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Regulation of sensitivity to 5-hydroxytryptamine in pulmonary supernumerary but not conventional arteries by a 5-HT_{1D}-like receptor

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Abstract

Bovine pulmonary supernumerary arteries are more sensitive to 5-hydroxtryptamine (5-HT) (pD_2 6.43 ± 0.25) than conventional arteries (pD_2 5.32 ± 0.16). This study investigated receptors for 5-HT in ring segments of these arteries. The 5-HT_2 receptor agonist, 2,5 dimethoxy-4-iodoamphetamine hydrobromide (DOI) constricts both arteries. The selective 5-HT_2 receptor antagonist ritanserin produced insurmountable antagonism of 5-HT concentration–response curves in both arteries, whereas the 5-$HT_{1B/1D}$ receptor antagonist *N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl[1,1,-biphenyl]-4-carboxamide hydrochloride (GR127935) produced much greater antagonism in supernumerary arteries. In rings preconstricted with 9,11-dideoxy-9,11-methanoepoxy prostalagdin $F_{2\alpha}$ (U46619) and relaxed with the adenylyl cyclase activator forskolin, the selective 5-HT_{1D} receptor agonist 2-[5-[3-(4-methylsulphonylamino) benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indole-3-yl] ethylamine (L694247) reversed the relaxation. Concentration–response curves for L694247-induced reversal of forskolin-relaxation were antagonised by GR127935 in supernumerary (pK_B 8.6) and conventional (pK_B 8.4) arteries, whereas concentration–response curves to 5-HT-were less sensitive to antagonism by GR127935T and this was more obvious in conventional (pK_B 7.6) than supernumerary (pK_B 8.1) arteries. Neither the selective 5-HT_{1D} receptor antagonist (1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(*S*,*R*) hydroxypropanyl)piperazine] hydrochloride (BRL15572) nor the 5-HT_{1B} receptor antagonist (2,3,6,7-tetrahydro-1′-methyl-5-[2′methyl-4′5-(methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl]furo[2,3-f]indole-3-spiro-4′-piperidine hydrochloride (SB224289) antagonised concentration–response curves induced by 5-HT or 5-HT_1-receptor-selective agonists. In addition to the 5-HT_{2A} receptor, 5-HT activates a GR127935-sensitive and a GR127935-insensitive receptor in these arteries. Supernumerary arteries have a greater proportion of GR127935-sensitive receptors, which display only some of the pharmacological characteristics of the cloned 5-HT_{1D} receptor. It is possible that the GR127935-sensitive receptor could be a species homologue of the human 5-HT_{1B} receptor that is insensitive to SB224289.

Keywords: Pulmonary supernumerary artery; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{1D}-like receptor

1. Introduction

The pulmonary circulation consists of two populations of arteries: conventional arteries, which divide with and accompany the airways, and supernumerary arteries, which branch from the conventional arteries at 90° and are not accompanied by an airway. Supernumerary arteries are small, muscular arteries that represent a substantial part of the cross-sectional area of the arterial system (Elliott and Reid, 1965; Shaw et al., 1999). Pathological findings have shown that supernumerary arteries, especially the region around their origin, are severely affected by prolonged pulmonary hypertension (Yaginuma et al., 1990; Ogata and Iijima, 1993). This suggests that a greater understanding of the role of supernumerary arteries and their parent conventional arteries in pulmonary physiology is essential.

Excess production/release of pulmonary vasoconstrictors such as 5-hydroxytryptamine (5-HT) has been implicated in the development of several forms of pulmonary hypertension (reviewed in MacLean, 1999). We have previously reported that bovine supernumerary arteries are much more sensitive than conventional arteries to the vasoconstrictor 5-HT (Bunton et al., 2000). In contrast both arterial populations display a similar sensitivity to the thromboxane A_2 mimetic U46619 (Bunton et al., 2000). The different sensitivities to 5-HT may be due to different receptor classes in these arterial populations. The purpose

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of the present study was to examine the receptor types for 5-HT, which are present in supernumerary arteries and their parent conventional arteries, and to establish if different receptors could account for the different sensitivities to 5-HT.

Currently, seven 5-HT receptor classes have been identified, designated as 5-HT_1 to 5-HT_7 receptor classes. Four 5-HT receptor classes are known to be present on pulmonary vascular smooth muscle of conventional arteries: 5-HT_1 (MacLean et al., 1996), 5-HT_2 (Frenken and Kaumann, 1984), 5-HT_4-like (Becker et al., 1992) and 5-HT_7 (Morecroft and Maclean, 1998). The 5-HT_2 and the 5-HT_1 receptor classes mediate contractile responses. This study used selective agonists and antagonists to investigate the receptors for 5-HT on bovine conventional and supernumerary arteries. Since the 5-HT_1 receptor class is negatively coupled to adenylyl cyclase we also investigated the ability of agonists to reverse forskolin-induced relaxation of preconstricted arteries.

2. Methods

2.1. Isolated arterial preparations

Bovine lungs were obtained from a local abattoir within 30 min of slaughter and transported on ice to the laboratory. Endothelium-intact ring segments of conventional artery (diameter 4–5 mm) and their associated supernumerary arteries (diameter 0.5–1 mm) were dissected from the lung and freed of surrounding connective tissue. The vessels were then weighed and suspended between stainless steel hooks in 20-ml Linton vessel chambers in Krebs' solution of composition (mM): NaCl (140), KCl (4.7), $NaCO_3$ (24.8), $MgSO_4$ (1.2), KH_2PO_4 (1.2), CaCl (2.5), glucose (11.1) at 37°C under a tension of $2\,g$ for conventional arteries and $1\,g$ for supernumerary arteries and gassed with a mixture of O_2:CO_2 (95%/5%, v/v). Isometric tension was measured by attaching one of the hooks via a thread to a UF1 dynamometer (Pioden Controls), the other hook being in a fixed position. The output from the transducer was amplified and displayed on a Servogor 400 chart recorder.

2.2. Agonists and antagonists

The receptor-selective agonists and antagonists used in this study are listed in Table 1.

2.3. Concentration–response curves

The tissues were allowed to equilibrate for 1 h before each experiment. Agonists were added to the organ baths cumulatively in 0.5 log units to construct cumulative concentration–response curves. For these studies, two consecutive concentration–response curves were constructed, the first to 5-HT and the second to 5-HT (time control) or the subtype-selective agonist under study. The data obtained from the second concentration–response curve are expressed as a percentage of the maximum response to 5-HT in the first concentration–response curve. For antagonist studies, two consecutive concentration–response curves were again constructed, the first to 5-HT and the second to 5-HT alone (time control) or 5-HT plus the antagonist under study. Antagonists were incubated for 30 min. The data from the second concentration–response curves are expressed as a percentage of the maximum response to 5-HT in the first concentration–response curves.

Table 1
Receptor-selective agonists and antagonists used in this study

	Receptor						
	1A	1B	1D	1E	1F	2A	
Agonists							
8-OH-DPAT	*						(Hoyer et al., 1994)
Sumatriptan		*	*		*		(Humphrey et al., 1994; Peroutka and McCarthy 1989; Adham et al., 1993)
RU24969		*					(Middlemiss and Tricklebank, 1992; Hoyer et al., 1994)
L694247			*				(Beer et al., 1993)
5-CT	*	*	*				(Hoyer et al., 1994)
DOI						*	(Glennon et al., 1986, 1988; Dabire et al., 1989)
Antagonists							
Ritanserin						*	(Hoyer et al., 1994)
methiothepin		*	*			*	(Hoyer et al., 1994)
GR127935		*	*				(Clitherow et al., 1994; Skingle et al., 1996; Pauwels, 1996)
BRL15572			*				(Price et al., 1997)
SB224289		*					(Gaster et al., 1998)
Cyanopindolol		*	*				(Hoyer et al., 1994)

2.4. Reversal of forskolin-induced relaxation

In these studies, tissues were equilibrated for 1 h as before. With the exception of the studies using the 5-HT_{2A} receptor agonist DOI, ritanserin (100 nM) was added for the final 30 min of the equilibration period. The tissues were then constricted with U46619 (100 nM, approx. EC80). Once the contraction had stabilised, forskolin (1 μM) was added producing a full relaxation. Concentration–response curves for contraction to 5-HT or the 5-HT_1 receptor agonists were constructed. For antagonist studies, paired tissues were used where one tissue received the antagonist under study, and the other acted as the time control and received the drug vehicle. Antagonists were incubated for 30 min.

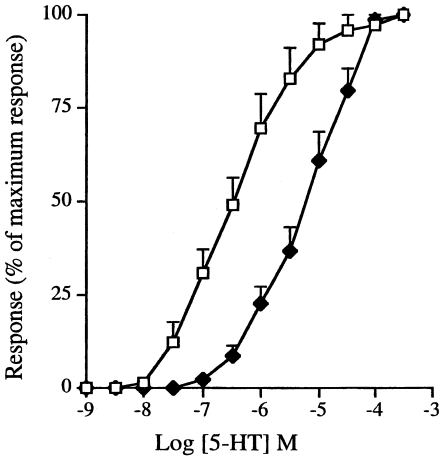

Fig. 1. Concentration–response curves for vasoconstriction to 5-HT in bovine isolated pulmonary supernumerary (□; $n = 17$) and conventional (♦; $n = 12$) artery rings.

