British Journal of Pharmacology (1996) 119, 277–282

Evidence for 5-HT_1-like receptor-mediated vasoconstriction in human pulmonary artery

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1 The 5-hydroxytryptamine (5-HT) receptors mediating contraction of human isolated pulmonary artery rings were investigated. Responses to the agonists 5-carboximidotryptamine (5-CT, non-selective 5-HT_1 agonist), sumatriptan (5-HT_{1D}-like receptor agonist), 5-HT and 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT, 5-HT_{1A} receptor agonist) were studied. Responses to 5-HT and sumatriptan in the presence of the antagonists, methiothepin (non-selective 5-HT_{1+2}-receptor antagonist), ketanserin (5-HT_{2A} receptor antagonist) and the novel antagonist, GR55562 (5-HT_{1D} receptor antagonist) were also studied.

2 All agonists contracted human pulmonary artery ring preparations in the following order of potency 5-CT > 5-HT = sumatriptan > 8-OH-DPAT. Maximum responses to 5-HT, 5-CT and sumatriptan were not significantly different.

3 Methiothepin 1 nM and 10 nM, but not 0.1 nM reduced the maximum contractile responses to 5-HT but did not alter tissue sensitivity to 5-HT. Methiothepin 0.1 nM, 1 nM and 10 nM had a similar effect on responses to sumatriptan.

4 The 5-HT_{2A} receptor antagonist ketanserin (10 nM, 100 nM and 1 μM) also reduced the maximum contractile response to both 5-HT and sumatriptan without affecting tissue sensitivity to these agonists.

5 The novel 5-HT_{1D} receptor antagonist, GR55562, inhibited responses to 5-HT and sumatriptan in a true competitive fashion.

6 The results suggest that the human pulmonary artery has a functional population of 5-HT_{1D}-like receptors which are involved in the contractile response to 5-HT.

Keywords: 5-Hydroxytryptamine receptors; human pulmonary arteries; vasoconstriction

Introduction

Recently, 5-hydroxytryptamine (5-HT) has been implicated in pulmonary hypertension, both primary and secondary (Hervé *et al.*, 1990; 1995) and it may be implicated in pulmonary hypertension caused by 5-HT uptake inhibitors used as anorectics (Brenot *et al.*, 1993). An understanding of the 5-HT receptors mediating vasoconstriction in the human pulmonary artery is therefore of interest. This was the aim of these studies.

Sumatriptan (GR43175) is a 5-HT_{1D}-like agonist showing a degree of selectivity for the 5-HT_{1D}-like receptors mediating smooth muscle contraction, for example, in the dog isolated saphenous vein (Humphrey *et al.*, 1988). In rabbit, cow and dog, sumatriptan is typically 4–10 fold less potent than 5-HT as a vasoconstrictor in systemic, cerebral and pulmonary vessels indicating that the vasoconstrictor effect of 5-HT is mediated mainly through 5-HT_{2A} receptors (Frenken & Kaumann, 1984; Humphrey *et al.*, 1988; Parsons & Whalley, 1989; MacLean *et al.*, 1994). In human basilar and coronary arteries, sumatriptan is similarly less potent than 5-HT (Parsons *et al.*, 1989; Connor *et al.*, 1989).

In Glasgow, McIntyre *et al.* (1992) studied the effect and duration of action of sumatriptan on coronary haemodynamics and systemic and pulmonary arterial systolic and diastolic pressures in patients undergoing diagnostic coronary angiography. They found that sumatriptan produced a more pronounced effect on pulmonary pressure when compared to overall systemic pressure. This led us to examine the effects of sumatriptan on human isolated pulmonary arteries. We reported preliminary data showing that sumatriptan is equipotent to 5-HT in human pulmonary arteries, suggesting the presence of 5-HT_{1D} receptors (Templeton *et al.*, 1993; 1994).

