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5-Hydroxytryptamine (5-HT) mediates potent relaxation in the sheep isolated pulmonary vein via activation of 5-HT_4 receptors

[1] T.M. Cocks & P.J. Arnold

Baker Medical Research Institute, Commercial Rd, Prahran, Victoria, 3181, Australia

1 We investigated the potent 5-hydroxytryptamine (5-HT)-mediated vasorelaxation of the sheep pulmonary vein. Here we present evidence that this response is due to activation of 5-HT_4 receptors.

2 5-HT (1–1000 nM) caused concentration-dependent, maintained relaxations ($pEC_{50} = 8.4 \pm 0.1$) of isolated rings of sheep pulmonary vein pre-contracted with endothelin-1 (3 nM).

3 The relaxation response to 5-HT was unaffected by either removal of the endothelium or by inhibition of NO-synthase by N^G-nitro-L-arginine (100 μM).

4 Ketanserin, methiothepin, methysergide and MDL 72222 at concentrations that selectively block 5-HT_2, 5-HT_1-like and 5-HT_3 receptors respectively, had no effect on the concentration-relaxation curve to 5-HT.

5 ICS 205-930 (1–10 μM) competitively antagonized the concentration-relaxation curve to 5-HT with a pA_2 of approximately 6.7.

6 Increasing the concentration of ICS 205-930 from 10 to 30 μM did not cause a further rightward shift of the 5-HT concentration-relaxation curve. The pEC_{50} of 6.50 for 5-HT in the presence of ICS 205-930 (30 μM) was taken as an estimate of the affinity of 5-HT for 5-HT_1-like receptors since methiothepin (10 nM) unmasked further competitive inhibition of 5-HT in the presence of this concentration of ICS 205-930.

7 Other 5-HT agonists including 5-carboxamidotryptamine (5-CT), α-methyl-5-HT and BIMU 8 (but not 2-methyl-5-HT) also relaxed the pulmonary vein. The response to 5-CT was inhibited by methiothepin (10 nM) and methysergide (100 nM) but unaffected by ICS 205-930 (30 μM), whilst that to α-methyl-5-HT and BIMU 8 was unaffected by methiothepin (10 nM) but blocked by ICS 205-930 (estimated pK_B values of 6.4 and 6.9 respectively). Relaxation curves to both 5-HT and BIMU 8 were unaffected by cocaine (6 μM).

8 In conclusion, these results indicate that the sheep pulmonary vein possesses 5-HT_4 receptors that mediate potent endothelium-independent relaxation. 5-HT_1-like relaxant receptors are also present in this tissue but 5-HT has a lower affinity at these receptors. This preparation may thus provide a robust and sensitive bioassay for future development of selective 5-HT_4 receptor agonists and antagonists.

Keywords: 5-Hydroxytryptamine (5-HT); vasorelaxation; sheep pulmonary vein; 5-HT_4 receptors

