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Further evidence that 5-HT-induced relaxation of pig pulmonary artery is mediated by endothelial 5-HT_{2B} receptors

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1 The endothelial 5-HT receptor mediating relaxation of pig pulmonary artery has been characterized using the selective 5-HT_{2B} receptor agonist BW 723C86 and a variety of structurally diverse 5-HT receptor antagonists.

2 If arterial rings with intact endothelium were precontracted with prostaglandin $F_{2\alpha}$ (3 μM), BW 723C86 caused concentration-dependent relaxation with a $pEC_{50}=8.21\pm0.03$ and $E_{max}=89\pm4\%$ relative to 5-HT. The relaxant responses to BW 723C86 were inhibited by the 5-HT_{2B} receptor antagonist SB 204741, the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 and the antimigraine drug pizotifen, yielding pA_2 values of 6.68, 7.20 and 8.32, respectively. The pA_2 values against BW 723C86 were similar to those determined against 5-HT.

3 The relaxant effect of 5-HT was antagonized by a variety of 22 compounds of diverse chemical structures. Based on the calculated mean pA_2 values the order of the most potent antagonists was ritanserin (9.38) > methysergide (8.86) > pizotifen (8.47) ⩾ methiothepin (8.32) > LY 53857 (7.84) ⩾ amoxapine (7.80) ⩾ loxapine (7.73) ⩾ metergoline (7.64) ⩾ mianserin (7.51) ⩾ rauwolscine (7.39). Compounds with weak blocking potency were yohimbine (6.37), spiperone (5.88) and ketanserin (5.85). Correlation analysis between the affinities of the antagonists in pig pulmonary artery and those from radioligand binding studies at human and rat 5-HT_{2B} receptors showed a highly significant correlation ($r=0.95$ and 0.84, $P<0.002$ and <0.005). Correlation with 5-HT_{2C} receptors was much lower ($r=0.57$, $P=0.035$), and no correlations were obtained with 5-ht_6 and 5-HT_7 receptors.

4 It is concluded that the 5-HT receptor mediating endothelium-dependent relaxation of pig pulmonary artery is of the 5-HT_{2B} subtype.

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Keywords: Pig pulmonary artery; 5-HT; BW 723C86; SB 204741; 5-HT_{2B} receptor; endothelium-dependent relaxation

Abbreviations: BW 723C86, (α-methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine); 5-HT, 5-hydroxytryptamine; L-NAME, N^G-nitro-L-arginine methyl ester; LY53857, (6-methyl-1-(1-methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester); NO, nitric oxide; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SB 204741, (N-(1-methyl-5-indolyl)-N′-(3-methyl-5-isothiazolyl)urea); SB 206553, (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole)

Introduction

It has been shown that the vasculature is endowed with a variety of specific 5-hydroxytryptamine (5-HT) receptors. Contraction of blood vessels to 5-HT is mediated *via* smooth muscle 5-$HT_{1B/1D}$ (formerly 5-HT_1-like) receptors and/or smooth muscle 5-HT_{2A} receptors (Saxena & Villalón, 1990; Hartig *et al*., 1996; Saxena *et al*., 1998). Relaxation to 5-HT is mediated *via* activation of both smooth muscle 5-HT_7 receptors or atypical endothelial 5-HT receptors coupled to the release of nitric oxide (see Hoyer *et al*., 1994; Martin, 1994 for reviews). The existence of relaxant 5-HT_7 receptors has been demonstrated in rabbit femoral vein (Martin & Wilson, 1995), cynomolgus monkey jugular vein (Leung *et al*., 1996), canine coronary artery (Terrón, 1996; Cushing *et al*., 1996), rabbit pulmonary artery (Morecroft & MacLean, 1998), and canine basilar and middle cerebral arteries (Terrón & Falcón-Neri, 1999). By contrast, there has been some controversy over the exact nature of endothelial 5-HT receptor subtype(s) (Sumner, 1991; Martin *et al*., 1993; Martin, 1994). At the present time there seems to be a fairly clear consensus that two distinct receptor types may be involved in the endothelium-dependent relaxant effect of 5-HT. In pig coronary artery (Schoeffter & Hoyer, 1990) and guinea-pig jugular vein (Gupta, 1992) the endothelial 5-HT receptors resemble the 5-$HT_{1B/1D}$ (formerly 5-HT_1-like) receptor subtype, whereas the relaxant 5-HT receptor in rat jugular vein (Ellis *et al*., 1995), canine vena cava (Grayson & Gupta, 1995), pig cerebral artery (Schmuck *et al*., 1996) and pig pulmonary artery (Glusa & Richter, 1993; Glusa & Roos, 1996) exhibits operational characteristics similar to the 5-HT_{2B} receptor.

