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# Characterization of 5-HT receptors on human pulmonary artery and vein: functional and binding studies

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**1** This study aimed to investigate the 5-hydroxytryptamine (5-HT) receptors mediating contraction of ring preparations isolated from human pulmonary arteries and veins. In functional studies, the responses to 5-HT, sumatriptan, ergotamine, serotonin-*O*-carboxymethyl-glycyl-tyrosinamide (SCMGT), α-methyl 5-HT (α-Me) and 2-methyl 5-HT (2-Me) were studied with WAY100635, GR127935, ritanserin, zacopride and SB204070 as antagonists.

**2** All agonists produced concentration-dependent contractions of human pulmonary artery and vein preparations. The order of potency ($-\log EC_{50}$ values) was ergotamine (6.88) > 5-HT (6.41) ⩾ SCMGT (6.20) = sumatriptan (6.19) ⩾ α-Me (6.04) in the artery, and ergotamine (7.84) > 5-HT (6.96) > sumatriptan (6.60) = α-Me (6.56) > SCMGT (6.09) in the vein. The potency of each agonist, except for SCMGT, was greater in vein than in artery preparations. Contractile responses to 5-HT were similar in intact and endothelium-denuded preparations but responses to sumatriptan were enhanced in artery rings without endothelium.

**3** GR127935 (1 nM to 0.5 μM) produced an unsurmountable antagonism of the response to 5-HT, sumatriptan, ergotamine and SCMGT. Ritanserin (1 nM to 1 μM) also reduced the maximum contractile responses to 5-HT, ergotamine and α-Me in artery and vein preparations without affecting those to sumatriptan and SCMGT. In endothelium-denuded preparations, surmountable antagonism of sumatriptan by GR127935 (in the presence of ritanserin) and of α-Me by ritanserin (in the presence of GR127935) allowed for the calculation of the apparent $pK_B$ values of GR127935 ($9.17 \pm 0.11$ in artery and $9.11 \pm 0.05$ in vein) and ritanserin ($8.82 \pm 0.09$ in artery and $8.98 \pm 0.12$ in vein).

**4** WAY100635 (1 nM to 1 μM), zacopride (1 nM to 1 μM), or SB204070 (1 nM) did not significantly alter the concentration-response curves for 5-HT, sumatriptan, ergotamine, SCMGT or 2-Me in human pulmonary artery or vein thus indicating that 5-$HT_{1A}$, 5-$HT_3$ and 5-$HT_4$ receptors are presumably not involved in the contractile response to these agonists.

**5** Binding studies using selective radioligands for different 5-HT receptors could not detect the presence of 5-$HT_{1A}$ receptor binding in human pulmonary blood vessels whereas the 5-$HT_{1B/1D}$ radioligand [$^3$H]-5-CT significantly labelled a population of specific binding sites in both vessel types. The presence of 5-$HT_{2A}$ receptors could also be inferred from the level of binding of [$^3$H]-ketanserin to membranes obtained from human pulmonary vessels, although significance could not be reached for arteries. 5-$HT_4$ specific receptor binding was scarce in veins and absent in the case of arteries.

**6** These findings indicate that the human pulmonary artery and vein have a mixed functional population of 5-$HT_{1B/1D}$ and 5-$HT_{2A}$ receptors mediating the contractile response to 5-HT which is consistent with results of the binding studies.

**Keywords:** 5-Hydroxytryptamine receptors; human pulmonary artery; human pulmonary vein; vasoconstriction

## Introduction

5-Hydroxytryptamine (5-HT, serotonin) has been implicated in both primary and secondary pulmonary hypertension (Hervé *et al*., 1995) including that caused by drugs which inhibit 5-HT uptake (Brenot *et al*., 1993). Moreover, chest disturbances have been often mentioned as side-effects associated to the treatment with the new generation of antimigraine drugs which are agonists at 5-$HT_1$ receptors (Ottervanger & Stricker, 1995). In the lungs, 5-HT may be released from pulmonary neuroendocrine cells and from platelets in pulmonary thrombi (Johnson & Georgieff, 1989) resulting in local concentrations presumably higher than those found in plasma (⩽30 nM; Hervé *et al*., 1995) and therefore sufficient to produce a vasoconstrictor effect on the pulmonary arteries and veins (Houghton & Phillips, 1973). Despite its interest, the characteristics of the pulmonary vascular 5-HT receptors have been less extensively studied in humans than in experimental animals.

Houghton & Phillips (1973) first reported a contractile response to 5-HT in spirally cut preparations of human isolated pulmonary artery and vein. Initially, results from animal and human studies indicate that the vasoconstrictor effect of 5-HT in pulmonary vascular preparations was mediated through 5-$HT_2$ receptors (Frenken & Kaumann, 1984; Raffestin *et al*., 1985; Selig *et al*., 1988), more recently classified as 5-$HT_{2A}$. However, recent studies revealed the involvement of 5-$HT_{1B/1D}$ receptors in vasoconstrictor responses to 5-HT in bovine and human isolated pulmonary arteries (Maclean *et al*., 1994, 1996).

We have extended these pharmacological studies further to characterize the types of 5-HT receptors mediating the vasoconstrictor response to 5-HT in human pulmonary artery and vein by using a number of ligands known for their 5-HT receptor agonist or antagonist properties (for a review on selectivity, see Hoyer *et al*., 1994). In functional studies we have examined the contractile responses to the endogenous agonist 5-HT, and in addition to sumatriptan, ergotamine and serotonin-*O*-carboxymethyl-glycyl-tyrosinamide (SCMGT). These agonists were studied in the absence and presence of the an-

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tagonists WAY100635, GR127935, ritanserin, zacopride and SB204070. Responses to the agonists α-methyl 5-HT (α-Me) and 2-methyl 5-HT (2-Me) were also studied in the absence and presence of ritanserin and zacopride respectively. In addition, the characterization of 5-HT receptors using selective radioligands has been carried out in pulmonary artery and vein membrane preparations.