2.5. Data analysis

Contractile responses to 5-HT and 5-HT receptor-selective agonists were expressed as a percentage of the maximum control response obtained for 5-HT. For experiments measuring agonist-induced reversal of forskolin-relaxation, agonist responses were expressed as a percentage reversal of the maximum relaxation to forskolin. pD_2 $\{-\log[EC_{50}(M)]\}$ values were calculated for individual concentration–response curves from which mean pD_2 values were obtained. Comparisons of data sets were carried out using Student's *t*-test; paired when two concentration–response curves were conducted in the one preparation, or unpaired when concentration–response curves were taken from different preparations. A *p*-value of less than 0.05 was considered significant. pK_B values were estimated for each of the antagonists according to the equation $pK_B = \log(CR - 1) - \log[B]$, where $CR = \text{antilog}(pD_2$ antagonist $- pD_2$ control) and $[B]$ is the antagonist concentration (M).

2.6. Chemicals

5-hydroxytryptamine creatinine sulphate (5-HT), Ritanserin tartrate, 2,5 dimethoxy-4-iodoamphetamine hydrobromide (DOI) were from Sigma-Aldrich. 5-carboxamidotryptamine maleate (5-CT), methiothepin maleate, sumatriptan succinate, GR127935T ((*N*-[4-methoxy-3-(4-methyl-1-piperazinyl0phenyl]-2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl[1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate, were gifts from Glaxo/Wellcome), BRL15572 (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(*S*, *R*) hydroxypropanyl)piperazine]hydrochloride and SB224289 (2,3,6,7-tetrahydro-1′-methyl-5-[2′methyl-4′5-(methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl]furo[2,3-*f*] indole-3-spiro-4′-piperidine hydrochloride were gifts from SmithKline Beecham), 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), RU24969 (5-methoxy-3-(1,2,3,6-te-

Table 2
Agonist pD_2 values and maximum responses in (a) conventional and supernumerary arteries, (b) conventional and supernumerary arteries preconstricted with U46619 (100 nM) and relaxed with forskolin (1 μM). Maximum responses in (a) are expressed as percentage of 5-HT maximum and in (b) as percentage of reversal of the forskolin relaxation. Values are means ± SEM from *n* experiments

Agonist	Conventional			Supernumerary		
	pD_2	Maximum	*n*	pD_2	Maximum	*n*
(a)						
5-HT	5.32 ± 0.16	100 ± 0	12	6.43 ± 0.25[a]	100 ± 0	17
5-CT	NE		4	7.19 ± 0.10	14 ± 3	8
8-OH-DPAT	NE		4	–	NA	8
RU24969	NE		4	6.23 ± 0.08	16 ± 5	6
L694247	NE		4	8.19 ± 0.10	28 ± 3	8
Sumatriptan	NE		4	5.90 ± 0.19	14 ± 4	10
DOI	8.0 ± 0.17	56 ± 11	8	8.12 ± 0.11	68 ± 9	8
(b) Reversal of forskolin-relaxation						
5-HT	5.75 ± 0.08	113 ± 9	6	6.27 ± 0.07[a]	97 ± 14	9
5-CT	7.38 ± 0.26	126 ± 19	6	7.30 ± 0.02	137 ± 18	17
8-OH-DPAT	5.81 ± 0.10	93 ± 15	6	5.71 ± 0.19	67 ± 15	6
RU24969	6.25 ± 0.11	78 ± 8	6	6.53 ± 0.11	68 ± 14	7
L694247	7.93 ± 0.30	64 ± 11	6	8.31 ± 0.07	69 ± 16	7
Sumatriptan	6.32 ± 0.10	92 ± 7	6	5.93 ± 0.12	123 ± 20	6
DOI	NE	–	4	NE	–	4

NE, no effect; NA, not achieved.
[a] $P > 0.001$.

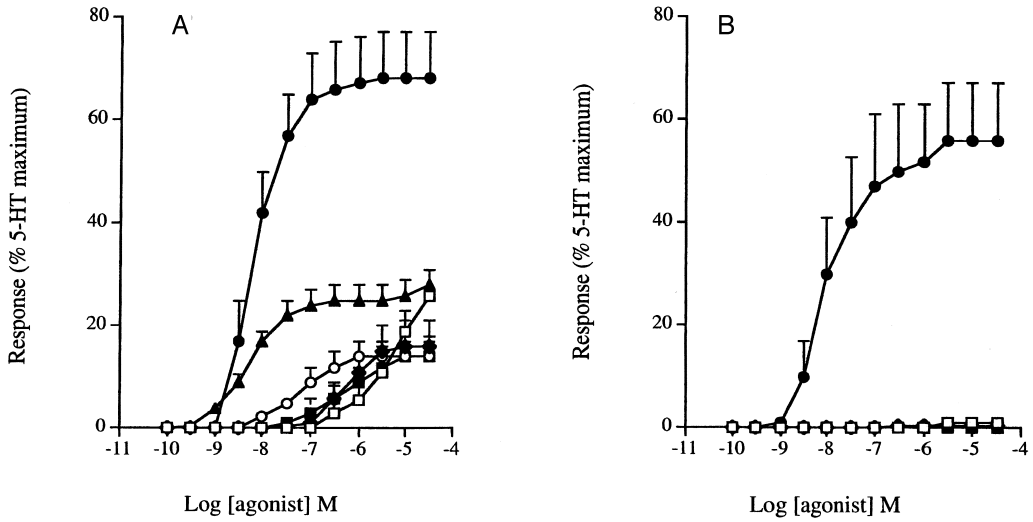

Fig. 2. Concentration–response curves for vasoconstriction to the 5-HT receptor selective agonists □, 8-OH-DPAT; ♦, RU24969; ○, 5-CT; ▲, L694247; ■, Sumatriptan; ●, DOI in isolated pulmonary supernumerary (A) and conventional (B) artery rings. With the exception of DOI all agonist responses were conducted in the presence of ritanserim (100 nM). Results are means ± SEM of six to eight experiments.

trahydro-4-pyrindinyl)-1*H*-indole), L694247 (2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indole-3-yl]ethylamine), cyanopindolol hemifumarate, (Tocris Cookson). U-46619 (9,11-dideoxy-9α,11α methanoepoxy prostaglandin $F_{2\alpha}$) was from Biomol Research Laboratories. Ritanserin and U46619 were dissolved in ethanol. BRL15572, L694247 and SB224289 were dissolved in dimethoxy sulfoxide (DMSO) and diluted in H_2O. GR127935 was dissolved in DMSO and diluted in DMSO. All other drugs were dissolved in H_2O.

Table 3
Agonist pD_2 values and estimated pK_B values for antagonists in (a) conventional and supernumerary arteries, (b) conventional and supernumerary arteries preconstricted with U46619 (100 nM) and relaxed with forskolin (1 μM)

(a) Agonist	Antagonist	Control pD_2	pD_2	Estimated pK_B (n)
5-HT	SA Ritanserin (100 nM)	6.34 ± 0.14	6.14 ± 0.24	NS (7)
	CA Ritanserin (100 nM)	5.61 ± 0.08	4.46 ± 0.09	NS (7)
	SA GR127935T (100 nM)	6.89 ± 0.06	6.12 ± 0.11[a]	7.9 (17)
	CA GR127935T (100 nM)	5.60 ± 0.10	5.12 ± 0.02	NC (12)
	SA BRL15572 (100 nM)	6.37 ± 0.09	6.36 ± 0.16	NE (5)
	CA BRL15572 (100 nM)	5.88 ± 0.22	6.02 ± 0.29	NE (5)
	SA SB224289 (100 nM)	6.48 ± 0.14	6.52 ± 0.14	NE (5)
	CA SB224289 (100 nM)	5.88 ± 0.22	5.94 ± 0.24	NE (5)
	SA SB224289 + BRL15572	6.26 ± 0.31	6.27 ± 0.16	NE (5)
(b) Reversal of forskolin-relaxation				
5-HT	SA GR127935T (100 nM)	6.21 ± 0.08	5.00 ± 0.06[a]	8.1 (12)
	CA GR127935T (100 nM)	5.66 ± 0.01	4.96 ± 0.03[a]	7.6 (4)
	SA Methiothepin (100 nM)	6.01 ± 0.42	4.79 ± 0.13[a]	8.4 (6)
	CA Methiothepin (100 nM)	5.69 ± 0.22	4.73 ± 0.07[a]	7.9 (6)
	SA BRL15572 (100 nM)	6.25 ± 0.10	6.40 ± 0.07	NE (8)
	CA BRL15572 (100 nM)	5.81 ± 0.31	5.70 ± 0.31	NE (4)
	SA SB224289 (100 nM)	6.44 ± 0.15	6.39 ± 0.23	NE (4)
	CA SB224289 (100 nM)	6.13 ± 0.12	6.10 ± 0.17	NE (4)
	SA Cyanopindolol (1 μM)	6.44 ± 0.15	6.50 ± 0.19	NE (4)
	CA Cyanopindolol (1 μM)	5.57 ± 0.21	5.32 ± 0.26	NE (4)
L-694247	SA GR127935T (100 nM)	8.01 ± 0.12	6.44 ± 0.18[a]	8.6 (6)
L-694247	CA GR127935T (100 nM)	7.92 ± 0.26	6.52 ± 0.14[a]	8.4 (5)
L-694247	SA BRL15572 (1 μM)	7.49 ± 0.38	7.74 ± 0.30	NE (5)
RU24969	SA SB224289 (1 μM)	5.76 ± 0.25	5.66 ± 0.13	NE (5)

NS, nonsurmountable; NC, noncompetitive; NE, no effect.
[a] $P < 0.001$.