An important advance in the characterization of 5-HT_{2B} receptor-mediated effects represent the tryptamine analogue BW 723C86. This compound is a potent partial agonist at 5-HT_{2B} receptors in rat stomach fundus (Ellis *et al*., 1994), canine vena cava (Grayson & Gupta, 1995), and rat jugular vein (Ellis *et al*., 1995). On the other hand, BW 723C86 displays lower potency at both 5-HT_{2A} and 5-HT_{2C} receptors in various functional assays (Ellis *et al*., 1994; Baxter *et al*., 1995; Baxter, 1996; Kennett *et al*., 1997).

The aim of the present study was to characterize the 5-HT receptor responsible for endothelium-dependent relaxation of pig pulmonary artery by means of the 5-HT_{2B} receptor agonist BW 723C86. Since agonist potencies by themselves do not allow definitive characterization of receptors, we also examined the effect of various 5-HT receptor antagonists generally used to characterize 5-HT_{2B}, 5-HT_{2C}, 5-ht_6 and 5-HT_7 receptors such as the selective 5-HT_{2B} receptor antagonist SB 204741, ergolines and tricyclic psychotropic agents (Table

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1) (Baxter, 1996; Monsma *et al*., 1993; Roth *et al*., 1994). Based on the potencies of the antagonists used, further evidence is presented that the relaxant receptor has the characteristics of the 5-HT_{2B} receptor subtype. A preliminary report of some of these data has been published previously (Roos & Glusa, 1998).

Methods

Experimental protocol

Pig lungs were obtained from the local slaughter-house. Small branches of pulmonary arteries were dissected and carefully cleaned of parenchyma and connective tissue. Up to six rings (2–3 mm long and 1.5–2 mm wide) were horizontally suspended between two L-shaped platinum hooks (150 μm diameter) and mounted in a 10 ml organ bath filled with modified Krebs-Henseleit-solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and D-glucose 11. The solution was continuously gassed with 95% O_2/5% CO_2 and warmed to a constant temperature of 37°C. Preparations were connected to an isometric force transducer (Hugo Sachs Elektronik, March, Germany) and changes in tension were recorded continuously. Resting tension was adjusted to 20 mN at the beginning of each experiment. During an initial stabilization period of 60 min, the bathing medium was changed every 20 min and the tension repeatedly readjusted to 20 mN. The tissues were stimulated at intervals of 45 min once with KCl (30 mM) and three times with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 3 μM) until the contractile response had become constant. The integrity of the endothelium was assessed functionally by measuring the extent of endothelium-dependent relaxation following application of bradykinin (10 nM).

The relaxant response to 5-HT (control) and BW 723C86 was studied after the third $PGF_{2\alpha}$ (3 μM)-induced contraction had stabilized, by constructing a cumulative concentration-response curve in the absence and presence of antagonist. Each successive agonist concentration was administered when the response had reached a plateau; this occurred generally after 2–4 min. Relaxant effects were expressed as a percentage of the $PGF_{2\alpha}$-induced contraction. Antagonists were added 30 min before the construction of agonist concentration-response curves. The effects of antagonists were investigated in ring segments adjacent to those used as controls.

Data presentation and statistical evaluation

Data are presented as mean ± s.e.mean for *n* separate experiments, using vessels from different animals. Agonist concentration-effect curves were fitted using the computer program GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, U.S.A.). Agonist potencies and maximum response were expressed as pEC_{50} values (negative logarithm of the molar concentration of agonist producing 50% of the maximum response) and E_{max} values, respectively. The potencies of the antagonists were expressed as either an apparent or a full pA_2 value. The apparent pA_2 value was calculated from the equation $pA_2 = -\log c(B) + \log (CR-1)$, where c(B) is the negative logarithm of the molar concentration of antagonist and CR the ratio of agonist EC_{50} measured in the presence of antagonist over that measured in the absence of antagonist (Furchgott, 1972). The full pA_2 value was determined using the method of Arunlakshana & Schild (1959). For the calculation of pA_2 values from Schild plot,

Table 1 Binding affinities (pK_i values) of the antagonist used in the present study at 5-HT_{2B}, 5HT_{2C} 5-ht_6 and 5-HT_7 receptors