## Methods

### *Preparation of human pulmonary blood vessels*

Lung tissue was obtained from patients who were undergoing surgery for lung carcinoma. The number of patients in the study was 84 (78 males; age range 41–78 years). None of the patients had a history of pulmonary hypertension. After the resection of one or more lung lobes, a piece of macroscopically normal tissue was excised and immersed in physiological salt solution (PSS; composition in mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, $KH_2PO_4$ 1.2, $NaHCO_3$ 25.0, glucose 11.1.) at 4°C for transport to the laboratory where pulmonary arteries and veins were dissected free from parenchymal tissue and the preparations were cut in rings (3–4 mm length × 2–5 mm internal diameter). Tissues were used within 3 h of surgery as we have previously shown that preparations stored overnight often failed to show an endothelium-dependent relaxation when stimulated with histamine (Ortiz *et al*., 1995). In some preparations the endothelium was removed by inserting into the lumen of each ring a roughened wooden stick and rubbing gently the intimal surface of the blood vessel. Intact or endothelium-denuded vascular rings were set up in 10 ml organ baths containing PSS, gassed with 5% $CO_2$ in $O_2$ at 37°C (pH 7.4). Each preparation was connected to a force displacement transducer (Grass FT03) and isometric tension changes were recorded by means of a computerized data acquisition system (Proto5, Letica, Barcelona, Spain). Some rings were cryopreserved (Sarria *et al*., 1995) and stored for subsequent radioligand studies (see below).

### *Functional studies*

These experiments were carried out as previously described (Ortiz *et al*., 1995). In brief, the preparations were allowed to equilibrate for 69–90 min (with changes in bath fluid every 20 min) before any pharmacological intervention occurred. A load of 2 g was maintained throughout the equilibration period and a stable resting level of tone was present at the end of this period. In all experiments, vascular ring preparations were initially challenged with a maximally effective concentration of 5-HT (10 μM) and, when the response to 5-HT had reached a plateau, histamine (1 μM) was added to assess endothelium-dependent relaxation in intact tissues or to confirm the lack of relaxation in endothelium-denuded preparations (Ortiz *et al*., 1992). After a further 60–90 min period of washout and resting, during which the tension was readjusted to 2 g, four protocols were followed.

In the first protocol, a cumulative concentration-response curve (CRC) for 5-HT or sumatriptan (each at concentrations from 10 nM to 10 μM) was obtained in intact and endothelium-denuded paired preparations from artery and vein. In the second protocol, a cumulative CRC for 5-HT, sumatriptan, ergotamine, SCMGT, α-Me or 2-Me (concentration range of 10 nM to 10 μM for each agonist) was constructed in intact artery and vein preparations by half logarithmic dosing increments. The first CRC to 5-HT was not altered by the previous challenge with 5-HT (10 μM) (Houghton & Phillips, 1973; Raffestin *et al*., 1985; this study) but a consecutive CRC in the same preparation showed a depression compared to the initial curve; therefore, a single CRC to an agonist was constructed in each preparation either in the absence (control) or presence (test tissue) of a single concentration of an antagonist. An equilibration time of 20 min for the antagonist was allowed before constructing the agonist CRC in the continued presence of the antagonist. Control tissues were incubated for 20 min in PSS. The third protocol was designed to evaluate the effects of GR127935 (in the presence of a high concentration of ritanserin), and of ritanserin (in the presence of a high concentration of GR127935) against contractile responses to selective 5-HT agonists obtained in endothelium-denuded artery and vein preparations. In the first group of experiments of this protocol, preparations were treated with ritanserin (100 nM) and a CRC for sumatriptan was obtained in the absence or presence of GR127935 (10 nM). In the second group of experiments of this protocol, endothelium-denuded preparations were treated with GR127935 (100 nM) and CRCs for α-Me were obtained in the absence or presence of ritanserin (10 nM). In additional experiments, a CRC for ergotamine (10 nM to 10 μM) was obtained in the absence or in the combined presence of GR127935 (100 nM) and ritanserin (100 nM). The incubation time for antagonists was as outlined in the second protocol. In the fourth protocol, we examined whether 5-HT produces an endothelium-independent relaxation. In these experiments, rings were preincubated with a high concentration (100 nM) of GR127935 and after 20 min the preparations were contracted with prostaglandin $F_{2\alpha}$ (2 μM); then, in the plateau phase of the contraction, 5-HT (1–100 μM) was added cumulatively. Time-matched control experiments (no 5-HT added) were carried out in paired preparations to check for time related changes in the plateau contraction.

### *Radioligand binding studies*

On the day of the experiment, partially thawed cryopreserved vascular tissues were immersed in buffer A (50 mM Tris-HCl, pH = 7.4, 4°C) and homogenized with a Polytron (24 000 r.p.m. 15 s, twice). The membranes were precipitated by centrifugation (20 000 *g*, 45 min, 4°C) and subsequently rinsed with buffer A. The final membrane pellet was resuspended in buffer B (buffer A containing 10 μM pargyline, 0.1% ascorbic acid and 4 mM $CaCl_2$, room temperature). Aliquots of membrane suspension were immediately frozen by immersion in liquid nitrogen and stored at −80°C until used. Protein content was measured according to Bradford (1976) using bovine serum albumin as standard.

The binding assays were performed in triplicate. A protein concentration of 150 μg $ml^{-1}$ in a sample volume of 1 ml was used for each radioligand. The radioligands were diluted in buffer B to the final concentration used for competition experiments (0.5 nM [$^3$H]-8-OH-DPAT, 2 nM [$^3$H]-ketanserin, 0.3 nM [$^3$H]-5-CT and 0.12 nM [$^3$H]-GR113808). Samples were incubated at 37°C for 30 min and rapidly filtered under reduced pressure through Whatman GF/B filters in a Brandel cell Harvester (MB-48R). The filters were previously soaked in 0.3% polyethylenimine and cooled in ice-cold buffer A. The filters were then rapidly rinsed in ice-cooled buffer A (4 ml, four times) and subsequently dried at 60°C for 30 min. The amount of radioactivity bound to the filters in tritiated samples was measured by liquid scintillation in 5 ml Optiphase Hisafe II (EG&G) per filter. 5-HT (10 μM) was used to define non-specific binding except for [$^3$H]-ketanserin experiments, where the same concentration of mianserin was used.