Here, we have extended these pharmacological studies further to characterize the 5-HT receptor populations in human pulmonary artery and, using the novel 5-HT_{1D} receptor antagonist, GR55562 (Connor *et al.*, 1995), to confirm our suggestion that 5-HT_{1D} receptors may play a role in 5-HT-induced vasoconstriction. We have studied the effects of 5-carboximidotryptamine (5-CT, non-selective 5-HT_1 agonist), sumatriptan (5-HT_{1D}-like receptor agonist), 5-HT and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 5-HT_{1A} receptor agonist) and 5-HT itself. We also examined the effects of the antagonists, methiothepin (5-HT_{1+2}-receptor antagonist being selective for 5-HT_{2A} at ~1 nM) and ketanserin (5-HT_{2A} receptor antagonist) (see Hoyer *et al.*, 1994 for details of selectivity).

Methods

Human lung tissue was obtained from patients undergoing surgery for bronchial carcinoma at the Royal Infirmary, Glasgow. The lung samples were stored in fresh Krebs solution and kept at 4°C until use, within 24 h. The arteries used were dissected from the 'healthy' tissue removed around the diseased areas of the lung. They were dissected free of adhering parenchymal tissue and cut into rings 2–3 mm in length. Vessels varied in size between 3–5 mm internal diameter.

Rings were set up between two stainless steel hooks in 40 ml Krebs-filled organ baths, under 2 g tension and maintained at 37°C. Due to their function in perfusion-ventilation matching, pulmonary arteries are extremely sensitive to changes in O_2 tension. These vessels are well supplied by the vasa-vasorum despite receiving mixed arteriovenous blood. Hence the vessels are exposed to O_2 tensions of ~120 mmHg *in vivo* and we chose to bubble the vessels such that equivalent Po_2s were present in the organ bath

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solution. They were bubbled with 16% O_2, 5% CO_2, balance N_2. This gave an organ bath Po_2 of ~120 mmHg and a Pco_2 of 35–40 mmHg, confirmed using an oxygen electrode situated in the organ bath as well as a blood gas analyser. As these vessels have walls only ~200 μm thick, O_2 diffusional problems are not encountered with active bubbling (Pittman & Duling, 1973). Tissues were allowed to equilibrate for 1 h before the addition of any drugs.

Cumulative concentration-response curves (CCRCs) to 5-HT and sumatriptan were not reproducible in each tissue, and declined markedly with time. Hence, CCRCs to the agonists were constructed either in the absence or presence of antagonist.

Due to the scarcity of human tissue, this project was conducted over a three year period. Hence, the experiments were handled by different investigators with time and the maximum concentration of agonists used varied slightly within the range 0.1 nM – 100 μM depending on the series of experiments being conducted. Control response EC_{50} values varied slightly (but not significantly) during the course of the study. These factors meant that experiments with the novel antagonist, GR55562, were conducted some time later than others and so the control curves to sumatriptan and 5-HT presented here (Figures 6 and 7) are different from those used to assess the effects of ketanserin and methiothepin. Antagonists were allowed a 45 min equilibrium period prior to constructing the CCRCs to the agonists.

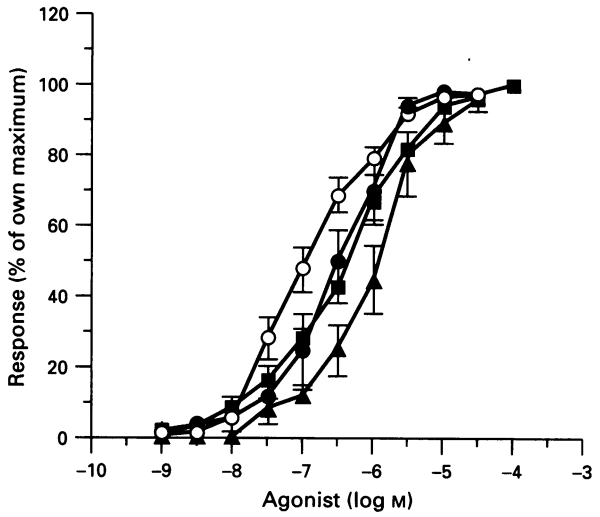

Figure 1 Vasoconstrictor responses to 5-carboximidotryptamine (○, $n=10,5$), 5-hydroxytryptamine (●, $n=8,5$), 8-hydroxy-2-(di-n-propylamino)tetralin (▲, $n=7,4$) and sumatriptan (■, $n=10,5$). Data are expressed as a percentage of the maximum response to the agonist in each preparation and shown as mean±s.e. n=number of rings, number of lungs.