Antagonists	*5-HT_{2B}*[a] (pK_i)	*5-HT_{2B}*[b] (pK_i)	*5-HT_{2C}*[c] (pK_i)	*5-ht_6*[d] (pK_i)	*5-HT_7*[e] (pK_i)
Ergolines					
LY53857		8.2	8.1		
Mesulergine		7.4	8.8	5.8	8.2
Methysergide	8.1	8.2	8.6	6.4	7.9
Metergoline			9.2	7.5	8.7
Rauwolfia alkaloids					
Rauwolscine		7.4	5.8		
Yohimbine	6.4	7.3	4.4		
Tricyclic psychotropic agents					
Amitriptyline				7.2	6.9
Amoxapine				8.2	7.4
Chlorpromazine			7.8	8.4	7.7
Chlorprothixine				8.5	8.3
Fluphenazine				7.8	8.1
Loxapine				7.8	7.4
Mianserin	7.7	7.3	8.0	7.3	7.0
Perphenazine				7.8	7.6
Spiperone	5.8	5.5	5.9	5.8	8.0
Thioridazine				8.2	7.2
Other reference drugs					
Ketanserin	6.2	5.5	7.0	<5.0	6.7
Methiothepin			7.6	8.7	9.0
Pizotifen			8.1		
Ritanserin	8.3	8.3	8.6	7.4	7.7
SB 204741	7.1		<6.0		

[a]Binding affinity (cloned human 5-HT_{2B} receptors expressed in Cos-7 cells; [^{3}H]-5-HT), data from Bonhaus *et al.* (1995). [b]Binding affinity (cloned rat 5-HT_{2B} receptors expressed in AV-12 cells; [^{3}H]-5-HT), data from Wainscott *et al.* (1996). [c]Binding affinity (native 5-HT_{2C} receptors from pig choroid plexus; [^{3}H]-mesulergine), data from Hoyer (1989) and Baxter (1996). [d]Binding affinity (cloned rat 5-ht_6 receptors expressed in Cos-7 cells or HEK-293 cells; [^{3}H]-LSD), data from Monsma *et al.* (1993) and Roth *et al.* (1994). [e]Binding affinity (cloned rat 5-HT_7 receptors expressed in Cos-7 cells; [^{3}H]-LSD), data from Shen *et al.* (1993) and Roth *et al.* (1994).

the slope was constrained to unity unless it was significantly different from unity ($P<0.05$).

For correlation of antagonist affinity estimates (pA_2 values) at the relaxant 5-HT receptor in pig pulmonary artery and binding affinities pK_i values for native or recombinant 5-HT$_{2B}$, 5-HT$_{2C}$, 5-ht$_6$ and 5-HT$_7$ receptors from human or rat were used (Table 1). Binding affinities for pig receptors were only available at 5-HT$_{2C}$ receptors. In all other cases binding data were taken from human or rat homologues without mixing data from different species (Table 1).

Where appropriate, differences between means were determined by Student's *t*-test (two-tailed), after checking the homogeneity of the variances. *P* values <0.05 were considered to indicate a significant difference between the responses being compared.

Drugs

The following compounds were either purchased or donated: amoxapine, BW 723C86 (α-methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine hydrochloride), fluphenazine dihydrochloride, loxapine succinate, LY53857 (6-methyl-1-(1-methylethyl)-ergoline-8β-carboxylic acid 2-hydroxyl-1-methylpropyl ester maleate), metergoline, methiothepin mesylate, N^G-nitro-L-arginine methyl ester (L-NAME), ritanserin, SB 206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) and thioridazine hydrochloride (all purchased from Research Biochemicals Int., Natick, MA, U.S.A.); rauwolscine (purchased from Roth, Karlsruhe, Germany); bradykinin triacetate, 5-hydroxytryptamine creatine sulphate (5-HT), and dinoprost tromethamine (PGF_{2a}) (all purchased from Serva, Heidelberg, Germany); chlorprothixene hydrochloride, perphenazine, and yohimbine hydrochloride (all purchased from Sigma-Aldrich, Deisenhofen, Germany); amitriptyline (purchased from Tropon, Köln, Germany); chlorpromazine hydrochloride and methysergide maleate (gifts from Arzneimittelwerk Dresden, Germany); ketanserin tartrate (gift from Janssen, Beerse, Belgium); mianserin (gift from Organon, Oberschleissheim, Germany); mesulergine maleate, pizotifen maleate, and spiperone hydrochloride (all gifts from Sandoz, Basle, Switzerland); SB 204741 (N-(1-methyl-5-indolyl)-N′-(3-methyl-5-isothiazolyl)urea) (gifts from SmithKline Beecham, Harlow, U.K.)

Results

Agonist studies

In the present study the functional integrity of the vascular endothelium was evaluated by allowing $PGF_{2\alpha}$ (3 μM)-precontracted arteries to relax following the addition of bradykinin (10 nM). The bradykinin-induced relaxation amounted to $86\pm4\%$ ($n=30$). The relaxation was abolished after mechanical removal of the endothelium or after preincubation with N^G-nitro-L-arginine methyl ester (L-NAME; 200 μM).