### *Statistics and analysis for functional and radioligand binding studies*

For functional studies values are given as means ± s.e.mean. The number of experiments is expressed as "n/p" where n represents the number of preparations examined, and p the number of patients from which those tissues were derived. Contractile responses are expressed in absolute values (g) or as a percentage of the response to the initial challenge with 5-HT (10 μM). The $EC_{50}$ of spasmogens was derived by interpolation in each concentration–effect curve. The $EC_{50}$ values were transformed into $pEC_{50}$ (i.e. $-\log EC_{50}$) values. For unsurmountable antagonism $-\log IC_{50}$ values (negative logarithm

of the molar concentration of the antagonist which reduces the maximal effect of the agonist by 50%) were calculated according to van Rossum (1963). When present, surmountable antagonism was assessed by determining the apparent antagonist dissociation constant ($K_B$) with the following equation: $K_B = [B]/(\text{dose ratio} - 1)$; where [B] is the concentration of the antagonist and the dose ratio is the $EC_{50}$ of the agonist in the presence of the antagonist divided by the $EC_{50}$ of the agonist in the control tissues. The results were then expressed as $pK_B$ (i.e. $-\log K_B$). Statistical analysis of results was carried out by analysis of variance (ANOVA) followed by Bonferroni's multiple comparison tests or by paired Student's *t* test as appropriate, with $P<0.05$ accepted as significant.

For binding studies at least three tissue samples, coming from different donors, were studied for each vessel type and radioligand. Each of these individual samples was assayed in triplicate. Specific binding and labelling density are expressed as mean ± s.e.mean. To determine the presence of specific binding, the values obtained for total and non-specific binding were compared using one-tail paired Student *t* test. In order to estimate an approximate density of receptors ($B_{max}$) in the membrane preparations obtained from arteries and veins a correction of the specific binding found was made to take into account the percentage of occupancy of each receptor at the radioligand concentration used. To do that, the binding isoterm equation was used. The following $K_d$ values for the binding of [$^3$H]-5-CT, [$^3$H]-ketanserin and [$^3$H]-GR 113808 to human 5-$HT_{1B/1D}$, 5-$HT_2$ and 5-$HT_4$ receptors, respectively, were used: 0.6 nM (Domènech *et al*., 1997), 4.9 nM (Pazos *et al*., 1985) and 0.59 nM (Domènech *et al*., 1994).

### *Drugs*

Drug concentrations are expressed as final bath concentrations of the active species. Drug sources were as follows: histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), and prostaglandin $F_{2\alpha}$ were from Sigma-Aldrich (Madrid, Spain); mianserin hydrochloride and ritanserin tartrate, from RBI (Research Biochemicals International, U.S.A.); ergotamine tartrate, GR127935 (*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), SB 204070 ((1-butyl-4-piperidinyl)methyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride, serotonin-*O*-carboxymethyl-glycyl-tyrosinamide (SCMGT), sumatriptan and zacopride (racemate) hydrochloride were synthesized at the Medicinal Chemistry Department of Almirall (Barcelona, Spain); (±)-α-methyl-5-HT maleate and 2-methyl-5-HT hydrochloride were from Tocris Cookson (U.K.); WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide.3HCl) was a generous gift of Wyeth Research (Maidenhead, U.K.). Radiochemical sources were as follows (specific activity Ci $mmol^{-1}$): [$^3$H]-8-OH-DPAT (163), [$^3$H]-ketanserin (80), [$^3$H]-5-CT (59) and [$^3$H]-GR 113808 (82) were obtained from Amersham (England). Other chemicals used were of analytical grade (E. Merck; Panreac). All drugs were dissolved in PSS just before use. Laboratory glassware was used at all times in the making up and dilution of SB 204070 as the compound may adhere to certain types of plastic (Wardle *et al*., 1994).

## Results

### *Contractile responses of 5-HT receptor agonists*

All the agonists tested produced a concentration-dependent contraction of ring preparations of human pulmonary arteries and veins (Figure 1). The rank order of agonist potencies (assessed as $pEC_{50}$ values) was ergotamine > 5-HT ≥ SCMGT = sumatriptan ≥ α-Me in artery, and ergotamine > 5-HT > sumatriptan = α-Me > SCMGT in vein preparations. The potency of each agonist, except for SCMGT, was greater in vein than in artery preparations (Table 1).

The absolute values of maximum responses to the agonists showed large interindividual variability, as previously reported (Haye-Legrand *et al*., 1987). Thus the maximum response to 5-HT obtained in control tissues at the end of the CRC was 1.63 ± 0.23 g (n/p = 28/20) in artery and 1.76 ± 0.21 g (n/p = 26/25) in vein preparations. These values did not significantly differ from each other nor were they significantly different from the response to the initial challenge with 5-HT (10 μM). Normalization of maximum effects as percentage of the response to the initial 5-HT (10 μM) reduced this variability. The

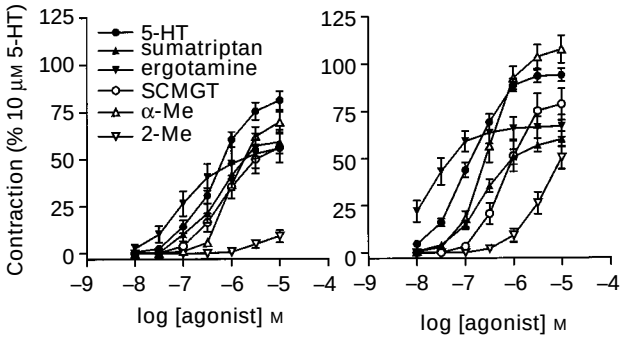


**Figure 1** Constrictor responses to 5-hydroxytryptamine (5-HT), sumatriptan, ergotamine, serotonin-*O*-carboxymethyl-glycyl-tyrosinamide (SCMGT), α-methyl 5-HT (α-Me) and 2-methyl 5HT (2-Me) in intact human pulmonary artery (left panel) and vein (right panel). Data are mean ± s.e.mean of n/p experiments as indicated in Table 1. For 2-Me experiments n/p = 5/3.