Analysis

The concentration which produced a maximum and reproducible response to KCl was 50 mM. Data are shown either as a % of the reference response to 50 mM KCl or as a % of the maximum response to the agonist under study. pEC_{50} values were calculated from individual concentration-response curves using computer-aided extrapolation of responses from each vessel. Statistical analysis was made by one-way analyses of variance (ANOVA). Due to the fact that sumatriptan was tested either in the presence of GR55562 or in its absence, the pA_2 values for GR55562 were calculated using the pEC_{50} values obtained for sumatriptan in the presence and absence of GR55562 and applying these to Schild analysis to give an estimation of the pA_2 and slope of the Schild plot.

Table 1 pEC_{50} values for sumatriptan, 5-hydroxytryptamine (5-HT), 5-carboximidotryptamine (5-CT) and 8-hydroxy-2-(di)-n-propylamino-tetralin (8-OH-DPAT) in human pulmonary artery rings

Agonist	*pEC_{50}*	
Sumatriptan	6.44±0.11	(10, 5)
5-HT	6.58±0.21	(8, 5)
5-CT	6.93±0.15	(10, 5)*
8-OHDPAT	5.93±0.16	(7, 4)***

Mean data±s.e.mean. Number of ring preparations, number of lungs in parentheses. Significance of difference from 5-HT; *$P<0.05$; ***$P<0.001$ (ANOVA).

Drugs and solutions

The following drugs were used: 5-hydroxytryptamine creatinine sulphate, 8-hydroxy-2-(di-n-propylamino) tetralin, (Sigma), 5-carboximidotryptamine (Research Biochemicals Int.), ketanserin bitartrate (Roth), methiothepin maleate (Hoffman La Roche), GR43175 (sumatriptan, Glaxo Group Research), GR55562 (3-[3-(dimethylamino)propyl]-4-hydroxy-N-[4-(4-pyridinyl)phenyl]enzamide, Glaxo Group Research). All drugs were dissolved in distilled water. The composition of the Krebs solution was as follows (mM): NaCl 140, KCl 4.7, $NaCO_3$ 24.8, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.1.

Table 2 pEC_{50} values for sumatriptan and 5-hydroxytryptamine (5-HT) in the human pulmonary artery and the effects of ketanserin, methiothepin and GR55562

Group	*pEC_{50} values for sumatripran*		*pEC_{50} values for 5-HT*	
Control	6.50±0.14	(10, 6)	6.59±0.13	(10, 6)
+Ketanserin 10 nM	6.27±0.15	(7, 6)	5.84±0.23	(9, 6)
+Ketanserin 100 nM	6.35±0.17	(7, 6)	6.26±0.29	(6, 6)
+Ketanserin 1 μM	6.38±0.13	(7, 6)	6.46±0.16	(7, 7)
+Methiothepin 0.1 nM	6.63±0.16	(7, 5)	6.53±0.10	(8, 6)
+Methiothepin 1 nM	6.55±0.11	(6, 4)	6.73±0.19	(7, 6)
+Methiothepin 10 nM	6.28±0.12	(5, 6)	6.38±0.14	(6, 6)
+GR55562 1 nM	6.20±0.11	(6, 6)	6.55±0.24	(4, 4)
+GR55562 10 nM	6.51±0.11	(8, 6)	6.61±0.29	(5, 5)
+GR55562 100 nM	5.68±0.11	(6, 5)***	6.08±0.09***	(7, 6)
+GR55562 300 nM	4.80±0.06	(4, 4)***	- - - - -	
+GR55562 1 μM	>>4.8	(8, 6)	5.55±0.12***	(6, 5)

Mean data±s.e.mean. Number of ring preparations, number of patient tissue samples in parentheses. Significance of difference from relevant control response in absence of antagonist: *$P<0.05$; ***$P<0.001$ (ANOVA).

