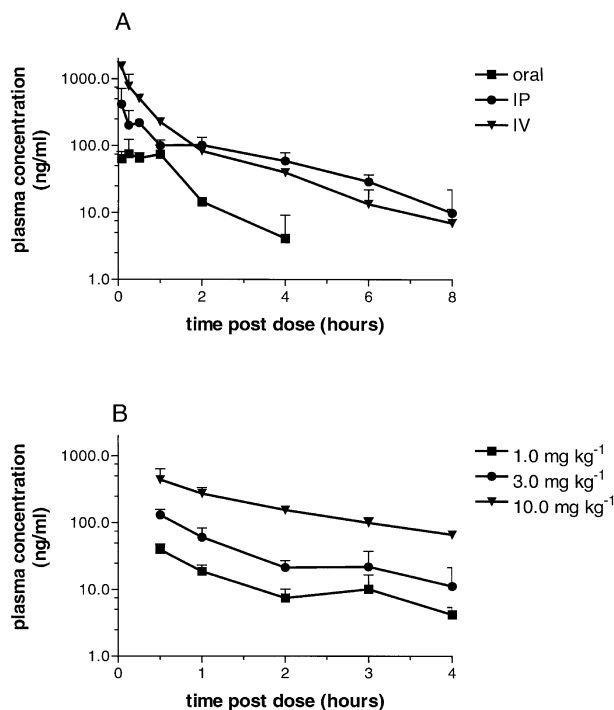
### Discussion

RS-127445 (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine) was discovered during the course of studies aimed at developing bioavailable, high affinity and selective 5-HT<sub>2B</sub> receptor antagonists. RS-127445 was found to have nM affinity and 1000 fold selectivity for the 5-HT<sub>2B</sub> receptor. RS-127445 is thus among the highest affinity, most selective 5-HT<sub>2B</sub> receptor ligands to be described (Audia *et al.*, 1996; Baxter, 1996).

The possibility that RS-127445 had agonist activity at 5-HT<sub>2B</sub> receptors was evaluated using cell-based assays and



**Figure 7** (A)  $\alpha$ -methyl-5-HT mediated relaxation of precontracted rat jugular vein in the presence of ascending concentrations of RS-127445. Each data point is the means  $\pm$  s.e. mean of at least 4–12 different determinations. (B) Schild regression of the data in Figure 7A.



**Figure 8** (A) Plasma concentrations of RS-127445 following dosing (5 mg kg<sup>-1</sup>) via various routes. Each data point represents the means  $\pm$  s.e. mean for three animals. The peak plasma concentrations of RS-127445 for these routes were 75.7  $\pm$  48.1, 417  $\pm$  298 and 1550  $\pm$  220 ng ml<sup>-1</sup> respectively. This entire experiment has been repeated at least three times with similar results. (B) Plasma concentrations of RS-127445 following intraperitoneal dosing with different doses of RS-127445. Each data point is the means  $\pm$  s.e. mean of eight animals.

HEK-293 cells expressing human recombinant 5-HT<sub>2B</sub> receptors. Transfected HEK-293 cells were used for these functional assays because the 5-HT<sub>2B</sub> receptor mediated responses were quantitatively greater in this cell line than in the transfected CHO-K1 cells. Both the phospholipase C-mediated conversion of myoinositol into inositol phosphates and the subsequent elevation in intracellular calcium concentrations were used as indices of 5-HT<sub>2B</sub> receptor activation (Foguet *et al.*, 1992; Schmuck *et al.*, 1994). In HEK-293 cells expressing human recombinant 5-HT<sub>2B</sub> receptors, 5-HT potently stimulated the formation of inositol phosphates and increased intracellular calcium concentrations. Whereas, RS-127445, at concentrations up to 10  $\mu$ M, had no stimulatory effect on the formation of inositol phosphates or on intracellular calcium concentrations. Thus, regardless of the endpoint used to measure receptor activation, RS-127445 had no detectable agonist activity at these human recombinant 5-HT<sub>2B</sub> receptors.