**Table 1** Values for potency ($pEC_{50}$) and maximal effect ($E_{max}$) of 5-hydroxytryptamine (5-HT), sumatriptan, ergotamine, serotonin-O-carboxymethyl-glycyl-tyrosinamide (SCMGT) and α-methyl 5-HT (α-Me) in intact preparations of human isolated pulmonary artery (HPA) and vein (HPV)

| *Agonist* | *tissue* | *n/p* | *$pEC_{50}$* | *$E_{max}$ (%)* |
|---|---|---|---|---|
| 5-HT | HPA | 28/20 | 6.41 ± 0.06 | 87.3 ± 4.5 |
| | HPV | 26/25 | 6.96 ± 0.06† | 93.9 ± 3.4 |
| Sumatriptan | HPA | 15/10 | 6.19 ± 0.13 | 59.1 ± 6.3* |
| | HPV | 18/12 | 6.60 ± 0.06†* | 60.0 ± 4.1* |
| Ergotamine | HPA | 11/7 | 6.88 ± 0.12* | 55.8 ± 7.5* |
| | HPV | 12/7 | 7.84 ± 0.16†* | 67.0 ± 5.9* |
| SCMGT | HPA | 10/8 | 6.20 ± 0.08 | 56.1 ± 8.0* |
| | HPV | 11/8 | 6.09 ± 0.12* | 78.5 ± 8.4 |
| α-Me | HPA | 7/5 | 6.04 ± 0.03* | 69.6 ± 5.4 |
| | HPV | 9/4 | 6.56 ± 0.07†* | 107.5 ± 7.2† |

Data are means ± s.e.mean of n/p (number of preparations/number of patients) experiments; $E_{max}$ is presented as percentage of maximal contraction to 5-HT (10 μM); †$P<0.05$ from corresponding values in artery; *$P<0.05$ from 5-HT values in the same tissue.

maximum response to 5-HT was similar in artery and vein preparations (Figure 1, Table 1). In artery ring preparations, the maximum effect of sumatriptan, ergotamine, and SCMGT were less than that of 5-HT while the difference failed to reach significance for α-Me. In vein preparations, the maximum effects of sumatriptan and ergotamine were less than that of 5-HT while the difference failed to reach significance for SCMGT and α-Me. We did not find significant differences in the maximum response to each agonist between artery and vein preparations, with the single exception of α-Me.

In additional experiments it was found that the CRC for 5-HT obtained in intact artery and vein preparations did not differ from those obtained in endothelial-denuded preparations ($pEC_{50}$ values were $6.66 \pm 0.16$ in intact vs $6.48 \pm 0.14$ in denuded arteries; $6.94 \pm 0.13$ in intact vs $6.73 \pm 0.16$ in denuded veins; values for $E_{max}$ (not shown), n/p = 5/3). By contrast, the potency and efficacy of sumatriptan were enhanced in endothelium-denuded arteries ($pEC_{50}$ and $E_{max}$ values were $6.23 \pm 0.06$ and $54.4 \pm 6.3\%$ in intact vs $6.67 \pm 0.03$ and $69.0 \pm 5.9\%$ in denuded rings, respectively; n/p = 5/3, $P < 0.05$) but not in endothelium-denuded veins ($pEC_{50}$ and $E_{max}$ values were $6.59 \pm 0.14$ and $51.7 \pm 7.4\%$ in intact vs $6.82 \pm 0.11$ and $50.5 \pm 6.8\%$ in denuded rings, respectively; n/p = 5/3).

The responses to 2-Me were concentration-related but did not reach a maximum in the concentration range studied (i.e. up to 10 μM; Figure 1); hence $pEC_{50}$ values were not calculated for this agonist. The contractile responses to 2-Me were not antagonized by zacopride (0.1 – 1 μM) in artery and vein preparations (data not shown).

### *Effects of GR127935 on agonist-induced responses*

Although GR127935 has been reported to exhibit intrinsic activity at cloned human $5\text{-}HT_{1B/1D}$ receptors (Pauwels & Colpaert, 1995) we observed no such activity in the pulmonary vascular preparations. GR127935 produced a concentration-dependent downward shift of the CRCs of 5-HT, sumatriptan, ergotamine and SCMGT in intact artery and vein preparations (Figure 2). This resulted in reduced maximum response to these agonists with no significant effects on their $pEC_{50}$ values (data not shown) except for 0.1 μM GR127935 against 5-HT in vein ($6.59 \pm 0.09$, n/p = 7/4, $P < 0.05$ vs control values in the absence of antagonist) and for 0.5 μM GR127935 against ergotamine in vein ($6.99 \pm 0.22$, n/p = 7/4, $P < 0.05$ vs control). As the antagonism was not surmountable, the $-\log IC_{50}$ values were calculated for this antagonist and the results are given in Table 2. In both preparations, GR127935 was most potent against sumatriptan and least potent vs ergotamine with 5-HT and SCMGT lying in between. GR127935 appeared to be less potent in vein compared to artery but the difference reached significance only for 5-HT.

In separate experiments, endothelium-denuded artery and vein preparations were treated with a high concentration of ritanserin (100 nM) to isolate pharmacologically the contractile $5\text{-}HT_{1B/1D}$ receptors. Under these experimental conditions, GR127935 (10 nM) produced a parallel shift of the CRC for sumatriptan without significant depression of its $E_{max}$ (control $pEC_{50}$ and $E_{max}$ values for sumatriptan in the absence of GR127935 were $7.07 \pm 0.06$ and $73.2 \pm 2.9\%$ in artery and $7.10 \pm 0.03$ and $59.0 \pm 1.9\%$ in vein; n/p = 5/3 for each group). This permitted the calculation of apparent $pK_B$ values of $9.33 \pm 0.13$ in artery and $9.27 \pm 0.26$ in vein preparations (n/p = 5/3 for each tissue).