The 5-HT$_{2B}$ receptor agonist BW 723C86 (1–100 nM) was found to induce concentration-dependent relaxation of $PGF_{2\alpha}$-precontracted pig pulmonary artery with intact endothelium. Relaxation was absent in endothelium-denuded arteries. BW 723C86 was a partial agonist relative to 5-HT ($pEC_{50}=8.21\pm0.03$, $E_{max}=89\pm4\%$; $n=22$). The 5-HT$_{2B}$ receptor antagonist SB 204741 (0.1–3 μM) produced a parallel concentration-dependent rightward shift of the concentration-response curve to BW 723C86 with no significant effect on maximum response (Figure 1). Schild analysis yielded a straight line with a full pA_2 value of 6.68 ± 0.05 (slope of the Schild plot 0.87 ± 0.06, not significantly different from unity). In a similar manner, endothelium-dependent relaxation to BW 723C86 was antagonized by the 5-HT$_{2B/2C}$ receptor antagonist SB 206553 (0.3–3 μM) yielding a full pA_2 value of 7.20 ± 0.11 (slope of the Schild plot 1.10 ± 0.10, not significantly different from unity) (Figure 2). Furthermore, relaxation to BW 723C86 was antagonized by pizotifen (10 nM) with an apparent pA_2 value of 8.32 ± 0.08 ($n=4$). Since the calculated pA_2 values for

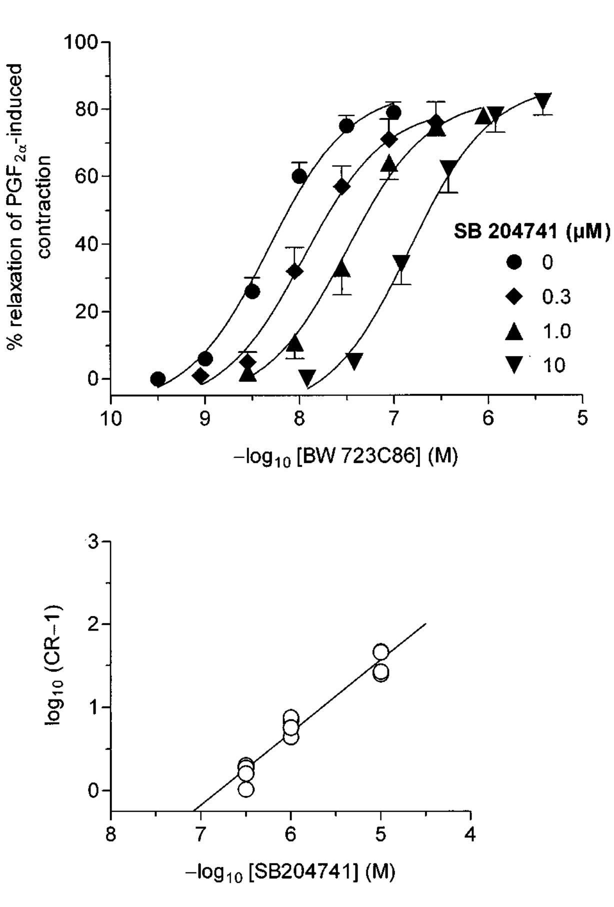

Figure 1 Antagonism of BW 723C86-induced relaxation by SB 204741 in pig pulmonary artery. The upper panel represents cumulative concentration-response curves to BW 723C86 in the absence and presence of SB 204741. The data are mean±s.e.mean (vertical bars) from 4–12 separate experiments. The lower panel represents the Schild regression analysis.

Table 2 Affinities (pA_2 values and Schild regression analysis) for antagonists against 5-HT- and BW 723C86-induced relaxation in pig pulmonary artery

Antagonist	*Concentration* (μM)	*pA_2 against 5-HT*	*slope*	*pA_2 against BW 723C86*	*slope*
SB 204741	0.3–10	6.59 ± 0.07 (12)	0.98 ± 0.16*	6.68 ± 0.05 (12)	0.87 ± 0.06*
SB 206553	0.1–3	7.23 ± 0.05 (14)	1.16 ± 0.08*	7.20 ± 0.11 (12)	1.10 ± 0.10*
Pizotifen	0.003–0.1	8.47 ± 0.07 (14)	0.96 ± 0.07*	8.32 ± 0.08 (4)	–

Values are mean±s.e.mean for *n* experiments in parenthesis. *The slope of the Schild plot was not significantly different from unity.