3. Results

3.1. Effect of 5-HT on contractile responses of supernumerary and conventional artery rings

5-HT produced concentration-dependent contractions in supernumerary and conventional artery rings (Fig. 1). Supernumerary arteries (10 nM–100 μM) were significantly more sensitive to 5-HT than conventional arteries (100 nM–100 μM) (Table 2).

3.2. Contractile responses to 5-HT receptor-selective agonists in supernumerary and conventional artery rings

pD_2 values and maximum responses of agonists are given in Table 2.

The 5-HT_2 receptor-selective agonist, DOI (1 nM–1 μM) produced similar concentration-dependent contractions in supernumerary and conventional artery rings (Fig. 2). In supernumerary arteries, the 5-HT_1 receptor-selective agonists (all 0.1 nM–50 μM) produced small contractile responses. The rank order of potencies were: L694247 > 5-CT > RU24969 > sumatriptan > 8-OH-DPAT. No contractile responses were obtained with 5-HT_1 receptor agonists in conventional arteries.

3.3. Effect of ritanserin and GR127935 on the 5-HT concentration–response curve in supernumerary and conventional arteries

pD_2 values and estimated pK_B values are given in Table 3.

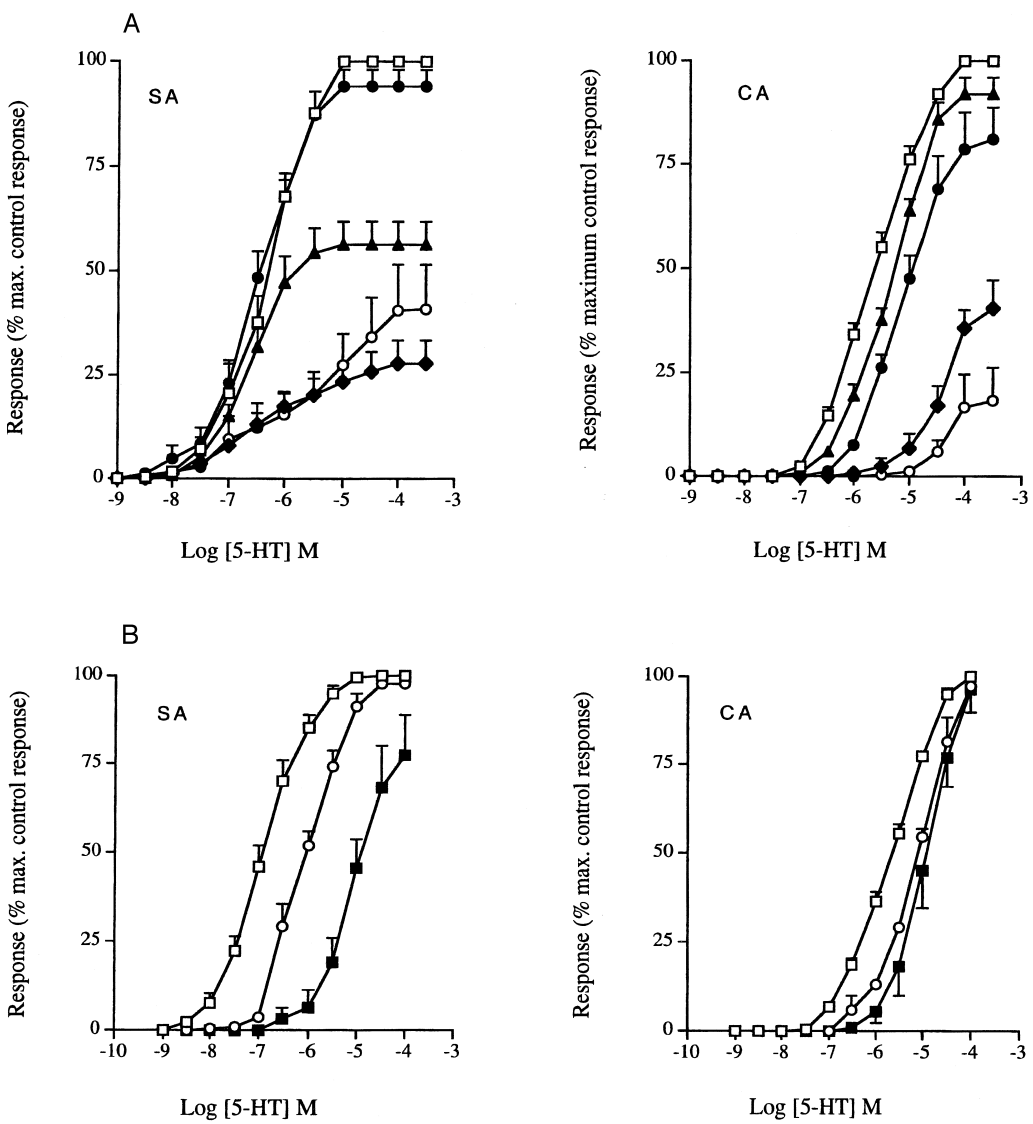

Fig. 3. Effect of increasing concentrations of ritanserin (A) and GR127935T (B) on concentration–response curves to 5-HT (□) in isolated supernumerary (SA) and conventional (CA) artery rings. Antagonist concentrations: ▲, 0.1 nM; ●, 1 nM; ◆, 10 nM; ○, 100 nM; ■, 1 μM. Results are means ± SEM of 8–10 experiments.

In supernumerary arteries ritanserin (1–100 nM) produced a concentration-dependent depression of the maximum contractile response without any rightward shift in the 5-HT concentration–response curve (Fig. 3A). The maximum inhibition by ritanserin was achieved at 10 nM, which reduced the maximum contraction by approximately 75%, leaving a ritanserin-resistant component. In conventional arteries ritanserin (1–100 nM) produced a concentration-dependent depression of the maximum contractile response and also produced a small rightward shift in the 5-HT concentration–response curve (Fig. 3A).

In supernumerary vessels GR127935 (100 nM and 1 μM) produced parallel rightward shifts in the 5-HT concentration–response curve (Fig. 3B). In conventional artery rings GR127935 (100 nM) produced a small threefold rightward shift of the 5-HT concentration–response curve (Fig. 3B). The addition of a higher concentration of GR127935T (1 μM) did not cause any further shift in the 5-HT concentration–response curve.

In supernumerary arteries, the combination of ritanserin (100 nM) and GR127935 (100 nM) abolished the contractile response to 5-HT (data not shown).

3.4. Effect of BRL15572, SB224289 and cyanopindolol on the 5-HT concentration–response curve in supernumerary and conventional artery rings

In supernumerary and conventional arteries BRL15572 (100 nM), SB224289 (100 nM), or cyanopindolol (10 nM–1 μM) did not alter the 5-HT concentration–response curve (Table 3). In supernumerary arteries, the combination of BRL15527 and SB242289 did not alter the 5-HT concentration–response curve.

3.5. Effect of 5-HT, 5-HT$_{2A}$ receptor-selective and 5-HT$_1$ receptor-selective agonists on reversal of forskolin-induced relaxation of preconstricted supernumerary and conventional artery rings

pD_2 values and maximum responses of agonists are given in Table 2.

The 5-HT$_{2A}$ receptor agonist DOI (1 nM–1 μM), in the absence of ritanserin, did not reverse forskolin-relaxation in either population (Fig. 4).

In both supernumerary and conventional artery rings, preconstricted with U46619 (100 nM) and relaxed with forskolin (1 μM) and in the presence of ritanserin (100 nM), 5-HT and the 5-HT$_1$ agonists (all 0.1 nM–50 μM) induced a concentration-dependent contraction (Fig. 4). The rank order of potencies were: supernumerary, L694247 > 5-CT > RU24969 > 5-HT > sumatriptan = 8-OH-DPAT; conventional, L694247 > 5-CT > 5-HT = sumatriptan = RU24969 > 8-OH-DPAT.

3.6. Effect of GR127935, methiothepin, BRL15572, SB224289, cyanopindolol or BRL15572 + SB224289A on 5-HT-induced reversal of forskolin-induced relaxation of preconstricted supernumerary and conventional artery rings

pD_2 values and estimated pK_B values are given in Table 3.