### *Effects of ritanserin on agonist-induced responses*

Ritanserin produced a concentration-related depression of the CRCs for 5-HT, ergotamine and α-Me in intact artery and vein preparations (Figure 3), i.e. it reduced maximum effect of these agonists without significant changes in their $pEC_{50}$ values (data not shown) except for 0.1 μM ritanserin against α-Me in artery ($5.60 \pm 0.08$, n/p = 7/3, $P < 0.05$ vs control) and vein ($6.22 \pm 0.04$, n/p = 8/3, $P < 0.05$ vs control). The calculated $-\log IC_{50}$ values for ritanserin are shown in Table 2. Ritanserin was equally potent against 5-HT and ergotamine and somewhat less potent against α-Me in the artery but differences in potency were not found in the vein. Ritanserin was less potent in vein compared to artery but the difference reached significance only for 5-HT. Ritanserin (10 nM), a concentration sufficient to produce a significant inhibition of the responses to 5-HT, did not alter the CRCs to sumatriptan and SCMGT in artery and vein preparations (data not shown).

In separate experiments, endothelium-denuded artery and vein preparations were incubated with GR127935 (100 nM) to

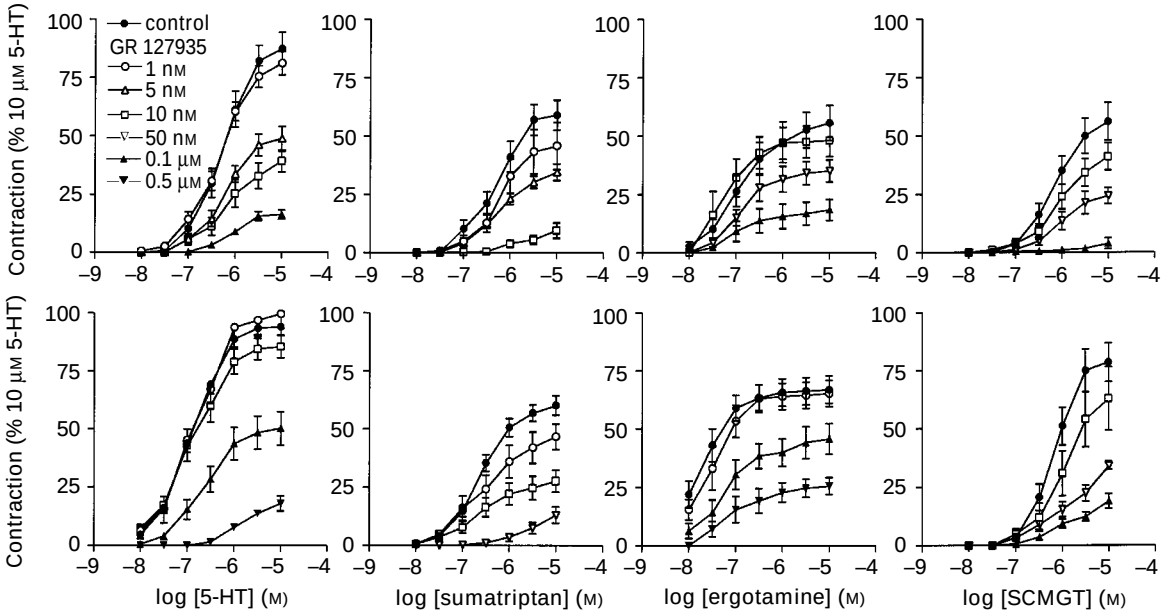


**Figure 2** Effect of GR127935 on vasoconstrictor responses to 5-hydroxytryptamine (5-HT), sumatriptan, ergotamine, and serotonin-*O*-carboxymethyl-glycyl-tyrosinamide (SCMGT) in intact human pulmonary artery (upper panels) and vein (lower panels) preparations. The responses to the contractile agonists were obtained in the absence (control tissues) and presence of the antagonist as shown. Data are mean ± s.e.mean of 5 to 8 experiments from 3 to 5 patients.

negate the influence of contractile 5-$HT_{1B/1D}$ receptors, and CRCs for α-Me were obtained in the absence (control) or presence of ritanserin (10 nM). In these experimental conditions, ritanserin produced a parallel shift of the CRC to α-Me without depressing its $E_{max}$ (control $pEC_{50}$ and $E_{max}$ values for α-Me were 7.06±0.05 and 73.6±2.1% in artery and 6.99±0.07 and 97.4±6.1% in vein; n/p = 5/3 for each group). This permitted the calculation of apparent $pK_B$ values of 8.82±0.09 and 8.98±0.12 for artery and vein preparations respectively (n/p = 5/3 for each tissue).

Further experiments showed that the combination of GR127935 and ritanserin (each at 100 nM) abolished the concentration (10 nM to 10 μM)-related contractions of artery and vein preparations in response to ergotamine (data not shown).

### *Effects of WAY100635, zacopride and SB204070 on agonist-induced responses*

WAY100635 (1 nM to 1 μM), zacopride (10 nM to 1 μM), and SB204070 (1–10 nM), each had no significant effect on the maximum effects of 5-HT, sumatriptan, ergotamine and SCMGT nor did they modify the $pEC_{50}$ values of these agonists in intact artery and vein preparations (data not shown).

### *Effect of 5-HT on endothelium-denuded rings precontracted with prostaglandin $F_{2\alpha}$*

Prostaglandin $F_{2\alpha}$ (2 μM) produced a plateau contraction of endothelium-denuded artery (1.64±0.15 g, n/p = 3/3) and vein (1.21±0.31 g, n/p = 3/3) preparations. Artery and vein contractions were not significantly different in size ($P > 0.05$) and time-matched control experiments showed that remained stable during the time necessary to make the CRC to 5-HT (not shown). 5-HT (up to 100 μM) did not significantly inhibit the tone of the preparations supported by prostaglandin $F_{2\alpha}$ (data not shown).