Introduction

Receptors for 5-hydroxytryptamine (5-HT) have been divided into three major classes; 5-HT_1-like, 5-HT_2 and 5-HT_3 (Bradley *et al.*, 1986). In addition to its action at 5-HT receptors (5-HT_2 or 5-HT_1-like) mediating vascular smooth muscle contraction, 5-HT also causes both endothelium-dependent and independent relaxation of a number of isolated blood vessels including porcine coronary artery, (Cocks & Angus, 1983) rabbit jugular vein, (Leff *et al.*, 1987) cat saphenous vein, (Feniuk *et al.*, 1983) porcine vena cava, (Sumner *et al.*, 1989; Sumner, 1991) and sheep and goat pulmonary veins (Eyre, 1975; Chand, 1981). 5-HT_1-like receptors have been found to mediate vasorelaxation in most of these cases (but see Sumner *et al.*, 1991; Leff *et al.*, 1987), and have been characterized by the following criteria: (1) susceptibility to blockade by methiothepin and methysergide; (2) resistance to blockade by 5-HT_2 and 5-HT_3 receptor selective antagonists; (3) a high agonist potency of 5-HT which is mimicked by 5-carboxamidotryptamine (5-CT) with an equal or greater potency (Bradley *et al.*, 1986). A novel 5-HT receptor site, positively coupled to adenylate cyclase, designated as 5-HT_4 has been identified in mammalian brain, (Dumuis *et al.*, 1988a,b; 1989; Bockaert *et al.*, 1990) guinea-pig ileum, (Craig & Clarke, 1989) rat oesophagus (Baxter *et al.*, 1991) porcine heart, (Villalon *et al.*, 1990; 1991) and human atria (Kaumann *et al.*, 1990; 1991). ICS 205-930 has been reported to be an antagonist at this receptor (Dumuis *et al.*, 1988b). This compound also acts as an antagonist at 5-HT_3 receptors with a pK_B of 10.2–10.6 (Richardson *et al.*, 1985). At higher concentrations, however, ICS 205-930 antagonizes the actions of 5-HT at 5-HT_4 receptors, (pK_B 6.0–6.7). Also a number of 5-HT_4 receptor agonists including 5-methoxytryptamine, α-methyl-5-HT, substituted benzamide derivatives and benzimidazolone derivatives (BIMU 8) have been described (Kaumann *et al.*, 1991; Villalon *et al.*, 1991; Schiantarelli *et al.*, 1990).

The receptor which mediates the potent vasorelaxation to 5-HT in the sheep pulmonary vein (Eyre, 1975) has not been characterized. Here we present evidence that this receptor is of the 5-HT_4 subclass which is the first account of the presence of this receptor in vascular tissue. The sheep pulmonary vein may thus provide a simple, reliable bioassay which can be used for future development of additional compounds as either 5-HT_4 receptor agonists or antagonists.

Methods

Lungs from healthy sheep, of mixed breed, age and sex were obtained from a local abattoir and placed into cold, oxygenated Krebs solution (see below) and transported quickly to the laboratory (15 min). The main pulmonary vein

[1] Author for correspondence.

and its first branches from all lungs were carefully dissected and 3 mm ring segments were cut with a fixed double-bladed scapel arrangement. Some rings had their endothelium removed by abrading the luminal surface with a tapered wooden stick (Cocks & Angus, 1983). All rings of vein were then mounted on parallel stainless steel wires (350 μm diameter) passed through the lumen and suspended in 25 or 30 ml organ baths containing Krebs solution (see below) kept at 37°C and continually gassed with carbogen (95% O_2:5% CO_2). One wire was fixed to a support leg which in turn was attached to a micrometer so that the leg could be easily moved in a vertical plane. The other wire was attached to a Grass FT.03 force-displacement transducer to record circumferential, isometric force, which was then amplified and displayed on a flat-bed chart recorder (see Angus *et al.*, 1986). The Krebs solution had the following composition (mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2 and glucose 11.

Protocol

The rings of vein were allowed to remain unstretched in the organ baths for 60 min after which time they were stretched to a passive force of 2 g. This passive force was previously determined as that required to stretch the veins such that their circumference was approximately 0.9 times that when the veins were subjected to a transmural distending pressure of 20 mmHg (see Angus *et al.*, 1986). After a further 30 min the force was reset to 2 g and the ring segments were then left to equilibrate for a further 60 min. They were then pre-contracted with endothelin-1 (ET-1) at a concentration (3 nM) which caused approximately 50% of the maximum contractile response to ET-1 (30 nM). When the contraction to ET-1 had reached its peak and either maintained a steady plateau or lost active force at a linear rate of 0.07 ± 0.01 g min^{-1}, cumulative concentration (0.5 log unit)-relaxation curves were constructed to the agonists under study. When antagonists were used they were allowed to equilibrate with the tissue for 30 min before relaxation curves were constructed. Only one agonist curve was constructed for each ring segment.

In tissues where 100% relaxation had not been obtained by the agonist, sodium nitroprusside (SNP) (10 μM) was added at the end of the experiment to show that the tissues were able to relax fully.