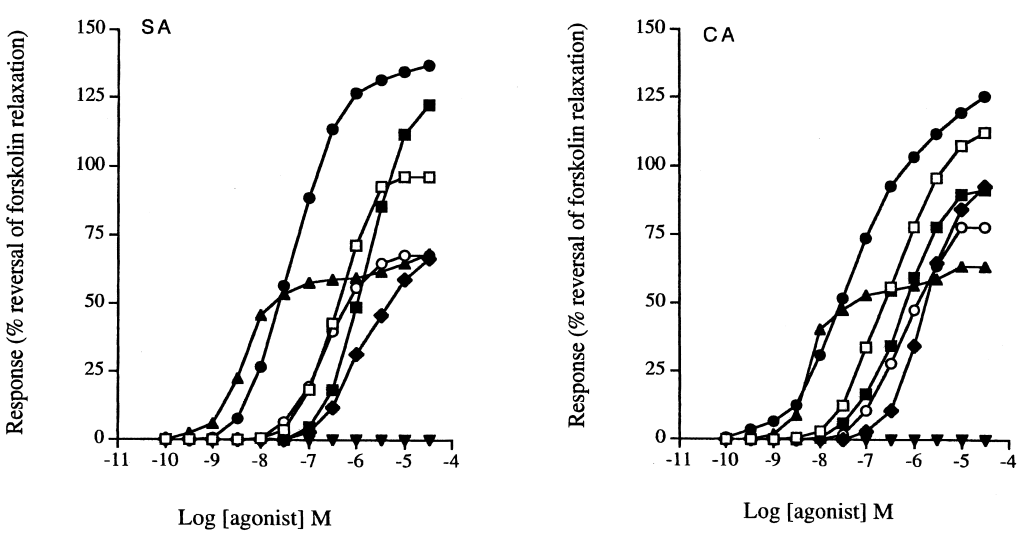

Fig. 4. Concentration–response curves for reversal of forskolin relaxed rings by 5-HT (□) and the selective agonists 8-OH-DPAT, ♦; RU24969, ○; 5-CT, ●; L694247, ▲; Sumatriptan, ■; DOI, ▼; in isolated pulmonary supernumerary (SA) and conventional (CA) artery rings. With the exception of DOI all experiments were conducted in the presence of ritanserin (100 nM). Artery rings were preconstricted with U46619 (100 nM) and relaxed with forskolin (1 μM); thereafter, agonists were added in a cummulative manner. Results are means ± SEM of six to eight experiments.

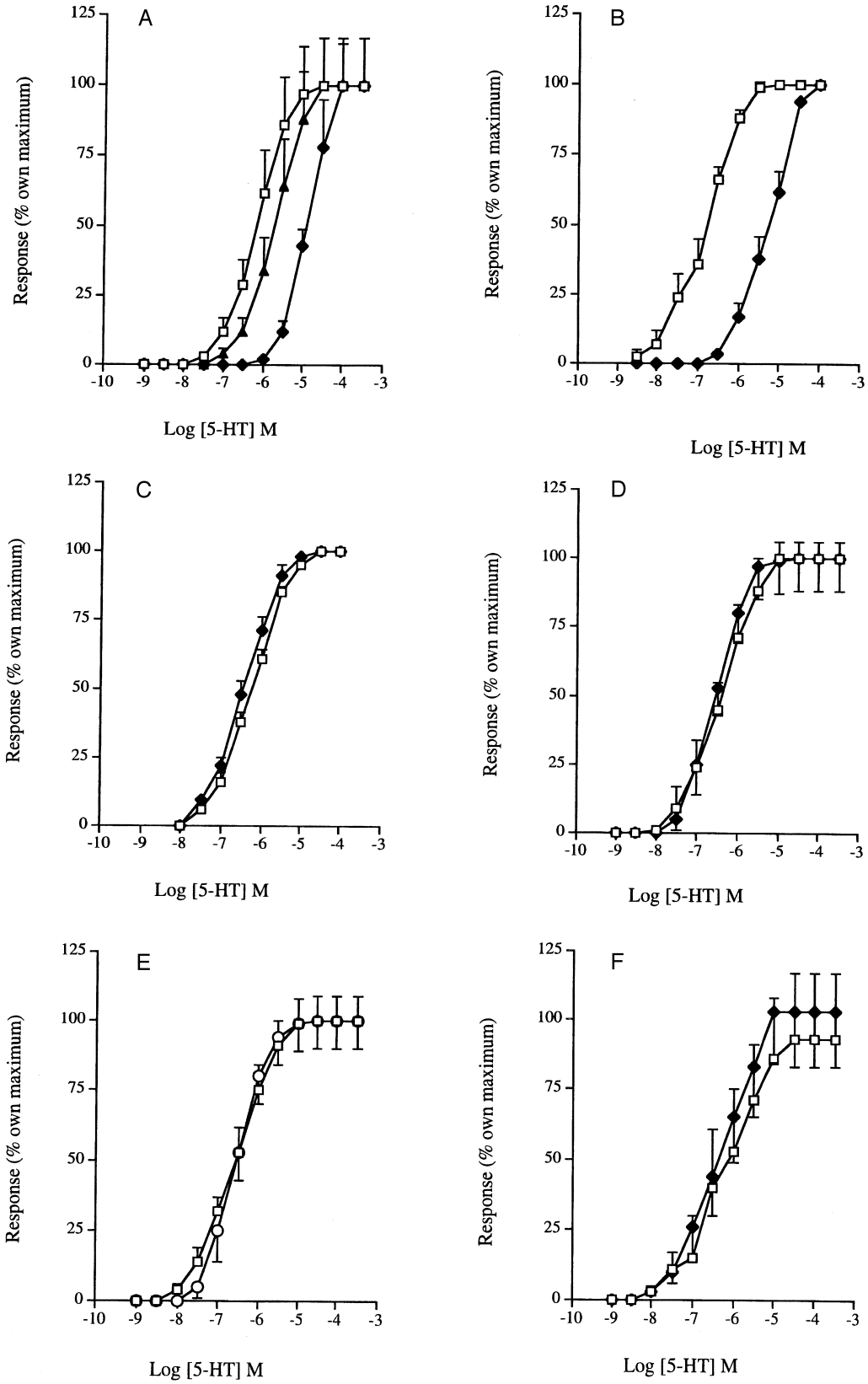

Fig. 5. Concentrations–response curves for vasoconstriction to 5-HT in forskolin relaxed supernumerary artery rings in the absence (□) and presence of (A) GR127935T; (B) methiothepin; (C), BRL15572; (D) SB224289; (E), cyanopindolol; (F) BRL15572 + SB224289. Antagonist concentrations: 10 nM, ▲; 100 nM, ◆; 1 μM, ○. All artery rings were preincubated with ritanserin (100 nM) alone or ritanserin plus one of the above antagonists for 30 min. Each ring was then constricted with U46619 (100 nM) and relaxed with forskolin (1 μM); thereafter, 5-HT was added in a cummulative manner. Results are means ± SEM of four to five experiments.