Results

All the agonists tested induced a concentration-dependent vasoconstriction (Figure 1). Table 1 summarises their pEC_{50} values and maximum responses. It can be seen that the order of potency of the agonists was 5-CT > 5-HT = sumatriptan > 8-OH-DPAT. There was no significant difference between the maximum responses to 5-CT, 5-HT and sumatriptan (% response to 50 mM KCl: 167±20%; 103±14%, 145±42% respectively) whilst that to 8-OH-DPAT was significantly less than that to 5-HT (70±8%, $P < 0.001$). The potency of the 5-HT$_1$-receptor agonists strongly indicated the prevalence of vasoconstrictor 5-HT$_1$ receptors in this tissue.

All pEC_{50} values for 5-HT and sumatriptan in the presence of specific antagonists are summarised in Table 2.

Ketanserin had no significant effect on the pEC_{50} value of 5-HT (Table 2); it did, however, reduce the maximum response to 5-HT (Figure 2).

Ketanserin had little effect on the sensitivity of the vessels to sumatriptan at the EC_{50} level (Table 2). It did increase the threshold concentration for contraction. For example, 18±2% of the maximum response was normally observed with 0.3 μM sumatriptan but in the presence of 0.1 and 1 μM

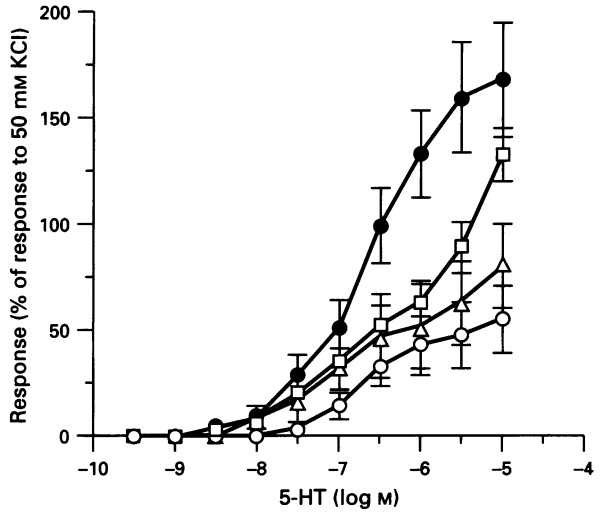

Figure 2 The effect of ketanserin on vasoconstrictor responses to 5-hydroxytryptamine (5-HT) in human isolated pulmonary artery rings. Maximum vasoconstrictor responses to 5-HT (●, $n = 10,6$) and in the presence of 10 nM (□, $n = 9,6$), 100 nM (△, $n = 6,6$) and 1 μM (○, $n = 7,7$) ketanserin. Data show the maximum response to each agonist expressed as a percentage of the response to 50 mM KCl in the same preparation and are shown as mean±s.e. n = number of rings, number of lungs.