Consistent with it being a high affinity antagonist, RS-127445 potently blocked the 5-HT evoked increase in inositol phosphate formation. The results of Schild regression analysis were consistent with RS-127445 acting in a competitive manner and having nM affinity for the receptor. Also consistent with it being a high affinity antagonist, RS-127445 blocked the 5-HT evoked increases in intracellular calcium

concentrations with a potency 1000 times greater than that of yohimbine.

Since species-species differences in the pharmacological properties of 5-HT<sub>2B</sub> receptors have been reported (Wainscott *et al.*, 1996), the potency and activity of RS-127445 at endogenous rat 5-HT<sub>2B</sub> receptors was also examined. The most extensively characterized 5-HT<sub>2B</sub> receptor mediated functional response, and indeed the response that was first used to define the 5-HT<sub>2B</sub> receptor, is contraction of the isolated rat stomach fundus (Clineschmidt *et al.*, 1985; Baxter *et al.*, 1994; Cox & Cohen, 1995). Consistent with it being a 'silent' antagonist RS-127445 produced no detectable response in the isolated stomach fundus but, potently antagonized the contractile response produced by 5-HT. A second functional response which has been attributed to 5-HT<sub>2B</sub> receptors is relaxation of the rat jugular vein (Bodelsson *et al.*, 1993; Ellis *et al.*, 1995). As was found in the studies using isolated stomach fundus, RS-127445 had no detectable intrinsic activity but acted as an antagonist, potently blocking the relaxation produced by the 5-HT<sub>2B</sub> receptor agonist  $\alpha$ -methyl-5-HT. These findings demonstrate that RS-127445 is a potent antagonist, devoid of intrinsic activity at both endogenous rat and human recombinant 5-HT<sub>2B</sub> receptors.

One anomalous finding to arise from the functional studies using rat stomach fundus or jugular vein was that the inhibitory actions of RS-127445 could not be fully surmounted with increasing concentrations of agonist. This raised the possibility that RS-127445 may be acting by a noncompetitive mechanism. However, a more plausible explanation is that the experimental conditions did not allow sufficient time for steady-state conditions to be achieved following addition of the agonist (Kenakin, 1993). This possibility is supported by the finding that inhibitory actions of RS-127445 were not fully reversed even after washing the tissues in drug-free buffer for an hour.

To evaluate the suitability of RS-127445 for studying the role of 5-HT<sub>2B</sub> receptors *in vivo*, its pharmacokinetic properties in the rat were characterized. RS-127445 was rapidly absorbed following both oral and intraperitoneal administration with peak plasma concentrations being achieved within 15 min of dosing. Moreover, the plasma concentrations of RS-127445 that were achieved were proportional to the administered dose. There were no obvious dose or route-dependent limitations to the absorption of RS-127445. Approximately 60% of an intraperitoneal dose and 14% of the oral dose of RS-127445 (5 mg kg<sup>-1</sup>) was bioavailable. The plasma concentrations of RS-127445 that were generated (following intraperitoneal administration of 5 mg kg<sup>-1</sup>) exceeded 20 nM for at least 4 h following dosing. Thus plasma concentrations of RS-127445 which would be predicted to fully saturate accessible 5-HT<sub>2B</sub> receptors can be readily achieved and maintained in the rat.

In summary RS-127445 is a novel high affinity, selective 5-HT<sub>2B</sub> receptor antagonist devoid of detectable intrinsic activity. Moreover, the compound is readily absorbed by various routes of administration. Thus RS-127445 may prove useful in further *in vivo* studies of 5-HT<sub>2B</sub> receptor mediated processes and will be further evaluated as a potential therapeutic agent for the treatment of disorders in which activation of 5-HT<sub>2B</sub> receptors may play a pathological role.