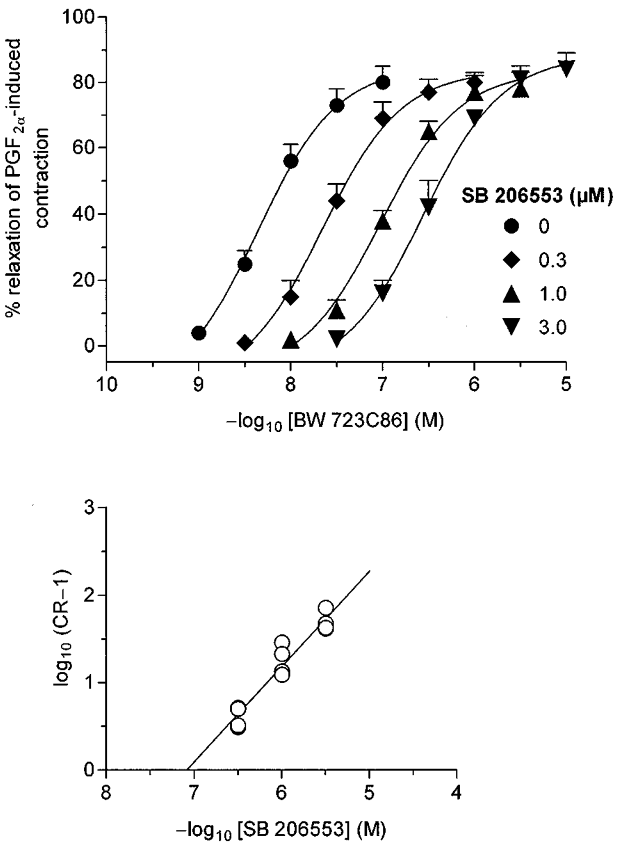

Figure 2 Antagonism of BW 723C86-induced relaxation by SB 206553 in pig pulmonary artery. The upper panel represents cumulative concentration-response curves to BW 723C86 in the absence and presence of SB 206553. The data are mean ± s.e.mean (vertical bars) from 4–8 separate experiments. The lower panel represents the Schild regression analysis.

SB 204741, SB 206553 and pizotifen against the relaxant effect of BW 723C86 matched the affinity of these antagonists against the relaxant effect of 5-HT, the involvement of a common receptor site can be suggested (Table 2). It should be emphasized that neither BW 723C86 (1–100 nM) nor 5-HT (0.1–100 nM) were able to induce contractile responses of quiescent rings of pig pulmonary artery, with and without endothelium.

Antagonist studies

A number of structurally different compounds were tested against the relaxant effect of 5-HT, the results of the antagonist potencies are given in Table 3. The Figures 3 and 4 demonstrate the inhibitory effects of two representatives from the compounds used, pizotifen and LY53857. The antagonists caused a concentration-dependent rightward shift of the concentration-response curve to 5-HT with little or no effect on maximum response with the exception of ritanserin, methysergide and methiothepin which antagonized the relaxant effect of 5-HT in an unsurmountable manner. Among the compounds tested ritanserin, methysergide, pizotifen and methiothepin proved to be the most potent antagonists (mean pA$_2$ values from 9.4–8.3). The tricyclic psychotropic drugs blocked the relaxant effect of 5-HT with mean pA$_2$ values of 7.8–6.5. Among both the Rauwolfia alkaloids rauwolscine (pA$_2$ 7.4) was more potent than yohimbine (pA$_2$ 6.4). Compounds with weak blocking potency were spiperone (5.9) and ketanserin (5.9). Attempts were made to correlate the affinity parameters determined in functional tests in pig

Table 3 Antagonist affinity estimates against 5-HT in pig pulmonary artery

Antagonists	*Conc.* (μM)	*pA$_2$ ± s.e.mean*	n
Ritanserin	0.01	9.38 ± 0.13†	5
Methysergide	0.01	8.86 ± 0.18††	4
Pizotifen	0.003–0.1	8.47 ± 0.017*	14
Methiothepin	0.01	8.32 ± 0.18†††	3
LY53857	0.03–1	7.84 ± 0.07*	15
Amoxapine	0.1–1	7.80 ± 0.04	12
Loxapine	0.1–1	7.73 ± 0.06	11
Metergoline	0.1–1	7.64 ± 0.09	10
Mianserin	0.1	7.51 ± 0.12	4
Rauwolscine	1.0	7.39 ± 0.15	3
Chlorprothixene	0.1–10	7.26 ± 0.07	9
SB 206553	0.1–3	7.23 ± 0.05*	14
Fluphenazine	1.0	7.10 ± 0.12	3
Perphenazine	1	6.78 ± 0.13	4
Amitriptyline	1	6.77 ± 0.12	4
SB 204741	0.3–10	6.59 ± 0.07*	12
Chlorpromazine	1	6.57 ± 0.13	3
Mesulergine	3	6.55 ± 0.04	4
Thioridazine	1	6.53 ± 0.11	5
Yohimbine	1–3	6.37 ± 0.04	9
Spiperone	3	5.88 ± 0.11	3
Ketanserin	1	5.85 ± 0.13	6

*pA$_2$ value from Schild regression analysis. †, ††, †††, Unsurmountable antagonism (E_{max} was 55 ± 5%, 51 ± 9% and 52 ± 7% relative to 5-HT).