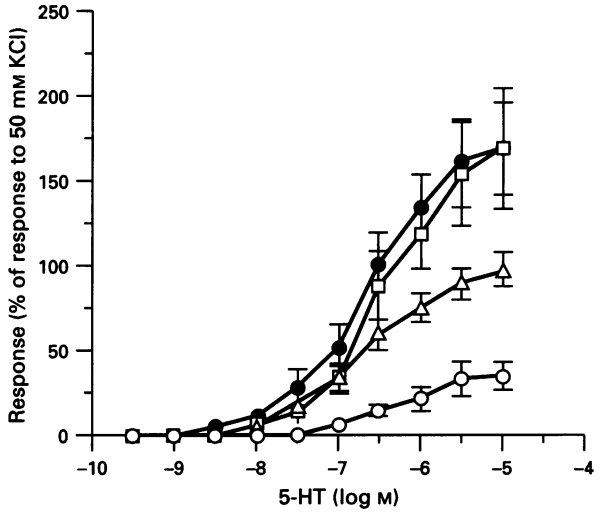

Figure 4 The effect of methiothepin on vasoconstrictor responses to 5-hydroxytryptamine (5-HT) in human isolated pulmonary artery rings. Maximum vasoconstrictor responses to 5-HT (●, $n = 10,6$) and in the presence of 0.1 nM (□, $n = 8,6$), 1 nM (△, $n = 7,6$) and 10 nM (○, $n = 6,6$) methiothepin. Data show the maximum response to each agonist expressed as a percentage of the response to 50 mM KCl in the same preparation and are shown as mean±s.e. n = number of rings, number of lungs.

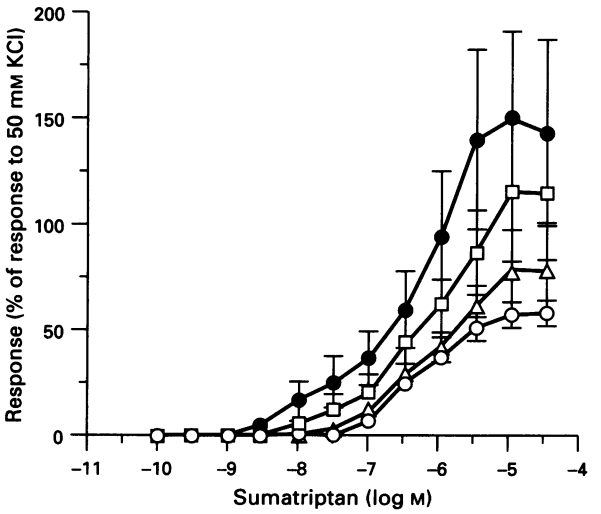

Figure 3 The effect of ketanserin on vasoconstrictor responses to sumatriptan in human isolated pulmonary artery rings. Maximum vasoconstrictor responses to sumatriptan (○, $n = 13,9$) and in the presence of 10 nM (□, $n = 7,6$), 100 nM (△, $n = 7,6$) and 1 μM (○, $n = 7,6$) ketanserin. Data show the maximum response to each agonist expressed as a percentage of the response to 50 mM KCl in the same preparation and are shown as mean±s.e. n = number of rings, number of lungs.

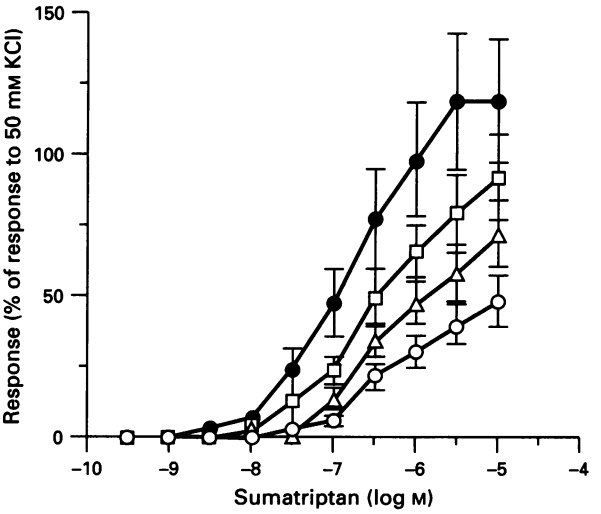

Figure 5 The effect of methiothepin on vasoconstrictor responses to sumatriptan in human isolated pulmonary artery rings. Maximum vasoconstrictor responses to sumatriptan (●, $n = 10,6$) and in the presence of 0.1 nM (□, $n = 7,5$), 1 nM (△, $n = 6,6$) and 10 nM (○, $n = 6,5$) methiothepin. Data show the maximum response to each agonist expressed as a percentage of the response to 50 mM KCl in the same preparation and are shown as mean±s.e. n = number of rings, number of lungs.