## References

- AUDIA, J.E., EVARD, D.A., MURDOCH, G.R., DROSTE, J.J., NISSEN, J.S., SCHENCK, K.W., FLUDZINSKI, P., LUCAITES, V.L., NELSON, D.L. & COHEN, M.L. (1996). Potent, selective tetrahydro-*B*-carboline antagonists of the serotonin<sub>2B</sub> (5HT<sub>2B</sub>) contractile receptor in the rat stomach fundus. *J. Med. Chem.*, **39**, 2773–2780.
- BAEZ, M., YU, L. & COHEN, M.L. (1990). Pharmacological and molecular evidence that the contractile response to serotonin in rat stomach fundus is not mediated by activation of the 5-hydroxytryptamine<sub>1C</sub> receptor. *Mol. Pharmacol.*, **38**, 31–37.
- BAXTER, G., KENNETT, G., BLANEY, F. & BLACKBURN, T. (1995). 5-HT<sub>2</sub> receptor subtypes: a family re-united? *Trends Pharmacol. Sci.*, **16**, 105–110.
- BAXTER, G.S. (1996). Novel discriminatory ligands for 5-HT<sub>2B</sub> receptors. *Behav. Brain Res.*, **73**, 149–152.
- BAXTER, G.S., MURPHY, O.E. & BLACKBURN, T.P. (1994). Further characterization of 5-hydroxytryptamine receptors (the putative 5-HT<sub>2B</sub>) in rat stomach fundus longitudinal muscle. *Br. J. Pharmacol.*, **112**, 323–331.
- BODELSSON, M., TORNEBRANDT, K. & ARNEKLO-NOBIN, B. (1993). Endothelial relaxing 5-hydroxytryptamine receptors in the rat jugular vein: similarity with the 5-hydroxytryptamine<sub>1C</sub> receptor. *J. Pharmacol. Exp. Ther.*, **264**, 709–716.
- BONHAUS, D.W., BACH, C.E., DESOUZA, A., SALAZAR, F.H., MATSUOKA, B.D., ZUPPAN, P., CHAN, H.W. & EGLIN, R.M. (1995). The pharmacology and distribution of human 5-hydroxytryptamine<sub>2B</sub> (5-HT<sub>2B</sub>) receptor gene products: comparison with 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. *Br. J. Pharmacol.*, **115**, 622–628.
- BORMAN, R.A. & BURLEIGH, D.E. (1995). Functional evidence for a 5-HT<sub>2B</sub> receptor mediating contraction of longitudinal muscle in human small intestine. *Br. J. Pharmacol.*, **114**, 1525–1527.
- CHENG, Y.C. & PRUSOFF, W.H. (1973). Relationship between inhibition constant (K<sub>i</sub>) and the concentration of inhibitor which causes 50 percent inhibition (IC<sub>50</sub>) of an enzymatic reaction. *Biochem. Pharmacol.*, **92**, 881–894.
- CHOI, D.S. & MAROTEAUX, L. (1996). Immunohistochemical localisation of the serotonin 5-HT<sub>2B</sub> receptor in mouse gut, cardiovascular system, and brain. *FEBS Letters*, **391**, 45–51.
- CLINESCHMIDT, B.V., REISS, D.R., PETTIBONE, D.J. & ROBINSON, J.L. (1985). Characterisation of 5-Hydroxytryptamine receptors in rat stomach fundus. *J. Pharmacol. Exp. Ther.*, **235**, 696–708.
- COX, D.A. & COHEN, M.L. (1995). 5-Hydroxytryptamine<sub>2B</sub> receptor signaling in rat stomach fundus: role of voltage-dependent calcium channels, intracellular calcium release and protein kinase C. *J. Pharmacol. Exp. Ther.*, **272**, 143–150.
- ELLIS, E.S., BYRNE, C., MURPHY, O.E., TILFORD, N.S. & BAXTER, G.S. (1995). Mediation by 5-hydroxytryptamine<sub>2B</sub> receptors of endothelium-dependent relaxation in rat jugular vein. *Br. J. Pharmacol.*, **114**, 400–404.
- FOGUET, M., HOYER, D., PARDO, L.A., PAREKH, A., KLUXEN, F.W., KALKMAN, H.O., STUHMER, W. & LUBBERT, H. (1992). Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J.*, **11**, 3481–3487.