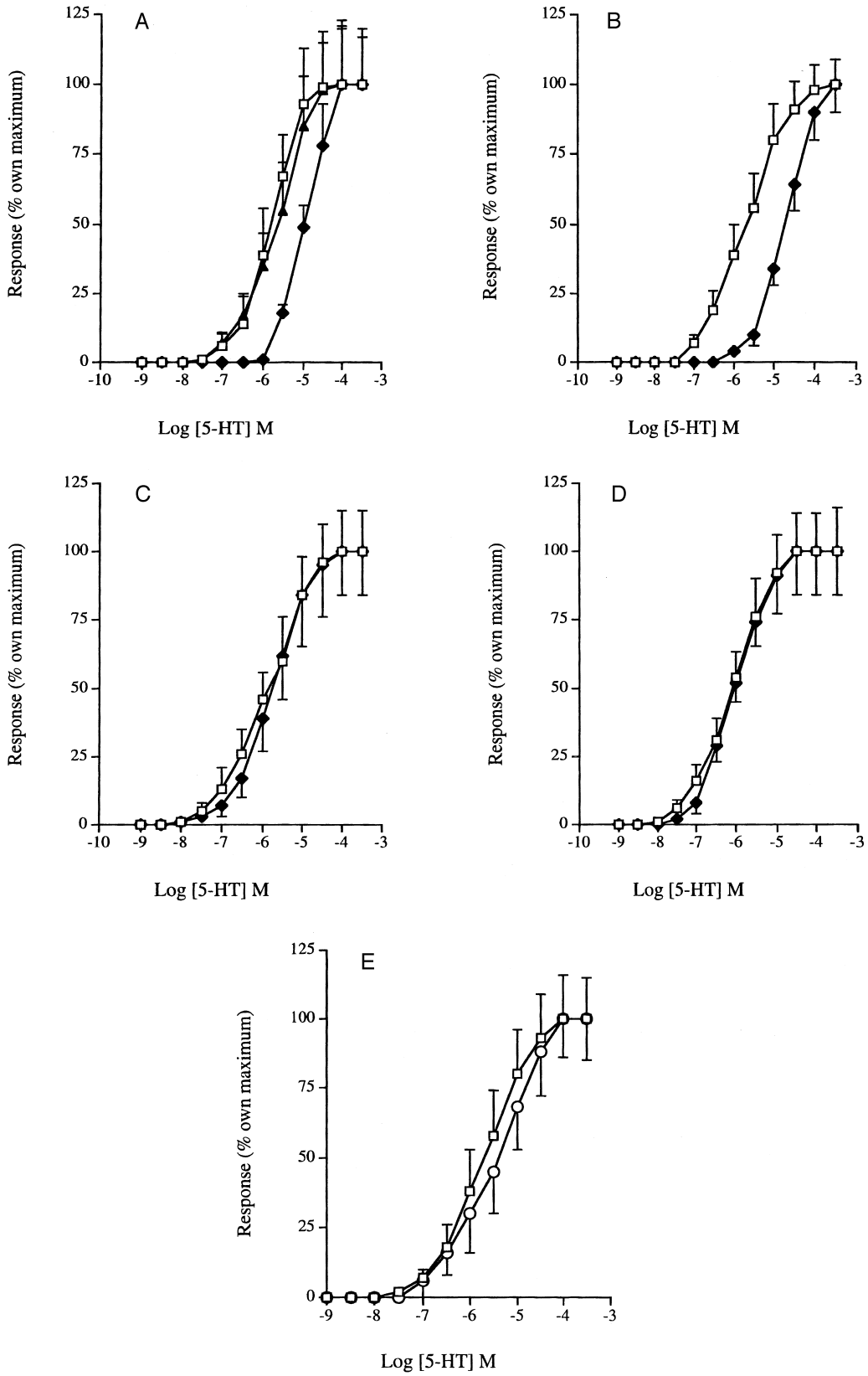

Fig. 6. Concentrations–response curves for vasoconstriction to 5-HT in forskolin relaxed conventional artery rings in the absence (□) and presence of (A) GR127935T; (B) methiothepin; (C), BRL15572; (D) SB224289; (E), cyanopindolol. Antagonist concentrations:10 nM, ▲; 100 nM, ♦; 1 μM, ○. All artery rings were pre-incubated with ritanserin (100 nM) alone or ritanserin plus one of the above antagonists for 30 min. Each ring was then constricted with U46619 (100 nM) and relaxed with forskolin (1 μM); thereafter, 5-HT was added in a cummulative manner. Results are means ± SEM of four to five experiments.

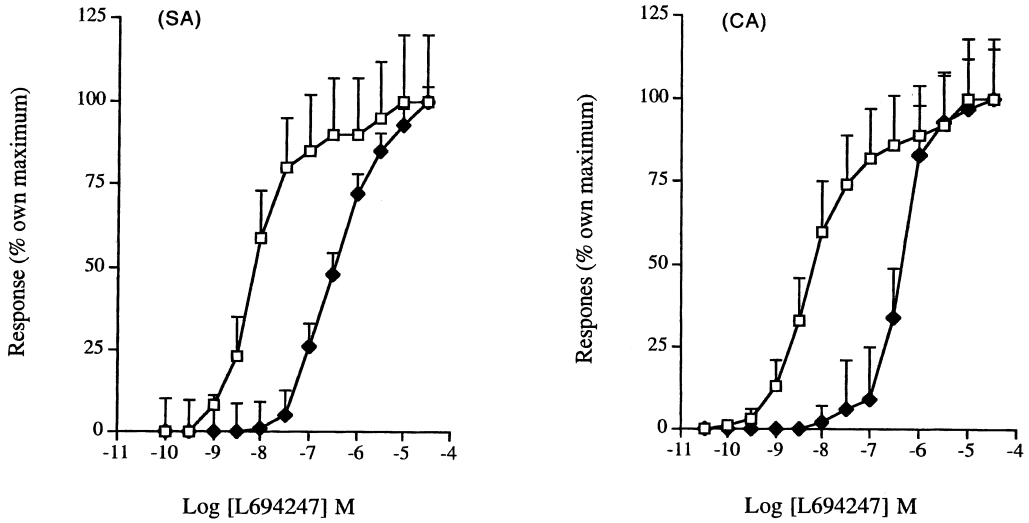

Fig. 7. Concentration–response curves for vasoconstriction to L-694247 in forskolin relaxed supernumerary (SA) and conventional (CA) artery rings in the absence (□) and presence of (A) GR127935T. Antagonist concentration: 100 nM, ◆. All artery rings were preincubated with ritanserin (100 nM) alone or ritanserin plus GR127935 for 30 min. Each ring was then constricted with U46619 (100 nM) and relaxed with forskolin (1 μM); thereafter, L-694247 was added in a cummulative manner. Results are means ± SEM of four to five experiments.

In supernumerary arteries GR127935 (Fig. 5A) and methiothepin (Fig. 5B) produced rightward shifts of the 5-HT concentration–response curve. In contrast BRL15572 (100 nM, Fig. 5C), SB224289 (100 nM, Fig. 5D), cyanopindolol (1 μM, Fig. 5E) or BRL15572 + SB224289 (both 100 nM, Fig. 5F) did not alter the 5-HT concentration–response curve.

In conventional arteries, GR127935T (100 nM) and methiothepin (100 nM) produced rightward shifts of the 5-HT concentration–response curve (Fig. 6A and B). BRL15572 (100 nM, Fig. 6C), SB224289 (100 nM, Fig. 6D) and cyanopindolol (1 μM, Fig. 6E) did not alter the 5-HT concentration–response curve.

3.7. Effect of GR127935T on L694247-induced reversal of forskolin relaxed supernumerary and conventional artery rings

pD_2 values and estimated pK_B values are given in Table 3.

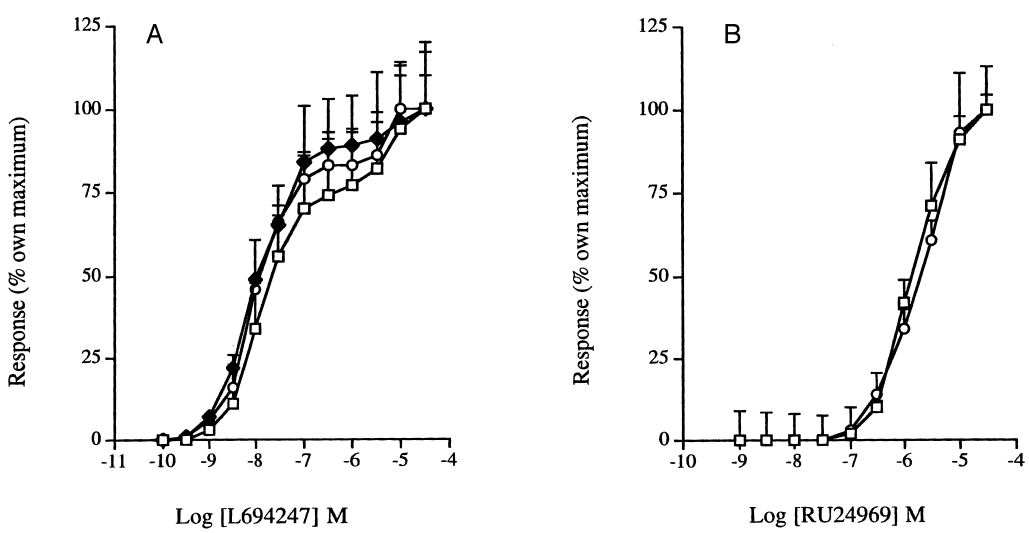

Fig. 8. Concentration–response curves for (A) L-694247 in the absence (□) and presence of BRL15572 and (B) RU24969 in the absence (□) and presence of SB224289. Antagonist concentrations: 100 nM, ◆; 1 μM, ○. All artery rings were preincubated with ritanserin (100 nM) alone or ritanserin plus one of the above antagonists for 30 min. Each ring was then constricted with U46619 (100 nM) and relaxed with forskolin (1 μM); thereafter, agonists were added in a cummulative manner. Results are means ± SEM of four to five experiments.

In supernumerary and conventional arteries GR127935 (100 nM), produced similar rightward shifts of the concentration–response curve for L694247 (Fig. 7).

3.8. Effect of BRL15572 and SB224289 on L694247- and RU24969-induced reversal of forskolin relaxed supernumerary artery rings

pD_2 values are given in Table 3. In supernumerary arteries the concentration–response curve for L694247 and RU24969 were unaffected by BRL15572 (100 nM and 1 μM) or SB224289 (1 μM), respectively (Fig. 8).

4. Discussion

4.1. Agonist studies

The present study confirms a previous report (Bunton et al., 2000) that bovine pulmonary supernumerary arteries display a greater sensitivity to 5-HT than their parent conventional artery.

That DOI produced similar maximum contractile responses in supernumerary and conventional arteries indicates that a 5-HT_2 receptor is present in both arterial populations. DOI displays selectivity for the 5-HT_{2A} and 5-HT_{2C} receptors; however, the 5-HT_{2C} receptor has not been reported to be present on peripheral tissue indicating a role for the 5-HT_{2A} receptor. This is in agreement with previous reports that the 5-HT_{2A} receptor is important in mediating contractile responses of pulmonary vascular smooth muscle (Uma and Kayaalp, 1987; Le Roux and Syce, 1989; MacLean et al., 1994). In both arterial populations the maximum response induced by DOI was less than the maximum response produced by 5-HT. This may suggest that activation of a 5-HT_{2A} receptor alone may not evoke the full contractile response. However, several studies report that DOI is a more potent agonist and displays a higher binding affinity for the 5-HT_2 receptors than 5-HT but displays a lower efficacy than 5-HT (Cohen et al., 1993; Dabire et al., 1989). Therefore, DOI may simply confirm the presence of the 5-HT_{2A} receptor in these vessels but may not truly reflect the contribution of the 5-HT_{2A} receptors to the contractile response to 5-HT.