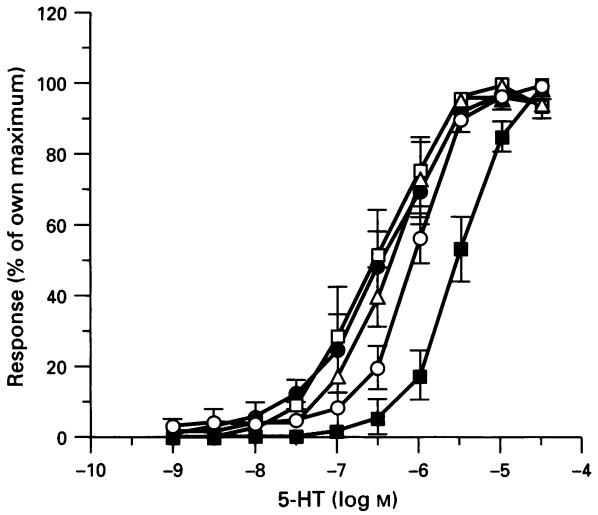

Figure 6 The effect of GR55562 on vasoconstrictor responses to 5-hydroxytryptamine (5-HT) in human isolated pulmonary artery rings. Control vasoconstrictor responses to 5-HT (●, $n=10,6$) and in the presence of 1 nM (□, $n=4,4$), 10 nM (△, $n=5,5$), 100 nM (○, $n=7,6$) and 1 μM (■, $n=6,5$) GR55562. Data are expressed as a percentage of the maximum response to the agonist in each preparation and shown as mean±s.e. n=number of rings, number of lungs.

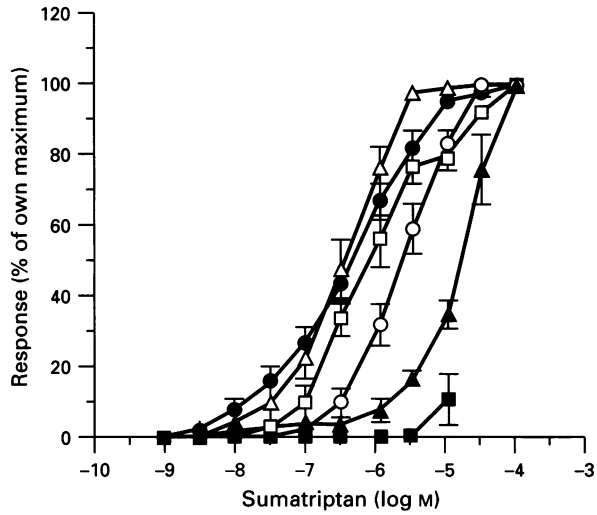

Figure 7 The effect of GR55562 on vasoconstrictor responses to sumatriptan in human isolated pulmonary artery rings. Control vasoconstrictor responses to sumatriptan (●, $n=10,6$) and in the presence of 1 nM (□, $n=6,6$), 10 nM (△, $n=8,6$), 100 nM (○, $n=6,5$), 300 nM (▲, $n=4,4$) and 1 μM (■, $n=8,6$) GR55562. Data are expressed as a percentage of the maximum response to the agonist in each preparation and shown as mean±s.e. n=number of rings, number of lungs.

ketanserin, the response to 0.3 μM sumatriptan was significantly reduced to 4±2 and 1±1% respectively, $P < 0.001$. This is demonstrated in Figure 3 which also shows that 0.1 μM and 1 μM ketanserin reduced the maximum response to sumatriptan ($P<0.001$).

Methiothepin had no significant effect on the sensitivity of the tissue to 5-HT at the EC_{50} level (Table 2); however it reduced responses to 0.3 μM 5-HT. For example, responses to 5-HT were 18±25% of maximum before, and only 5±1% after, 10 nM methiothepin ($P<0.001$). This is demonstrated in Figure 4 which also shows that 1 and 10 nM methiothepin inhibited the maximum responses to 5-HT ($P<0.001$).