- FORBES, I.T., JONES, G.E., MURPHY, O.E., HOLLAND, V. & BAXTER, G.S. (1995). N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl) urea: a novel, high-affinity 5-HT<sub>2B</sub> receptor antagonist. *J. Med. Chem.*, **38**, 855–857.
- GLUSA, E. & RICHTER, M. (1993). Endothelium-dependent relaxation of porcine pulmonary arteries via 5-HT<sub>1C</sub>-like receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **347**, 471–477.
- HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). VII. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, **46**, 157–204.
- HOYER, D. & MARTIN, G. (1997). 5-HT receptor classification and nomenclature: Towards a harmonization with the human genome. *Neuropharmacology*, **36**, 419–428.
- KENAKIN, T. (1993). *Pharmacologic analysis of drug-receptor interaction*. New York: Raven Press, Ltd.
- KENNETT, G.A., WOOD, D.M., BRIGHT, F., CILIA, J., PIPER, D.C., GAGER, T., THOMAS, D., BAXTER, G.S., FORBES, I.T., HAM, P. & BLACKBURN, T.P. (1996). *In vitro* and *in vivo* profile of SB 206553, a potent 5-HT<sub>2C</sub>/5-HT<sub>2B</sub> receptor antagonist with anxiolytic-like properties. *Br. J. Pharmacol.*, **117**, 427–434.
- KURSAR, J.D., NELSON, D.L., WAINSCOTT, D.B. & BAEZ, M. (1994). Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine<sub>2B</sub> receptor. *Mol. Pharmacol.*, **46**, 227–234.
- KURSAR, J.D., NELSON, D.L., WAINSCOTT, D.B., COHEN, M.L. & BAEZ, M. (1992). Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine<sub>2B</sub>) from rat stomach fundus. *Mol. Pharmacol.*, **42**, 549–557.
- NOZULAK, J., KALKMAN, H.O., FLOERSHEIM, P., HOYER, D., SCHOEFFTER, P., BUERKI, H.R. (1995). (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo[1,7-bc][2,6]-naphthyridine: a 5-HT<sub>2C/2B</sub> receptor antagonist with low 5-HT<sub>2A</sub> receptor affinity. *J. Med. Chem.*, **38**, 28–33.
- SCHMUCK, K., ULLMER, C., ENGELS, P. & LUBBERT, H. (1994). Cloning and functional characterization of the human 5-HT<sub>2B</sub> serotonin receptor. *FEBS Letters*, **342**, 85–90.
- ULLMER, C., BODDEKE, H.G.W.M., SCHMUCK, K. & LUBBERT, H. (1996). 5-HT<sub>2B</sub> receptor-mediated calcium release from ryanodine-sensitive intracellular stores in human pulmonary artery endothelial cells. *Br. J. Pharmacol.*, **117**, 1081–1088.
- ULLMER, C., SCHMUCK, K., KALKMAN, H.O. & LUBBERT, H. (1995). Expression of serotonin receptor mRNAs in blood vessels. *FEBS Letters*, **370**, 215–221.
- VANE, J.R. (1959). The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. *Br. J. Pharmacol.*, **14**, 78–98.
- WAINSCOTT, D.B., LUCAITES, V.L., KURSAR, J.D., BAEZ, M. & NELSON, D.L. (1996). Pharmacologic characterization of the human 5-hydroxytryptamine<sub>2B</sub> receptor: Evidence for species differences. *J. Pharmacol. Exp. Ther.*, **276**, 720–727.
- WATTS, S.W., BAEZ, M. & WEBB, R.C. (1996). The 5-hydroxytryptamine<sub>2B</sub> receptor and 5-HT receptor signal transduction in mesenteric arteries from deoxycorticosterone acetate-salt hypertensive rats. *J. Pharmacol. Exp. Ther.*, **277**, 1103–1113.

(Received December 23, 1998)

Accepted April 6, 1999)