The 5-HT_1 receptor-selective agonists (in the presence of ritanserin) produced little or no contractile response in conventional arteries, in agreement with MacLean et al. (1994). In supernumerary arteries, these agonists produced contractile responses that were generally less than 25% of the maximum response to 5-HT. This would indicate that a 5-HT_1 receptor mediating contraction is present in supernumerary arteries but not conventional arteries. Since the selective 5-HT_{1D} receptor agonist L694247 was the most potent and efficacious of the 5-HT_1 agonists, this may suggest that the 5-HT_{1D} receptor is present on supernumerary arteries.

4.2. Antagonist studies

Ritanserin caused insurmountable antagonism of the 5-HT concentration–response curve in both arterial populations, reducing the magnitude of the responses to 5-HT. In supernumerary arteries, this occurred without changing the tissue sensitivity to 5-HT, whereas in conventional arteries there was also a rightward shift of the 5-HT concentration–response curve. A ritanserin-resistant component remained in supernumerary arteries accounting for approximately 25% of the maximum response to 5-HT. Since the ritanserin resistant component is similar to the maximum response evoked by the 5-HT_1 selective agonists and because this component was abolished by GR127935 (data not shown), this suggests that the ritanserin-resistant component is mediated by a 5-HT_1 receptor. The inhibitory action of ritanserin supports the view that the 5-HT_{2A} receptor has an important role in the contractile response to 5-HT in these arteries. Insurmountable antagonism has been reported previously for ritanserin in pulmonary arteries (Marwood, 1994) and in other tissues especially at the 5-HT_{2A} receptor (Leff and Martin, 1989; Frenken and Kaumann, 1984; Leff and Martin, 1986). There is no clear explanation for this phenomenon, however several theoretical suggestions have been proposed (De Chaffoy de Courcelles et al., 1986; Frenken and Kaumann, 1987a,b; Leff and Martin, 1989).

In supernumerary arteries the 5-$HT_{1B/1D}$ receptor antagonist GR127935 produced parallel rightward shifts of the 5-HT concentration–response curve. In contrast, in conventional arteries GR127935 (100 nM) produced a small rightward shift which was not altered by the addition of a higher concentration of GR127935 (1 μM).

Since the selective 5-HT_{1D} receptor agonist L694247 was much more potent than the 5-HT_{1B} receptor agonist RU24969, these observations suggest that a 5-HT_{1D} receptor is involved in mediating the contractile response to 5-HT. The greater antagonism of the 5-HT concentration–response curve, by GR127935, in supernumerary arteries suggests that this receptor is present to a greater extent in these arteries. The pK_B (7.9) estimated for GR127935 (100 nM) in supernumerary arteries is lower than the value reported for the cloned receptors (8.6, Table 3). One explanation for the lower affinity of GR127935 in supernumerary arteries may be that a component of the 5-HT concentration–response curve is mediated by the 5-HT_{2A} receptor.

In the presence of 1 μM GR127935 the sensitivities of both arterial populations to 5-HT are similar (Fig. 3B). Based on the reported affinity at the 5-HT_{1D} receptor this concentration of GR127935 would be expected to occupy greater than 99% of the 5-HT_{1D} receptors. Therefore, the response to 5-HT in the presence of 1 μM GR127935 can be assumed to be mediated almost entirely by the 5-HT_{2A} receptor class. This would suggest that the 5-HT_{2A} receptor in conventional and supernumerary arteries exhibits a

low affinity for the natural agonist compared with the 5-HT_{1D} receptor. Under these conditions, 5-HT_{2A} receptors appear capable of producing the maximum response, despite being less sensitive to 5-HT. The fact that the 5-HT concentration–response curves in supernumerary and conventional arteries were approximately two log units and one-half log unit (respectively) more sensitive than the responses of these arteries in the presence of GR127935 (1 μM) would suggest that the greater sensitivity in the absence of GR127935 is due to the presence of the 5-HT_{1D} receptor. The fact that the pD_2 of the response to 5-HT in conventional arteries is closer to the apparent affinity of the 5-HT_{2A} receptor for 5-HT may suggest that the 5-HT concentration–response curve in conventional arteries involves predominantly 5-HT_{2A} receptors. Therefore, it is possible that the relative densities of the 5-HT_{1D} receptor might explain the difference in the sensitivity of these two arterial populations to 5-HT.

4.3. Interaction between the 5-HT_{2A} and 5-HT_{1D} receptors

This explanation of a high affinity 5-HT_{1D} receptor and a low affinity 5-HT_{2A} receptor is an over simplification because it is apparent that ritanserin substantially reduced the magnitude of the response to 5-HT in supernumerary arteries over a concentration range of 5-HT (Fig. 3A and B), which the previous argument suggests produces minimal activation of the 5-HT_{2A} receptors. It is also clear that 5-HT (1 μM), which normally produced 80% of the maximum response to 5-HT in supernumerary arteries, only evoked approximately 20% (in the presence of ritanserin) and 10% (in the presence of GR127935) of the maximum response to 5-HT by independent activation of the 5-HT_{1D} and 5-HT_{2A} receptors, respectively. These observations indicate that the contractile response produced by 5-HT is much greater than the response produced by activation of the individual 5-HT_{2A} or 5-HT_{1D} receptors alone or the simple addition of their responses. This suggests that there is some positive interaction between the 5-HT_{1D} and the 5-HT_{2A} receptors. We have reported previously that sumatriptan and DOI display synergy in supernumerary arteries (Bunton et al., 1997). Because activation of the 5-HT_{2A} receptors alone can, at high concentrations, produce a maximum response, whereas activation of the 5-HT_{1D} receptors alone can only produce a small contractile response in supernumerary arteries, this may suggest that the role of the 5-HT_{1D} receptors, within this interaction, is to influence potency while the 5-HT_{2A} receptors determine the magnitude of the response.

4.4. Reversal of forskolin-induced relaxation, agonist studies

When we examined the effect of the selective 5-HT_{1D} receptor antagonist (BRL15572) to confirm the presence of the 5-HT_{1D} receptor in supernumerary arteries we found no effect of this or other 5-HT_1 subtype-selective antagonists (Table 3). We therefore adopted a methodology that would permit a more direct investigation of the 5-HT_1 receptor class mediating contraction. Because the 5-HT_1 receptor class is reported to be negatively coupled to adenylyl cyclase, a technique that is commonly used to characterise 5-HT_1 receptors has been to examine the ability of 5-HT_1 selective agonists to inhibit forskolin-activated adenylyl cyclase (Levy et al., 1992; McAllister et al., 1992; Beer et al., 1993) or forskolin-elevated cyclic AMP (Adham et al., 1993; Gudermann et al., 1993; Price et al., 1997). In a preliminary report we have shown that 5-HT inhibits forskolin-elevated cAMP in supernumerary arteries (Brown et al., 2000).

We used a similar approach (agonist-induced reversal of forskolin-relaxed artery rings preconstricted with U46619 (approx. EC80) in the presence of ritanserin (100 nM)) to investigate the 5-HT_1 receptor. Using this approach 5-HT and the 5-HT_1 receptor agonists reversed both forskolin-relaxed supernumerary and conventional arteries. These studies were conducted in the presence of ritanserin to block the 5-HT_{2A} receptor. However, it seems unlikely that the 5-HT_{2A} receptor is involved in this response because the concentration of forskolin used abolishes responses mediated by this receptor (data not shown), moreover DOI (in the absence of ritanserin) did not reverse the forskolin-relaxation in either arterial population and the concentration–response curves for 5-HT-induced reversal of forskolin-relaxation were unaffected by ritanserin (up to 1 μM) in either population (data not shown).

This suggests that a 5-HT receptor apparently negatively coupled to adenylyl cyclase is present in both arterial populations. Not all of the 5-HT_1 receptor agonists produced 100% reversal of the forskolin relaxation. L694247 and 5-CT were the most potent of the 5-HT_1 receptor agonists in both arterial populations. However, whereas 5-CT consistently produced 100% reversal, L694247 only produced around 50–60% reversal. Although this may suggest that L694247 is a partial agonist, the previous section shows that, in unmanipulated supernumerary arteries (not forskolin-relaxed), L694247 was the most efficacious of the 5-HT_1 receptor agonists, producing a contractile response that was approximately twice as great as 5-CT (Fig. 2). Therefore, if the ability of the 5-HT_1 receptor agonists to reverse the forskolin-relaxation is a reflection of their ability to inhibit adenylyl cyclase then these observations suggest that inhibition of adenylyl cyclase alone cannot account for the contractile responses (not forskolin-relaxed) mediated by L69427 and 5-CT in supernumerary arteries. Interestingly, L694247 consistently produced a biphasic concentration–response curve consisting of an initial reversal of the forskolin-relaxation resulting in a plateau around 50–60% of the maximum reversal. Concentrations above 100 nM produced a further reversal. This could indicate the presence of different

receptors, which display a high and low affinity for L694247.