Methiothepin had no significant effect on the sensitivity of the tissue to sumatriptan at the EC_{50} level (Table 2). Both 1 and 10 nM methiothepin reduced the sensitivity of the tissue to 0.3 μM sumatriptan. Responses to 0.3 μM sumatriptan were 18±2% before, 5±5% and 2±2% after 1 and 10 nM methiothepin respectively ($P<0.001$). This is demonstrated in Figure 5 which also shows that methiothepin reduced the maximum response to sumatriptan in a concentration-dependent fashion.

GR55562 competitively antagonized responses to 5-HT (Figure 6, Table 2). It had no significant effect on the maximum response to 5-HT.

GR55562 inhibited responses to sumatriptan in a competitive fashion with 1 μM almost totally abolishing the responses to sumatriptan (Figure 7, Table 2). It had no significant effect on the maximum response to sumatriptan. The pA_2 for GR55562 was 8.88±0.45 and the slope of the Schild plot = 0.87±0.12.

Discussion

Recently, several studies have suggested a role for 5-HT in the aetiology of pulmonary hypertension (PHT) and this is the reason for studying the pulmonary arterial response to 5-HT. Increased plasma levels of 5-HT can be caused by altered platelet storage of 5-HT and may be associated with the development of pulmonary hypertension (Hervé *et al.*, 1990; 1995). Hervé *et al.* (1995) have reported that plasma 5-HT levels are increased in primary PHT. Elevated 5-HT levels, associated with altered platelet 5-HT storage have also been reported in systemic sclerosis (Klimiuk *et al.*, 1989), in patients using the appetite suppressant, fenfluramine (Brenot *et al.*, 1993) and in platelet storage pool disease (Hervé *et al.*, 1990). It has been suggested that there may be a link between 5-HT and the PHT often associated with these diseases (Hervé *et al.*, 1995).

5-HT is released from pulmonary neuroendocrine cells as well as platelets (Johnson & Georgieff, 1989). Hervé *et al.* (1995) have described plasma concentrations of 5-HT up to 30 nM in primary pulmonary hypertensive patients and it is possible that release from pulmonary neuroendocrine cells may result in higher concentrations locally. The results of this study suggest that such concentrations would exert a vasoconstrictor effect on the pulmonary artery. The exact role of 5-HT in pulmonary hypertension does, however, need to be clarified.

Ketanserin, the 5-HT$_{2A}$ receptor antagonist has been of limited use in the treatment of PHT, for example secondary to platelet storage pool disease (Hervé *et al.*, 1990). The use of ketanserin assumes, however, that 5-HT$_{2A}$ receptors mediate vasoconstriction in the pulmonary circulation. This study indicates that it is a 5-HT$_{1D}$ receptor which predominates. The evidence for this conclusion lies mainly in the effects of the specific agonists and the specific 5-HT$_{1D}$ receptor antagonist, GR55562. The order of potency for the agonists was 5-CT > 5-HT = sumatriptan > 8-OH-DPAT. A high potency of 5-CT as compared with 5-HT satisfies one of the criteria for classification of a 5-HT$_1$-like receptor (Bradley *et al.*, 1986) and the competitive nature of GR55562 suggests the presence of 5-HT$_{1D}$ receptors. Recently, two distinct 5-HT$_{1D}$ receptors have been characterized in the human genome and termed the 5-HT$_{1D}\alpha$ and 5-HT$_{1D}\beta$ receptor (Weinshank *et al.*, 1992). The order of potency for the agonists in the human pulmonary artery is similar to the order of binding affinities of these agonists to the 5-HT$_{1D}\alpha$ receptor (Weinshank *et al.*, 1992). It is not, however, clear if the order of potency can be directly related to binding affinities. We, and others, have attempted binding assays to pulmonary artery preparations without success.