### *Binding studies*

Membrane preparations obtained from arteries and veins were labelled with a single concentration of 5-HT receptor radioligands as described under Methods. No statistically significant levels of specific binding could be found with [$^3$H]-8-OH-

**Table 2** −log $IC_{50}$ values of GR127935 and ritanserin against 5-hydroxytryptamine (5-HT) and other agonists of 5-HT receptors

| | *GR127935* | | *Ritanserin* | |
|---|---|---|---|---|
| | *Artery* | *Vein* | *Artery* | *Vein* |
| 5-HT | 7.95±0.07 | 7.14±0.17$^{\#}$ | 7.82±0.12 | 6.99±0.11$^{\#}$ |
| Sumatriptan | 8.40±0.08* | 8.16±0.13* | | |
| Ergotamine | 7.13±0.13*† | 6.85±0.17† | 7.67±0.19 | 7.43±0.10 |
| SCMGT | 7.62±0.09†‡ | 7.42±0.13† | | |
| α-Methyl-5-HT | | | 7.23±0.08* | 7.08±0.05 |

Data are means±s.e.mean of 5/3–9/6 (number of preparations/number of patients) experiments. *$P < 0.05$ compared to 5-HT values in the same column; †‡$P < 0.05$ vs sumatriptan and ergotamine in the same column, respectively; $^{\#}P < 0.05$ from corresponding artery values.

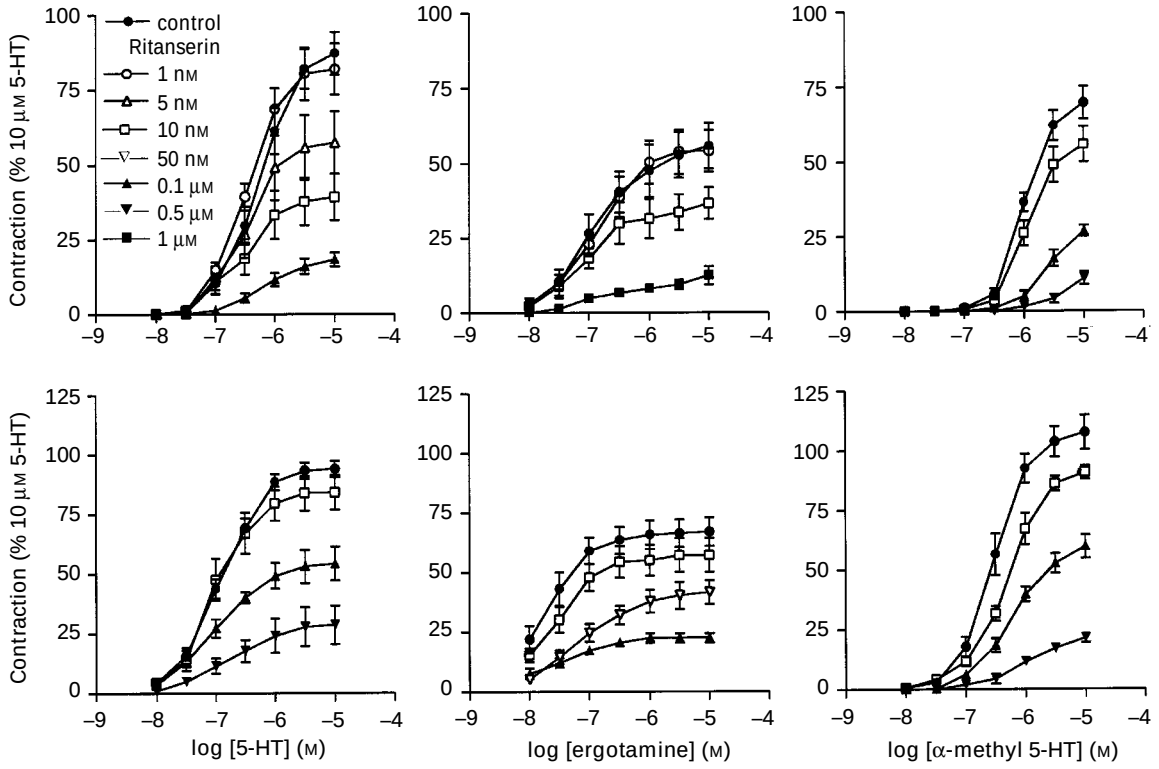


**Figure 3** Effect of ritanserin on vasoconstrictor responses to 5-hydroxytryptamine (5-HT), ergotamine and α-methyl 5-HT in intact human pulmonary artery (upper panels) and vein (lower panels) preparations. The responses to the contractile agonists were obtained in the absence (control tissues) and presence of the antagonist as shown. Data are mean±s.e.mean of 5 to 9 experiments from 3 to 6 patients.

**Table 3** Presence of 5-hydroxytryptamine (5-HT) receptor specific binding and estimated receptor density in membrane preparations of human pulmonary artery and vein

| *Radioligand* | *n* | *Specific binding as % of total* | *Estimated $B_{max}$* (fmols $mg^{-1}$ protein) |
|---|---|---|---|
| [$^3$H]-ketanserin | | | |
| Artery | 3 | 16.0±9.4 | 380.0±250.0 |
| Vein | 3 | 19.0±1.2‡ | 370.0±83.0 |
| [$^3$H]-5-CT | | | |
| Artery | 8 | 42.0±8.0‡ | 51.0±12.0 |
| Vein | 7 | 35.0±17.0* | 39.0±18.0 |
| [$^3$H]-GR 113808 | | | |
| Artery | 7 | 5.5±3.0 | NC |
| Vein | 7 | 31.0±4.0† | 20.0±5.3 |
| [$^3$H]-8-OH-DPAT | | | |
| Artery | 3 | 6.0±2.0 | NC |
| Vein | 3 | 6.0±7.0 | NC |

Data are means±s.e.mean of *n* preparations obtained from the same number of patients assayed in triplicate. NC: not calculated. *$P<0.05$, †$P<0.01$, ‡$P<0.001$ from non-specific binding.

DPAT in artery and vein preparations (Table 3). [$^3$H]-5-CT labelled a small population of specific binding sites in membranes obtained from both arteries and veins. With [$^3$H]-ketanserin a larger population of specific binding sites was found in both vessel types, although in the case of arteries the specific binding found was not significantly different from non-specific binding, due to the high variability found with this kind of samples. [$^3$H]-GR 113808 labelled a small but significant population of binding sites in membranes obtained from pulmonary veins, whereas in the case of arteries the signal found was negligible.