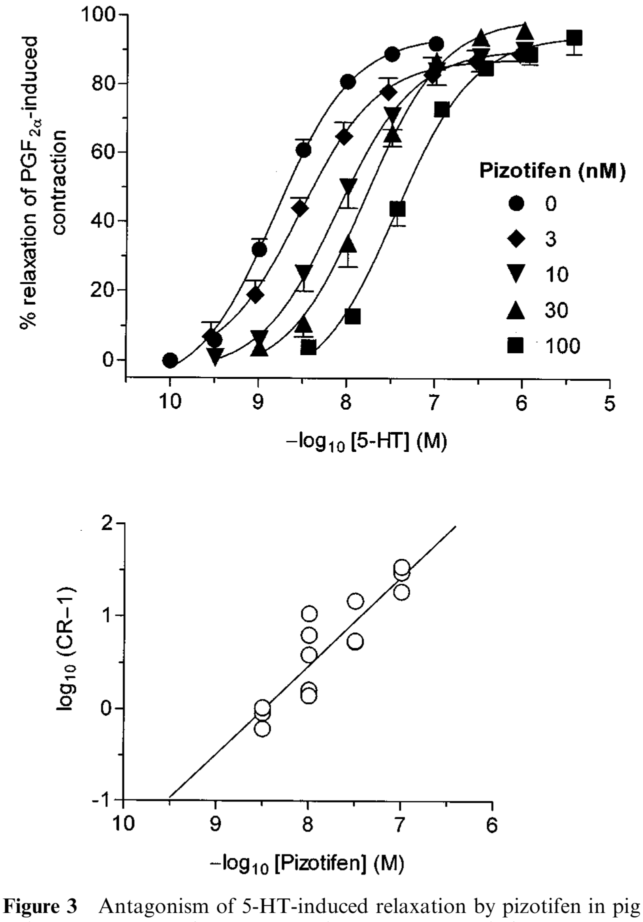

Figure 3 Antagonism of 5-HT-induced relaxation by pizotifen in pig pulmonary artery. The upper panel represents cumulative concentration-response curves to 5-HT in the absence and presence of pizotifen. The data are mean ± s.e.mean (vertical bars) from 3–14 separate experiments. The lower panel represents the Schild regression analysis.

pulmonary arteries with their affinities (pK_i values) at native or recombinant 5-HT_{2B}, 5-HT_{2C}, 5-ht_6 and 5-HT_7 receptors (Table 1). Affinities found in pig pulmonary artery fitted best with radioligand binding data at the human and rat 5-HT_{2B} receptor (r = 0.95 and r = 0.84, $P<0.002$ and <0.005) (Figure 5). Correlation with 5-HT_{2C} receptors was much lower than that obtained with the 5-HT_{2B} type (r = 0.57, $P=0.035$) and non-significant correlations were obtained with 5-ht_6 and 5-HT_7 receptors (Figure 6).

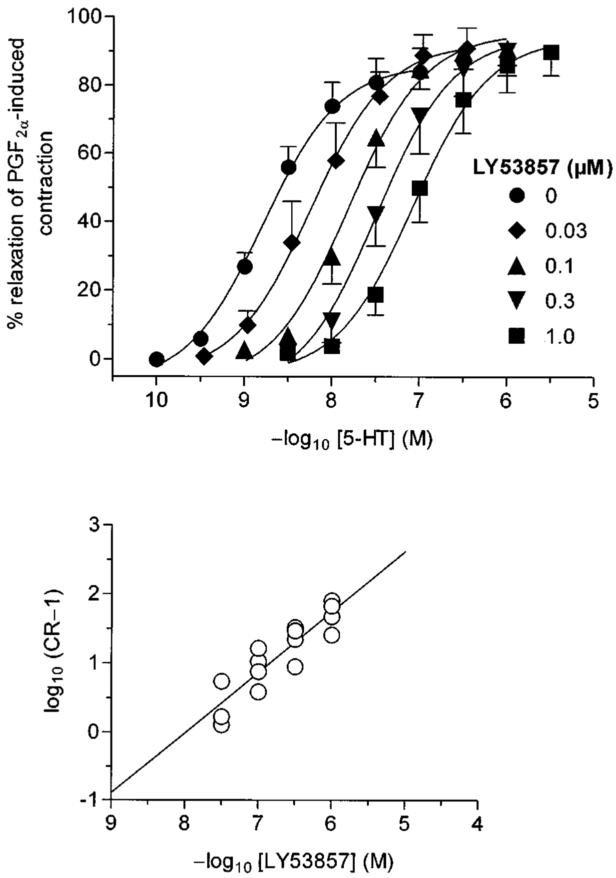

Figure 4 Antagonism of 5-HT-induced relaxation by LY53857 in pig pulmonary artery. The upper panel represents cumulative concentration-response curves to 5-HT in the absence and presence of LY53857. The data are mean ± s.e.mean (vertical bars) from 3–6 separate experiments. The lower panel represents the Schild regression analysis.