Statistical analysis

All relaxation responses were expressed as percentages of the contraction to ET-1. The concentration-relaxation curves were fitted to a logistic equation to determine maximum response, (E_{max}) and the negative logarithm of the molar concentration of drug that elicited 50% of the maximum relaxation, (pEC_{50}) (Nakashima *et al.*, 1982). All pEC_{50} values quoted are mean values ± s.e.mean with the number of experiments (n) given in parentheses. The effects of the antagonists and agonists on both pEC_{50} and E_{max} in different treatment groups were compared by use of the unpaired Student's t test and a P value of 0.05 or less was considered statistically significant.

Competitive antagonism of the vasodilator effects of 5-HT by ICS 205-930 was analysed by the equation of Arunlakshana & Schild (1959). pK_B estimates for antagonist experiments in which only one concentration of antagonist was used were determined from concentration-ratios by the equation for competitive inhibition at equilibrium, $pK_B = \log_{10}(x-1) - \log_{10}[A]$, where x is the concentration-ratio and [A] the applied antagonist concentration.

Drugs

Drugs and their sources (in parentheses) were: 5-hydroxytryptamine creatinine sulphate, acetylcholine bromide and N^G-nitro-L-arginine (Sigma, U.S.A.); ionomycin (Calbiochem, Australia); ketanserin (gift from Janssen-Cilag, Australia); endothelin-1 (Peninsula Laboratories, U.S.A.); 5-carboxamidotryptamine maleate, α-methyl-5-hydroxytryptamine, 2-methyl-5-hydroxytryptamine and sumatriptan (gifts from Dr P. Humphrey, Glaxo Group Research, Ware, U.K.); methiothepin (gift from Hoffman-La Roche, U.S.A.); MDL72222 (1αH,3αω5αH-tropan-3yl-3,5-dichlorobenzoate) (gift from Dr J. Fozard, formerly at Merrell Dow, Strasbourg, France); methysergide hydrochloride and ICS 205-930 ((3α-tropanyl)-1H-indole-3-carboxylic acid ester) (gifts from Dr J. Fozard, Sandoz, Basle, Switzerland); sodium nitroprusside dihydrate (Roche, Australia); BIMU 8 (endo-N-(8-methyl-8-azabicyclo [3.2.1.] oct-3-yl) -2,3-dihydro-(1-methyl) ethyl-2-oxo-1H-benzimidazole-1-carbo-oxamide hydrochloride) gift from Dr C. Rizzi, Boehringer Ingelheim, Italy); cocaine hydrochloride (gift from Alfred Hospital, Melbourne, Australia).

Results

Characterization of 5-hydroxytryptamine-mediated relaxation

5-HT (1–1000 nM) caused concentration-dependent, stable relaxations in the sheep pulmonary vein contracted with ET-1 (see Figure 1), with a pEC_{50} value of 8.4 ± 0.1 ($n = 26$) and an E_{max} of $88.7 \pm 4.7\%$ ($n = 13$). At higher concentrations (1–10 μM), 5-HT caused concentration-dependent contractions, whcih were antagonised by ketanserin (1 μM). Neither mechanical removal of the endothelium nor treatment of endothelium-intact rings with N^G-nitro-L-arginine (L-NNA; 100 μM) affected the pEC_{50} or E_{max} of the relaxation curve to 5-HT. Respective pEC_{50} and E_{max} values were 8.3 ± 0.1, $98.0 \pm 2.0\%$ ($n = 5$) and 8.2 ± 0.1, $99.5 \pm 1.7\%$ ($n = 7$) which were not significantly different from the 5-HT control ($P > 0.05$). Functional removal of the endothelium, however, proved to be difficult in this tissue since not all tissues with intact endothelium relaxed in response to acetylcholine. If relaxation was observed it was abolished by either luminal abrasion to remove the endothelial cells or L-NNA (100 μM).