4.5. Reversal of forskolin-induced relaxation, antagonist studies

In these studies GR127935 and methiothepin produced rightward shifts of the 5-HT concentration–response curve for reversal of forskolin-relaxation in both arterial populations. However, both GR127935 and methiothepin produced greater antagonism in supernumerary arteries compared with conventional arteries (Figs. 5 and 6). In contrast, when the forskolin-relaxation was reversed by the selective 5-HT_{1D} receptor agonist L694247, GR127935 produced a greater antagonism, which was similar in both arterial populations (Fig. 7, Table 3). This may suggest that the reversal of forskolin-relaxation mediated by L694247 involves a single receptor type, which is present on both supernumerary and conventional arteries. The estimated pK_B values for GR127935 at this receptor in conventional (8.4) and supernumerary arteries (8.6) are similar to the reported affinity of GR127935 at the cloned 5-HT_{1D} receptor (8.6, Table 4) providing further support for the presence of a 5-HT_{1D} receptor subtype. The lower pK_B values for GR127935 when the natural agonist 5-HT was used may indicate that 5-HT, but not L694247, has the ability to activate an additional receptor, which is less sensitive to antagonism by GR127935. These data suggest that at least two receptors, apparently negatively coupled to adenylyl cyclase, are present in supernumerary and conventional arteries. The fact that the pK_B for GR127935 (using 5-HT as the agonist) was significantly lower in conventional arteries compared with supernumerary arteries (Table 3) may indicate that the GR127935-insensitive receptor is more abundant in conventional arteries and the GR127935-sensitive receptor is in greater abundance in supernumerary arteries. If 5-HT is less potent at the GR127935-insensitive receptor, this may also explain the lower pD_2 (Table 2) for 5-HT-induced reversal of forskolin-relaxation in conventional arteries. In unmanipulated arteries, GR127935 produced greater antagonism of the 5-HT concentration–response curve in supernumerary than conventional arteries. This supports the view that supernumerary arteries have a greater proportion of GR127935-sensitive receptors than conventional arteries, but also suggests that although both GR127935-sensitive and GR127935-insensitive receptors are apparently negatively coupled to adenylyl cyclase, only the GR127935-sensitive receptor has the capacity to promote constriction in unmanipulated arteries.

Although the pK_B values estimated for GR127935 (using L694247) are consistent with the presence of a 5-HT_{1D} receptor (Table 4), the selective 5-HT_{1D} receptor antagonist BRL15572 had no effect on either the 5-HT- or L694247-induced reversal of forskolin-relaxation. The absence of any involvement of the 5-$HT_{1A/1B}$ receptor subtypes is indicated by the lack of effect of cyanopindolol and also by the lack of effect of the selective 5-HT_{1B} receptor antagonist SB224289 against the concentration–response curves to 5-HT or the selective 5-HT_{1B} receptor agonist RU24969. GR127935 has a low affinity for the 5-HT_{1E} and 5-HT_{1F} receptor subtypes (Table 4). Therefore, because the GR127935-sensitive receptor exhibits only some of the pharmacological characteristics of the cloned 5-HT_{1D} receptor, we have referred to this receptor as a 5-HT_{1D}-like receptor. These observations in the bovine pulmonary arteries differ from the human pulmonary arteries where the 5-HT_{1B} receptor has been reported to mediate contractile responses (MacLean, 1999). It is possible that the GR127935-sensitive receptor is the bovine 5-HT_{1B} receptor but it differs from the human 5-HT_{1B} receptor in that it is sensitive to SB224289.

Table 4
Reported pK_i values at cloned receptors taken from Hoyer et al. (1994), Price et al. (1997), Leysen et al. (1996), Gaster et al. (1998), Skingle et al. (1996) and Pauwels (1996)

	pK_i (cloned receptors)					
	1A	1B	1D	1E	1F	2A
Ritanserin	–					9.3
Methiothepin	7.1	7.6	7.9	6.7	6	8.8
GR127935	7.2	9	8.6	5.4	6.4	6.6
Cyanopindolol	8.4	8.6	6.9	–	–	4.5
BRL15572	7.7	6.1	7.9	5.2	6	–
SB224289	<5.5	8.2	6.3	<5	<5	–

In summary these studies suggest that the 5-HT_{2A} receptor is involved in the contractile response to 5-HT in bovine supernumerary and conventional arteries. In supernumerary and conventional arteries there are at least two receptors, GR127935-sensitive and GR127935-insensitive, that appear to be negatively coupled to adenylyl cyclase. Supernumerary arteries appear to have a greater proportion of GR127935-sensitive receptors whereas conventional arteries have a higher proportion of GR127935-insensitive receptors. The GR127935-sensitive receptor displays some pharmacological characteristics of the cloned 5-HT_{1D} receptor subtype but is not affected by BRL15572. We have therefore referred to this receptor as a 5-HT_{1D}-like receptor. Of the two receptors apparently negatively coupled to adenylyl cyclase the 5-HT_{1D}-like receptor appears to have the capacity to promote contractile responses.

4.6. Pulmonary hypertension

5-HT elevates pulmonary vascular resistance and is associated with some forms of pulmonary hypertension (MacLean, 1999). Conventional arteries accompany the airway to supply the terminal respiratory unit from within the lobule. In contrast to this circuitous route supernumerary arteries arise from the conventional arteries at 90° unaccompanied by an airway to take a shorter route,

entering the lobule from its edge (Reid, 1994). Supernumerary arteries account for 40% of the cross-sectional area of all branches from the axial artery alone in the human (Elliott and Reid, 1965). Since they increase in number towards the periphery, they are likely to account for a substantial part of the total cross-sectional area of the pulmonary arterial bed (Elliott and Reid, 1965). Therefore, the sensitivity of these vessels to vasoconstrictors such as 5-HT is likely to have an important influence on pulmonary vascular resistance. Moreover, it has been shown that in pulmonary hypertensive models the 5-HT_1 receptor plays a greater role in contractile responses to 5-HT (MacLean, 1999). As well as influencing pulmonary vascular resistance, 5-HT is also a powerful mitogen; some of the most marked pathological changes in pulmonary hypertension are reported to occur at the origin of the supernumerary arteries. For example, medial proliferation appears to be particularly pronounced in this region (Reid, 1994; Yaginuma et al., 1990; Ogata and Iijima, 1993).

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References

Adham, N., Kao, H.-T., Schechter, L.E., Bard, J., Olsen, M., Urquhart, D., Durkin, M., Hartig, P.R., Weinshank, R.L., Branchek, T.A., 1993. Cloning of another human serotonin receptor (5-HT_{1F}): a fifth 5-HT_1 receptor subtype coupled to the inhibition of adenylyl cyclase. Proc. Natl. Acad. Sci. U. S. A. (Neurobiology) 90, 408–412.

Becker, B.N., Gettys, T.W., Middleton, J.P., Olsen, C.L., Albers, F.J., Lee, S.-L., Fanburg, L., Raymond, J.R., 1992. 8-Hydroxy-2-(di-*n*-propylamino)tetralin—responsive 5-hydroxytryptamine-4-like receptor expressed in bovine pulmonary artery smooth muscle cells. Mol. Pharmacol. 42, 817–825.

Beer, M.S., Stanton, J.A., Bevan, Y., Heald, A., Reeve, A.J., Street, L.J., Matassa, V.G., Hargreaves, R.J., Middlemiss, D.N., 1993. L-964,247: a potent 5-HT_{1D} receptor agonist. Br. J. Pharmacol. 110, 1196–1200.

Brown, T., Bunton, D., MacDonald, A., Shaw, A.M., 2000. 5-HT_{1D}-like receptor in bovine pulmonary supernumerary arteries. Br. J. Pharmacol. 129, 186.

Bunton, D., MacDonald, A., Shaw, A.M., 1997. Receptor subtypes for 5-HT in bovine pulmonary supernumerary arteries. Br. J. Pharmacol. 122, 313 pp.

Bunton, D., MacDonald, A., Brown, T., Tracey, A., McGrath, J.C., Shaw, A.M., 2000. 5-Hydroxytryptamine- and U46619-mediated vasoconstriction in bovine pulmonary conventional and supernumerary arteries. Clin. Sci. 98 (1), 81–89.

Clitherow, J.W., Scopes, D.I., Skingle, M., Jordan, C.C., Feniuk, W., Campbell, I.B., Carter, M.C., Collington, E.W., Connor, H.E., Higgins, G.A., Beattie, D., Kelly, H.A., Mitchell, W.L., Oxford, A.W., Wadsworth, A.H., Tyers, M.B., 1994. Evolution of a novel series of [(*N*,*N*-dimethylaminno)propyl]- and piperazinylbenzanilides as the first selective 5-HT_{1D} antagonists. J. Med Chem. 37, 2253–2257.