Discussion

It has been proposed that endothelial 5-HT_{2B} receptors mediate vascular relaxation by the release of NO (see Baxter *et al.*, 1995 for review). In previous studies on pig pulmonary artery it was shown that the 5-HT-induced relaxation was due to the release of endothelial NO followed by an increase in cyclic GMP in smooth muscle cells (Glusa & Richter, 1993). However, it was not unequivocally clarified whether the relaxation is mediated *via* endothelial 5-HT_{2C} or 5-HT_{2B} receptors (Glusa & Richter, 1993; Glusa & Roos, 1996). The existence of the 5-HT_{2B} mRNA transcript in pig pulmonary artery implies the expression of the 5-HT_{2B} receptor protein in this tissue (Ulmer *et al.*, 1995). The present study demonstrates that the selective 5-HT_{2B} receptor agonist BW 723C86 caused an endothelium-dependent relaxation of pig pulmonary artery. The potent partial agonist activity of BW 723C86 in pig pulmonary artery ($pEC_{50}=8.21$) is consistent with the findings at 5-HT_{2B} receptors in rat stomach fundus (Ellis *et al.*, 1994), rat jugular vein (Ellis *et al.*, 1995) and dog vena cava (Grayson & Gupta, 1995). Further evidence for the involvement of 5-HT_{2B} receptors in relaxation of pig pulmonary artery was provided by the antagonist profile of SB 204741, a highly selective 5-HT_{2B} receptor antagonist. SB 204741 acted as competitive antagonist against the relaxant effects of BW 723C86 and 5-HT, respectively. The affinity for SB 204741 ($pA_2=6.7$ against BW 723C86 and 6.6 against 5.HT) was in the same concentration range as determined in rat jugular vein ($pA_2=7.3$; Baxter, 1996), rat stomach fundus ($pA_2=7.6$;

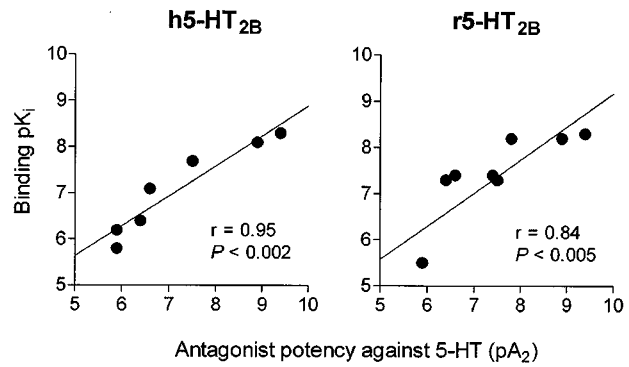

Figure 5 Correlation of antagonist affinity estimates (pA_2) against 5-HT at the relaxant 5-HT receptor in pig pulmonary artery and binding affinity (pK_i values) at human and rat 5-HT_{2B} receptors.

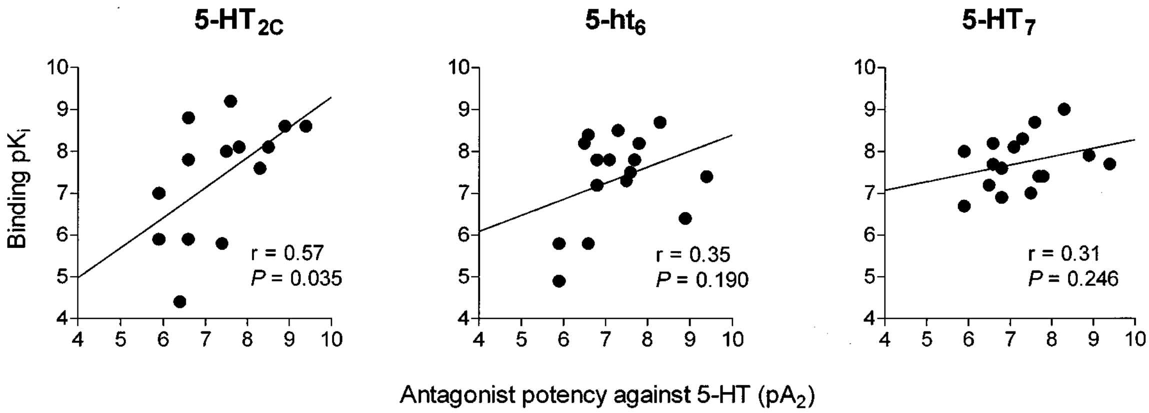

Figure 6 Correlation of antagonist affinity estimates (pA_2) against 5-HT at the relaxant 5-HT receptor in pig pulmonary artery and binding affinity (pK_i values) at pig 5-HT_{2C} receptors, rat 5-ht_6 receptors and rat 5-HT_7 receptors.