The effect of 5-HT_1-like, 5-HT_2 and 5-HT_3 antagonists on the relaxation curve to 5-HT is shown in Table 1. Methiothepin (100 nM) and methysergide (100 nM) did not significantly affect the 5-HT concentration-response curve, the estimated pEC_{50} values being 8.8 ± 0.2 ($n = 8$) and 7.9 ± 0.4 ($n = 3$) respectively. Similarly, the pEC_{50} values for 5-HT in the presence of the 5-HT_2 receptor antagonist ketanserin (1 μM)

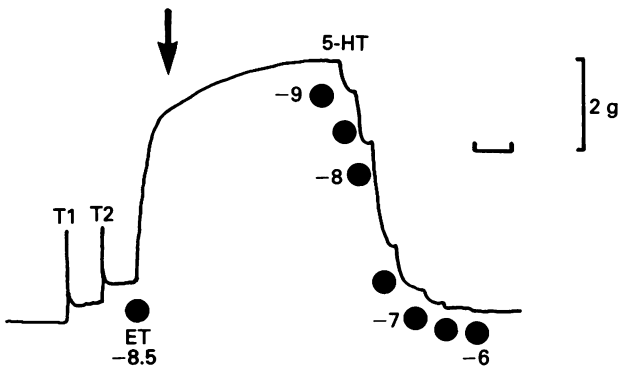

Figure 1 Tracing of a representative chart recording showing relaxation responses of the sheep isolated pulmonary vein to cumulative (log M) additions of 5-hydroxytryptamine (5-HT) contracted with endothelin-1 (ET) (3 nM). T_1 and T_2 correspond to the initial stretches of the vein to 2 g passive force. The horizontal bar represents 20 min before the arrow and 6 min after the arrow.

Table 1 Effect of 5-HT$_1$-like, 5-HT$_2$ and 5-HT$_3$ receptor antagonists on the relaxation response curve to 5-hydroxytryptamine (5-HT) in sheep isolated pulmonary vein preparations contracted with endothelin-1 (3 nM)

Antagonist	EC_{50}	n	*Maximal relaxation (%)*	n
5-HT alone	8.4 ± 0.1	26	88.7 ± 4.7	13
Methiothepin 100 nM	8.8 ± 0.2	8	94.2 ± 3.8	6
Methysergide 100 nM	7.9 ± 0.4	3	86.7 ± 6.9	3
Ketanserin 1 μM	8.3 ± 0.1	18	95.3 ± 2.8	16
MDL 72222 100 nM	7.9 ± 0.1	4	87.0 ± 6.6	4

Results are mean ± s.e.mean from *n* experiments.

and the 5-HT$_3$ receptor antagonist MDL72222 (100 nM) were 8.3 ± 0.1 ($n = 18$) and 7.9 ± 0.1 ($n = 4$) respectively, which were not significantly different from the pEC_{50} of 5-HT in the absence of antagonists ($P > 0.05$ in all cases) (see Table 1).

5-HT$_4$ receptor antagonism

ICS 205-930 (1–10 μM) caused a concentration-dependent, parallel rightward shift of the relaxation curve to 5-HT with no significant depression of the maximum response in the presence of ketanserin (1 μM) to block the contractile effects of 5-HT at higher concentrations (see Figure 2a). However, at a concentration of 30 μM ICS 205-930, no further rightward shift was observed. To test the hypothesis that the response to 5-HT in the presence of ICS 205-930 (30 μM) was due to 5-HT$_1$-like receptor activation at the higher concentrations of 5-HT, the effect of this concentration of ICS 205-930 (30 μM) was determined in the presence of methiothepin (10 nM). Under these conditions ICS 205-930 now produced a further significant approximate three fold rightward shift of the relaxation curve to 5-HT (see Figure 2a).

Figure 2b shows the Schild plot for ICS 205-930 against 5-HT, mean pEC_{50} values being used to calculate the concentration-ratios for concentrations of ICS 205-930 up to 10 μM. The pA_2 estimate obtained by Schild analysis was 6.7 (95% confidence limits 6.5, 7.0) with a slope of the regression line of 1.0 (95% confidence limits 0.8, 1.2).