## Discussion

### *Contraction of human pulmonary vascular preparations in response to 5-HT agonists*

The present study was designed to investigate the 5-HT receptor types involved in the vasoconstrictor response to 5-HT receptor agonists in human pulmonary artery and vein ring preparations. In functional studies, we show that the endogenous ligand 5-HT, the antimigraine drugs sumatriptan and ergotamine, and the agonists structurally related to 5-HT, SCMGT, α-Me and 2-Me, each produced concentration-related contractions of human pulmonary artery and vein ring preparations. These results confirm and extend previous reports in human isolated pulmonary blood vessels (Houghton & Phillips 1973; Boe *et al*., 1980; Raffestin *et al*., 1985; MacLean *et al*., 1996).

The vasoconstrictor effect of 5-HT in pulmonary vascular preparations isolated from different animal species including human has been ascribed to activation of 5-$HT_{2A}$ receptors (Frenken & Kaumann, 1984; Raffestin *et al*., 1985; Selig *et al*., 1988; MacLean *et al*., 1994). This assumption was mainly based in experiments where ketanserin or other 5-$HT_{2A}$ receptor antagonists were used. However, recent studies have unveiled the participation of 5-$HT_{1B/1D}$ receptors. Thus, in bovine isolated pulmonary arteries under conditions of basal tone, responses to 5-HT are exclusively mediated by activation of 5-$HT_{2A}$ receptors while when tone was pharmacologically increased, a 5-$HT_{1B/1D}$ response emerged to both 5-HT and sumatriptan (MacLean *et al*., 1994). The human isolated pulmonary artery is known to have very little or no spontaneous tone (Boe, 1982; Greenberg *et al*., 1987) although it responded with a strong contraction to 5-$HT_{1B/1D}$ agonists like sumatriptan (MacLean *et al*., 1996). Sumatriptan was found to be equipotent to 5-HT in contracting human isolated pulmonary artery ring preparations (MacLean *et al*., 1996; this study). In our hands, the maximal effect of sumatriptan was somewhat less than that found for 5-HT. This result is in contrast with MacLean *et al*. (1996) that found no difference between maximum responses to 5-HT and sumatriptan. A reduced size (about 70%) of the maximum response to sumatriptan compared to 5-HT has been also reported in other human vascular tissues endowed with 5-$HT_1$-like receptors (Parsons *et al*., 1989; Kaumann *et al*., 1994).

### *Role of the endothelium in the responses to 5-HT and sumatriptan*

We found that removal of endothelium did not alter the CRC to 5-HT in artery and vein preparations. This finding is consistent with a previous study in human pulmonary artery (Greenberg *et al*., 1987) and with the report that *in vitro* human pulmonary vascular responses to 5-HT (10 μM) were not modified by the NO inhibitors L-NMMA and L-NOARG (Ortiz *et al*., 1992). By contrast, the contractile responses to sumatriptan were enhanced in denuded arteries but not in denuded veins (this study). Consistent with this finding. MacLean *et al*. (1993) reported that treatment with L-NAME enhances responses to sumatriptan in human isolated pulmonary artery. Loss of endothelial integrity in human pulmonary artery preparations subjected to cold storage overnight (Ortiz *et al*., 1995) may explain the finding of greater $E_{max}$ values for sumatriptan by MacLean *et al*. (1996) compared to other studies including this one (see above).

We have not investigated the influence of endothelial integrity on the contractile responses to other 5-HT agonists or the type of 5-HT receptor involved but human pulmonary artery endothelial cells in culture express 5-$HT_{1D\beta}$ and 5-$HT_{2B}$ receptors (Ullmer *et al*., 1996). Differences in the modulatory role of endothelium may be also a factor contributing to explain the greater sensitivity to some 5-HT agonists found in vein compared to artery preparations (this study).

### *Participation of 5-$HT_{1B/1D}$ receptors in agonist-induced pulmonary vasoconstriction*

Besides sumatriptan, artery and vein preparations responded with concentration-related contractions to another 5-$HT_{1B/1D}$ receptor agonist, SCMGT (Boulenguez *et al*., 1991), and also to ergotamine, a 5-$HT_{1/2}$-receptor agonist. The proposed selectivity of sumatriptan and SCMGT was confirmed by the observation that effective concentrations of the 5-$HT_{2A}$-receptor antagonist ritanserin did not affect their contractile response in artery and vein preparations.

GR127935, a potent and selective 5-$HT_{1B/1D}$ receptor antagonist (Skingle *et al*., 1993), antagonized the responses to 5-HT, sumatriptan, SCMGT and ergotamine in intact artery and vein preparations. However, the antagonism produced by GR127935 on the responses to the agonists studied was not of a surmountable, competitive nature. This finding is consistent with other *in vitro* studies. Thus, sumatriptan-induced contraction of dog basilar artery (Connor *et al*., 1989) and dog and rabbit saphenous vein (Clitherow *et al*., 1994; Razzaque *et al*., 1995), were also antagonized by low concentrations of GR127935 (1–10 nM) with reduced maximum effect. In additional experiments, we show that preincubation of denuded preparations with a high concentration of ritanserin to block contractile 5-$HT_{2A}$ receptors unveiled a surmountable antagonism of the contractile responses of sumatriptan by GR127935 (10 nM) which permitted calculation of $pK_B$ values of 9.17 in artery and 9.11 in vein. These values are consistent with reported $pA_2$ and $pK_B$ values for GR127935 at 5-$HT_{1D}$ receptors (Razzaque *et al*., 1995).

In addition to the results of functional studies with selective agonists and antagonists, we demonstrated the presence of 5-$HT_{1B/1D}$ receptors in human pulmonary blood vessels by showing specific binding with the selective radioligand [$^3$H]-5-CT (Novak *et al*., 1993).