Baxter *et al*., 1994), dog vena cava ($pA_2 = 7.5$; Grayson & Gupta, 1995), and cloned human 5-HT_{2B} receptors ($pK_i = 6.7$; Thomas *et al*., 1996).

A significant correlation between the affinity estimates in pig pulmonary artery with those in radioligand binding studies was obtained with cloned human 5-HT_{2B} receptors and cloned rat 5-HT_{2B} receptors, respectively. In contrast, the correlation was less favourable at the 5-HT_{2C} receptor subtype ($r = 0.57$). A variety of studies has demonstrated a high degree of pharmacological similarity between 5-HT_{2B} and 5-HT_{2C} receptors. Unfortunately, there are only few drugs which are able to discriminate between both closely related subtypes (see Baxter *et al*., 1995). In addition to SB 204741 which displays at least 10 fold selectivity for 5-HT_{2B} versus 5-HT_{2C} receptors, both subtypes may be further delineated by the use of yohimbine, rauwolscine and ketanserin. Yohimbine and rauwolscine have been shown to possess moderately high affinity for 5-HT_{2B} receptors but a much lower affinity for 5-HT_{2C} receptors, whereas ketanserin has been shown to possess higher affinity for 5-HT_{2C} than for 5-HT_{2B} receptors (Baxter *et al*., 1995; Baxter, 1996; see also Table 1). The affinities estimated in the present study for rauwolscine, yohimbine and ketanserin argue for a role of 5-HT_{2B} receptors in endothelium-dependent relaxation of pig pulmonary artery. The good correlation between antagonist pA_2 values and pK_i values for human and rat 5-HT_{2B} binding sites supports this finding (Figure 5). Pig and human 5-HT_{2B} receptors have been shown to possess 95% sequence homology, while the sequence homology of the pig 5-HT_{2B} receptor with the rat homologue is 87% (Ulmer *et al*., 1995). Although it could be demonstrated that the human 5-HT_{2B} receptor has an overall pharmacological profile consistent with its rat homologue, some differences have been detected which suggest that the 5-HT_{2B} receptors are not a pharmacologically homogeneous class of receptors Schmuck *et al*., 1996; Wainscott *et al*., 1996). In this connection, yohimbine justifies special mention since this drug showed appreciably lower affinity for 5-HT_{2B} receptors in pig pulmonary artery than in rat jugular vein ($pA_2 = 7.3$; Ellis *et al*., 1995), rat stomach fundus ($pA_2 = 6.9-7.8$; Audia *et al*., 1996; Baxter *et al*., 1994) and cloned rat 5-HT_{2B} receptors ($pK_i = 7.3$; Wainscott *et al*., 1996). The discrepancy between our observations in the pig and those in the rat provides further evidence that species homologues exist in pharmacology of 5-HT_{2B} receptors (see also Bonhaus *et al*., 1995; Baxter *et al*., 1995 for review).

It has been shown that several drugs from structurally different classes (e.g., ergolines, tricyclic psychotropic drugs) possess high affinity for 5-HT_{2C} receptors and also for 5-ht_6 and 5-HT_7 receptors (Monsma *et al*., 1993; Shen *et al*., 1993; Roth *et al*., 1994). These compounds were used to demonstrate whether 5-ht_6 and 5-HT_7 receptors, respectively, might be involved in endothelium-dependent relaxation of pig pulmonary artery. In this regard, it is worth pointing out that mRNA for 5-HT_7 receptors has been found to be expressed in this tissue (Ulmer *et al*., 1995). By contrast, 5-ht_6 mRNA has not been detected in peripheral organs studied (Kohen *et al*., 1996). The present findings suggest that there is no role for 5-ht_6 and 5-HT_7 receptors in endothelium-dependent relaxation in blood vessels.

In conclusion, by using the selective 5-HT_{2B} receptor agonist BW 723C86 and a large number of structurally different antagonists, the present data provide further evidence that the endothelial receptor mediating relaxation of pig pulmonary artery is a 5-HT_{2B} receptor.

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