Effects of other agonists

Concentration-response curves obtained to two other agonists which mediated relaxation in the sheep pulmonary vein are shown in Figure 3. 5-Carboxamidotryptamine (5-CT) (1–1000 nM) caused only relaxation but was significantly less potent than 5-HT with a similar E_{max} (Table 2), whereas α-methyl-5-HT produced relaxation at lower (1–1000 nM), and contraction at higher (1–10 μM) concentrations due to stimulation of 5-HT$_2$ receptors. Thus for the relaxation responses to α-methyl-5-HT a pEC_{50} value of 6.6 ± 0.3 ($n = 5$) and E_{max} of 62.0 ± 5.0% ($n = 3$) were obtained. In the presence of ketanserin (1 μM) the pEC_{50} estimate for α-methyl-5-HT was 6.5 ± 0.1 ($n = 4$) which was not significantly different from that observed without ketanserin but the E_{max} was significantly increased to 93.0 ± 5.0% ($n = 4$). Relaxation-response curves to α-methyl-5-HT were therefore obtained in the presence of the 5-HT$_2$ receptor antagonist, ketanserin (1 μM), to block the latter contractile response. BIMU 8 (1–1000 nM) caused concentration-dependent relaxation in the sheep pulmonary vein with a pEC_{50} value of 8.3 ± 0.1 and an E_{max} of 84.5 ± 4.1% ($n = 4$). Two other 5-HT agonists were also studied: sumatriptan (a 5-HT$_1$-like receptor agonist, see Humphrey *et al.*, 1988) and 2-methyl-5-HT (a partially selective 5-HT$_3$ receptor agonist, see Richardson *et al.*, 1985). Both agents failed to produce an effect (either relaxation or contraction) in this tissue at concentrations up to 10 μM (Table 2).

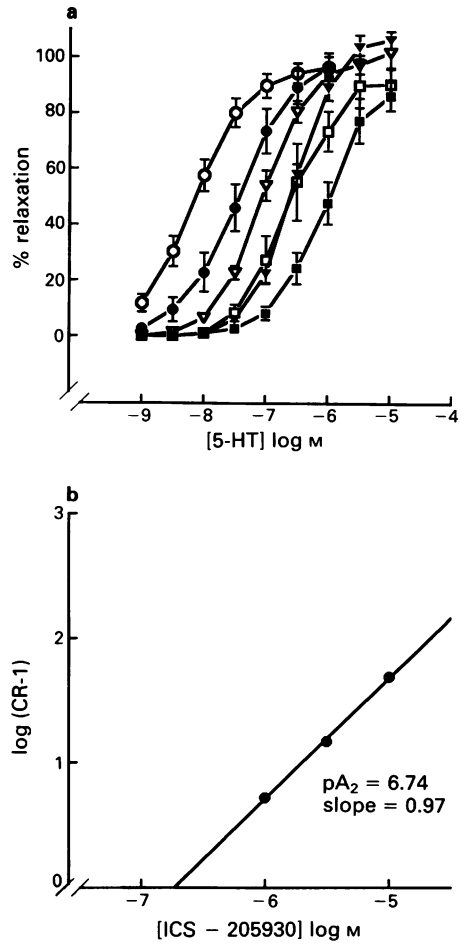

Figure 2 (a) Relaxation responses to 5-hydroxytryptamine (5-HT) alone (○; $n = 12$) or in the presence of ICS 205-930 1 μM (●; $n = 9$), 3 μM (▽; $n = 7$), 10 μM (▼; $n = 9$), 30 μM (□; $n = 7$) or 30 μM in the presence of methiothepin 10 nM (■; $n = 5$). All concentration-response curves were carried out in the presence of ketanserin (1 μM). Data points are means ± s.e.mean (vertical bars). (b) Schild plot depicting competitive antagonism by ICS 205-930 of the relaxation response curve to 5-HT. Concentration-ratios (CR) were calculated from the mean pEC_{50} values. The line through the data points was obtained by linear regression.