Cohen, M.L., Johnson, M.P., Schenck, K.W., Susemichel, A., Wainscott, D.B., Robertson, D.W., Nelson, D.L., 1993. DOI and α-methylserotonin: comparative vascular and nonvasculature smooth muscle effects and central 5-hydroxytryptamine-2 receptor affinities. J. Pharmacol. Exp. Ther. 266, 943–949.

Dabire, H., Chaouche-Teyara, K., Cherqui, C., Fournier, B., Laubie, M., Schmitt, H., 1989. Characterisation of DOI, a putative 5-HT_2 receptor agonist in the rat. Eur. J. Pharmacol. 168 (3), 369–374.

De Chaffoy de Courcelles, D., Leysen, J.E., Roevens, P., van Belle, H., 1986. The serotonin-S2 receptor: a receptor–transducer coupling model to explain insurmountable antagonistic effects. Drug Dev. Res. 8, 173–178.

Elliott, F.M., Reid, L.M., 1965. Some new facts about the pulmonary artery and its branching pattern. Clin. Radiol. 16, 193–198.

Frenken, M., Kaumann, A.J., 1984. Interaction of ketanserin and it's metabolite ketanserinol with 5-HT_2 receptors in pulmonary and coronary arteries of calf. Naunyn-Schiemdeberg's Arch. Pharmacol. 326, 334–339.

Frenken, M., Kaumann, A.J., 1987a. Allosteric properties of the 5-HT_2 receptor system of the rat tail artery-ritanserin and methysergide are not competitive antagonists but allosteric modulators. Naunyn-Schmiedeberg's Arch. Pharmacol. 335, 359–366.

Frenken, M., Kaumann, A.J., 1987b. Allosteric properties of the 5-HT receptors system of the rat tail artery. Naunyn-Schiemdeberg's Arch. Pharmacol. 326, 35–366.

Gaster, L.M., Blaney, F.E., Davies, S., Duckworth, D.M., Ham, P., Jenkins, S., Jennings, A.J., Joiner, G.F., King, F.D., Mulholland, K.R., Wyman, P.A., Hagan, J.J., Hatcher, J., Jones, B.J., Middlemiss, D.N., Price, G.W., Riley, G., Roberts, C., Routledge, C., Selkirk, J., Slade, P.D., 1998. The selective 5-HT_{1B} receptor inverse agonist 1′-Methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7,-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB224289) potently blocks terminal 5-HT autoreceptor function both in vitro and in vivo. J. Med. Chem. 41, 1218–1235.

Glennon, R., McKenney, J.D., Lyon, R.A., Titeler, M., 1986. 5-HT_1 and 5-HT_2 binding characteristics of 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane analogs. J. Med. Chem. 29, 194–199.

Glennon, R.A., Seggel, M.R., Soine, W.H., Herrick-Davis, K., Lyon, R.A., Titeler, M., 1988. [125I]-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane: an iodinated radioligand that specifically labels the agonist high affinity state of 5-HT_2 serotonin receptors. J. Med. Chem. 31, 5.

Gudermann, T., Levy, F.L., Birnbaumer, M., Birnbaumer, L., Kaumann, A.J., 1993. Human serotonin receptor clone encodes a 5-hydroxytryptamine$_{1E}$-like serotonin receptor. Mol. Pharmacol. 43, 412–418.

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. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Humphrey, P.P.A., Hartig, P., Hoyer, D., 1994. A proposed new nomenclature for 5-HT receptors. Trends Pharmacol. Sci. 14, 233–236.

Leff, P., Martin, G.R., 1986. Peripheral 5-HT_2-like receptors. Can they be classified with the available antagonists? Br. J. Pharmacol. 88, 585–593.

Leff, P., Martin, G.R., 1989. Quantification of the actions of 5-hydroxytryptamine receptor agonists and antagonists. In: Fozard, J.R. (Ed.), The Peripheral Actions of 5-Hydroxytryptamine. Oxford Medical Publications, pp. 26–44.

Le Roux, M.G.J., Syce, J.A., 1989. The effect of ketanserin on serotonin-induced vascular responses in the isolated perfused rat lung. Eur. J. Pharmacol. 169, 269–273.

Levy, F.O., Gudermann, T., Birnbaumer, M., Kaumann, A.J., Birnbaumer, L., 1992. Molecular cloning of a human gene (S31) encoding a novel serotonin receptor mediating inhibition of adenylyl cyclase. FEBS 296, 201–206.

Leysen, J.E., Gommeren, W., Heylen, L., Luyten, W.H.M.L., Van de Weyer, I., Vanhoenacker, P., haegeman, G., Schotte, A., Van Gom-

pel, P., Wouters, R., lesage, A., 1996. Alniditan, a new 5-hydroxytryptamine$_{1D}$ agonist and migraine abortive agent: ligand-binding properties of human 5-hydroxytryptamine$_{1D\alpha}$, human 5-hydroxytryptamine$_{1D\beta}$, and calf 5-hydroxytryptamine$_{1D}$ receptors investigated with [^{3}H]5-hydroxytryptamine and [^{3}H] alniditan. Mol. Pharmacol. 50, 1567–1580.

MacLean, M.R., 1999. Pulmonary hypertension, anorexigens and 5-HT: pharmacological synergism in action. TIPS 20, 490–495.

MacLean, M.R., Clayton, R.A., Hillis, S.W., McIntyre, P.D., Peacock, A.J., Templeton, A.G.B., 1994. 5-HT$_1$-receptor-mediated vasoconstriction in bovine isolated pulmonary arteries: influence of vascular endothelium and tone. Pulm. Pharmacol. 7, 65–72.

MacLean, M.R., Sweeney, G., Baird, M., McCulloch. K, M., Houslay, M., Morecroft, I., 1996. 5-hydroxytryptamine receptors mediating vasoconstriction in pulmonary arteries from control and pulmonary hypertensive rats. Br. J. Pharmacol. 119, 917–930.

Marwood, J.F., 1994. Influence of alpha 1-adrenoceptor antagonism of ketanserin on the nature of its 5-HT$_2$ receptor antagonism. Clin. Exp. Pharmacol. Physiol. 21, 955–961.

McAllister, G., Charlesworth, A., Snodin, C., Bee, M.S., Noble, A.J., Middlemiss, D.N., Iversen, L.L., Whiting, P., 1992. Molecular cloning of a serotonin receptor from human brain (5-HT$_{1E}$): a fifth 5-HT$_1$-like subtype. Proc. Natl. Acad. Sci. U. S. A. (Neurobiology) 89, 5517–5521.

Middlemiss, D.N., Tricklebank, M.D., 1992. Centrally active agonists and antagonists. Neurosci. Biobehav. Rev. 16, 75–82.

Morecroft, I., Maclean, M.R., 1998. 5-Hydroxtryptamine receptors mediating vasconstriction and vasodilation in perinatal and adult rabbit small pulmonary arteries. Br. J. Pharmacol. 125, 69–78.

Ogata, T., Iijima, T., 1993. Structure and pathogenesis of plexiform lesions in pulmonary hypertension. Chin. Med. J. (Engl.) 106, 45–48.

Pauwels, P.J., 1996. Pharmacological properties of a putative 5-HT$_{1B/1D}$ receptor antagonist GR127935. CNS Drug Rev. 2, 415–428.

Peroutka, S.J., McCarthy, B.G., 1989. Sumatriptan (GR43175) interacts selectively with 5-HT$_{1B}$ and 5-HT$_{1D}$ binding sites. Eur. J. Pharmacol. 163, 133–136.

Price, G.W., Burton, M.J., Collin, L.J., Duckworth, M., Gaster, L., Gothert, M., Jones, B.J., Roberts, C., Watson, J.M., Middlemiss, D.N., 1997. SB-216641 and BRL15572-compounds to pharmacologically discriminate h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors. Naunyn-Schmiedeberg's Arch Pharmacol. 356, 312–320.

Reid, L.M., 1994. Structural remodelling of the pulmonary vasculature by environmental change and disease. In: Wagner, W.W., Weir, E.K. (Eds.), The Pulmonary Circulation and Gas Exchange. Futura, New York, pp. 77–110.

Shaw, A.M., Bunton, D., Fisher, A., Daly, C., McGrath, J.C., MacDonald, A., 1999. V-shaped cushion at the origin of bovine pulmonary supernumerary arteries: structure and putative function. J. Appl. Physiol. 87, 2348–2356.

Skingle, M., Beattie, D.T., Scopes, D.I.C., Starkey, S.J., Connor, H.E., Feniuk, W., Tyers, M.B., 1996. GR127935: a potent and selective 5-HT$_{1D}$ receptor antagonist. Behav. Brain Res. 73, 157–161.

Uma, S., Kayaalp, S.O., 1987. The subtype of serotonin (5-HT) receptors in the rat pulmonary artery. Arch. Int. Pharmacodyn. 288, 248–255.

Yaginuma, G., Mohri, H., Takahashi, T., 1990. Distribution of arterial lesions and collateral pathways in the pulmonary hypertension of congenital heart disease: a computer-aided reconstruction study. Thorax 45, 586–590.