### *Participation of 5-HT$_{2A}$ receptors in agonist-induced pulmonary vasoconstriction*

The involvement of 5-HT$_{2A}$ receptors in the vasoconstrictor response to 5-HT in human pulmonary blood vessels is well established. Raffestin *et al.* (1985) showed that methysergide and ketanserin inhibited the contractile responses to 5-HT in human isolated pulmonary artery and vein but they did not examine further this antagonism. MacLean *et al.* (1996) showed that the 5-HT$_{2A}$ receptor antagonist ketanserin (10 nM to 1 $\mu$M) produced a non-surmountable antagonism of the contractile responses to 5-HT and sumatriptan in human pulmonary artery. In the present study, ritanserin, a 5-HT$_{2A}$ receptor antagonist (Hoyer, 1988), produced a non-surmountable antagonism of 5-HT, ergotamine and $\alpha$-Me in intact artery and vein preparations. However, when $\alpha$-Me was tested in denuded preparations pretreated with a high concentration of GR127935 to block contractile 5-HT$_{1B/1D}$ receptors, a surmountable antagonism was shown for ritanserin with pK$_B$ values of 8.82 for artery and 8.98 for vein. These values are consistent with reported values for ritanserin as antagonist of 5-HT$_{2A}$ receptors in other vascular tissues (Rinaldi-Carmona *et al.*, 1992).

Binding studies also suggested the presence of 5-HT$_{2A}$ receptors in human pulmonary blood vessels. A relatively high density of putative 5-HT$_{2A}$ receptors was found in membrane preparations of human pulmonary vein, as measured by the presence of specific binding for the 5-HT$_{2A}$ radioligand [$^3$H]-ketanserin (Pazos *et al.*, 1985). Membrane preparations from pulmonary arteries also showed a similar density of receptors, although the high variability found in these preparations impeded the obtention of significance in this case. The density of 5-HT$_{2A}$ receptors was approximately eight-fold higher than that found for 5-HT$_{1B/1D}$ with [$^3$H]-5-CT (this study). However, caution is needed to derive from these data the relative weight of 5-HT$_{2A}$ vs 5-HT$_{1B/1D}$ receptors in the pulmonary vasoconstrictor response to 5-HT as agonists (5-CT) label a smaller population of receptors than antagonists (ketanserin) in most systems (Hoyer & Boddeke, 1993).

### *Participation of 5-HT$_{1A}$, 5-HT$_3$ and 5-HT$_4$ receptors in agonist-induced pulmonary vasoconstriction*

The investigation of the presence of other subtypes of 5-HT$_1$ receptors was limited in the present study to 5-HT$_{1A}$ receptors. Sumatriptan and ergotamine have also affinity for 5-HT$_{1A}$ receptors as well as 5-HT itself (Hoyer *et al.*, 1994). WAY100635, a potent and selective antagonist of 5-HT$_{1A}$ receptors (Fletcher *et al.*, 1994) did not reduce the responsiveness and sensitivity of artery and vein preparations to the agonists studied. Furthermore, results from the binding study could not demonstrate the presence of specific binding using [$^3$H]-8-OH-DPAT, a known selective radioligand of 5-HT$_{1A}$ receptors (Gozlan *et al.*, 1983).

The pulmonary vasoconstrictor response to 5-HT is apparently not mediated through activation of 5-HT$_3$ or 5-HT$_4$ receptors. This assumption is based on the following experimental evidences. First, 2-Me, a 5-HT$_3$ receptor agonist (Hoyer *et al.*, 1994), produced, in concentrations up to 10 $\mu$M, relatively weak contractions of artery and vein preparations. These contractions were resistant to effective concentrations of zacopride, a 5-HT$_3$ selective antagonist (Smith *et al.*, 1988), and therefore are probably mediated through activation of a different type of 5-HT receptors (i.e. 5-HT$_{1B/1D}$) (Hoyer, 1989). Second, zacopride did not reduce either the efficacy and potency of 5-HT itself or that of the 5-HT$_{1/2}$ receptor agonists tested in this study. Third, SB204070, a highly potent and selective 5-HT$_4$ receptor antagonist (Wardle *et al.*, 1994), failed to alter the responsiveness and sensitivity of the tissues to the agonists studied. Fourth, binding studies with a potent and selective radioligand of 5-HT$_4$ receptors, [$^3$H]-GR113808 (Domènech *et al.*, 1994), showed a low specific signal in membrane preparations from veins, whereas no specific binding could be found in artery preparations (this study).

### *Absence of endothelium-independent 5-HT-induced relaxation*

The existence of an endothelium-independent relaxation produced by relatively high concentrations of 5-HT and mediated by 5-HT$_7$ receptors has been reported in canine coronary artery (Terron, 1996). These receptors may be present in human pulmonary blood vessels as we have shown specific binding with [$^3$H]-5-CT, a ligand that also displays affinity for 5-HT$_7$ receptors. However, functional experiments failed to support this view as indicated by the absence of relaxation with 5-HT (up to 100 $\mu$M) in precontracted denuded preparations pretreated with GR127935 to exclude the influence of contractile 5-HT$_{1B/1D}$ receptors.

In conclusion, 5-HT contracts human pulmonary arteries and veins via both 5-HT$_{1B/1D}$ and 5-HT$_{2A}$ receptors. Although the 5-HT$_{1B/1D}$ receptor population was found smaller in binding studies than the 5-HT$_{2A}$ receptor population, the findings of functional studies indicate that the 5-HT$_{1B/1D}$ receptors make a significant contribution to pulmonary vasoconstriction. Similar heterogeneity of contractile 5-HT receptors has also been encountered in human isolated coronary arteries (Kaumann *et al.*, 1994), umbilical arteries (MacLennan *et al.*, 1989), omental arteries (Wallerstedt *et al.*, 1996), saphenous vein (Docherty & Hyland, 1986) and cutaneous hand veins (Bodelsson *et al.*, 1992), and might therefore be a common characteristic of capacitance human arteries and veins. The present results may have clinical interest since lung tissue levels of 5-HT and sumatriptan achievable in the clinical setting are sufficient to produce a significant vasoconstriction of pulmonary arteries and veins. In addition, selective 5-HT$_{1B/1D}$ receptor antagonists may have therapeutic value in pulmonary hypertension.

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