The receptor antagonist, GR55562, is selective for the 5-HT$_{1D}$ receptor (Connor *et al.*, 1995) and was the only antagonist used in this study which demonstrated competitive antagonism. The pA_2 value of 8.88 against sumatriptan is higher than that of GR55562 against sumatriptan-induced

contractions of the dog and monkey isolated basilar artery (~7.9, Connor *et al.*, 1995). This demonstrates a high potency of this antagonist against the 5-HT$_{1D}$ receptor in the human pulmonary artery. This may reflect the uniquely large population of 5-HT$_{1D}$ receptors which mediate vasoconstriction in this human artery. As the slope of the Schild plot was not significantly different from unity this suggests competitive antagonism of only one receptor.

Methiothepin and ketanserin are competitive antagonists usually used to classify 5-HT receptor populations. They did not, however, act in a competitive fashion against either 5-HT or sumatriptan in the human pulmonary artery. Parsons & Whalley (1989) have reported a similar effect of ketanserin against responses to sumatriptan in the rabbit basilar artery. This, along with the non-specific nature of these antagonists makes their effects difficult to interpret. Ketanserin has some selectivity for the 5-HT$_{1D\alpha}$ receptor at ~0.1–0.3 μM (Weinshank *et al.*, 1991; Thomas *et al.*, 1995) and methiothepin is selective for the 5-HT$_{2A}$ receptor at ~1 nM, being non-selective at higher concentrations (Hoyer *et al.*, 1994). Ketanserin demonstrated a non-competitive antagonism against both 5-HT and sumatriptan in that it failed to alter the pEC$_{50}$ values significantly but did reduce the maximum responses achieved. It did, however suggest a heterogeneous population of receptors in that lower concentrations of 5-HT were relatively resistant to ketanserin compared with higher concentrations and the 5-HT CCRC appeared biphasic in nature in the presence of ketanserin (Figure 2). The non-competitive nature of ketanserin in this preparation meant that pA$_2$ values could not be calculated and hence could not be used to characterize the receptor subtype. The biphasic nature of the 5-HT CCRC in the presence of ketanserin suggests, however that non-5-HT$_{2A}$ receptors are activated by low concentrations of 5-HT and that, at higher concentrations, 5-HT may be activating 5-HT$_{2A}$-like receptors. Ketanserin also inhibited responses to sumatriptan in a non-competitive fashion. It had no effect on the pEC$_{50}$ values of sumatriptan although lower concentrations of sumatriptan were apparently reduced. This could either be due to sumatriptan activating 5-HT$_{2A}$ receptors at higher concentrations as suggested by Parsons and Whalley (1989) or to the ability of ketanserin to interact with the 5-HT$_{1D\alpha}$ receptor subtype.

Methiothepin has a pA$_2$ against 5-HT$_{2A}$ receptors of ~9 and against 5-HT$_1$ receptors of ~7.7–8.1 (Hoyer *et al.*, 1994). Methiothepin, 1 nM, caused a significant reduction of only higher concentrations of 5-HT, again suggestive of activation of 5-HT$_{2A}$ receptors only at higher concentrations of 5-HT. Methiothepin, 10 nM, reduced responses of all concentrations of 5-HT indicating activation of 5-HT$_1$ receptors at all concentrations of 5-HT. However, at 1 nM, methiothepin did reduce responses to all concentrations of sumatriptan which suggests some activity against 5-HT$_{1D}$ receptors by methiothepin at this concentration. The effect of 10 nM methiothepin was more profound, consistent with its ability to antagonize 5-HT$_{1D}$ receptors at this concentration.

In summary, the rank order of agonist potency in the human pulmonary artery strongly suggests the presence of 5-HT$_1$ receptors in this preparation. The competitive nature of GR55562 further suggests the presence of 5-HT$_{1D}$ receptors in this preparation and indicates that 5-HT activates these receptors.

In conclusion, this study suggest that the human pulmonary artery has a functional population of 5-HT$_{1D}$-like receptors which may play a role in the contractile response to 5-HT.

The authors thank the Medical Research Council U.K., the Wellcome Trust U.K. and the Royal Society, London for support. The authors also thank Glaxo for the donation of sumatriptan and GR55562 and Dr Helen Connor for her continuous support and advice throughout these studies.

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(Received March 13, 1996
Revised May 28, 1996
Accepted June 19, 1996)