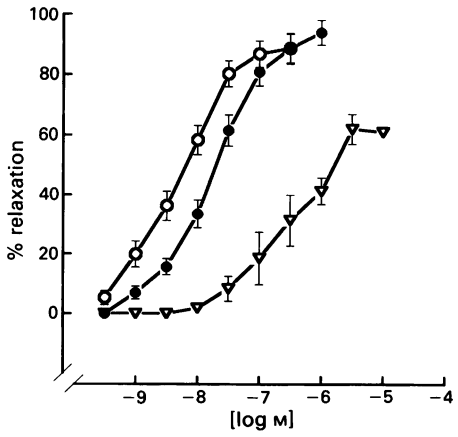

Figure 3 Relaxation response curves to 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT) and α-methyl-5-hydroxytryptamine (α-methyl-5-HT) in sheep pulmonary vein. 5-HT (○; $n = 26$), 5-CT (●; $n = 13$), and α-methyl-5-HT (▽; $n = 5$). Data points are means ± s.e.mean (vertical bars).

Table 2 Relaxant activity of 5-hydroxytryptamine (5-HT) receptor agonists in sheep isolated pulmonary vein preparations contracted with endothelin-1 (3 nM)

Agonist	EC_{50}	n	*Maximal relaxation (%)*	n
5-HT	8.4 ± 0.1	26	88.7 ± 4.7	13
5-CT	7.7 ± 0.1	13	94.0 ± 4.2	9
α-Methyl-5-HT	6.6 ± 0.3	5	62.0 ± 5.0	3
BIMU 8	8.3 ± 0.1	4	84.5 ± 4.1	4
2-Methyl-5-HT	< 5.0	2	0	2
Sumatriptan	< 5.0	3	0	3

Results are mean ± s.e.mean from *n* experiments. For abbreviations, see text.

Characterization of 5-carboxamidotryptamine-mediated relaxation

Both methiothepin (10 nM) and methysergide (100 nM) produced significant rightward shifts of the relaxation curve to 5-CT without affecting E_{max} (see Figure 4). pEC_{50} estimates were 7.7 ± 0.1 ($n = 13$) for the 5-CT control, 5.9 ± 0.2 ($n = 6$) in the presence of methiothepin ($P < 0.001$) and 6.9 ± 0.2 ($n = 3$) in the presence of methysergide ($P = 0.005$). A pEC_{50} estimate was also obtained for 5-CT in the presence of ICS 205-930 (30 μM), giving a value of 7.9 ± 0.1 ($n = 2$) which was not significantly different from the control (data not illustrated). Also, in endothelium-denuded rings of vein, the mean pEC_{50} was 7.5 ± 0.1 ($n = 4$) which was not significantly different from the control.

Characterization of α-methyl-5-HT mediated relaxation

Methiothepin (10 nM) did not significantly affect the concentration-response curve to α-methyl-5-HT (obtained in the presence of ketanserin (1 μM)), where the pEC_{50} was 6.4 ± 0.1 ($n = 3$) and E_{max} was unchanged. ICS 205-930 (3 μM), however, caused a significant rightward shift of the relaxation curve to α-methyl-5-HT with a pEC_{50} value of 5.6 ± 0.1 ($n = 6$; $P < 0.05$), the estimated pK_B value being 6.4. E_{max} was not significantly different from the control (see Figure 5).

Characterization of BIMU 8-mediated relaxation

Neither methiothepin (10 nM) nor ketanserin (1 μM) had a significant effect on the concentration-response curve to BIMU 8. Respective pEC_{50} values were 8.8 ± 0.4 ($n = 3$) and 8.4 ± 0.3 ($n = 3$), (control $pEC_{50} = 8.3 \pm 0.1$). ICS 205-930 (10 μM), however, caused a significant rightward shift of the relaxation curve to BIMU 8 with a pEC_{50} value of 6.4 ± 0.1 ($n = 4$; $P < 0.01$). The estimated pK_B value was 6.9. E_{max} was not significantly different from the control (see Figure 6).

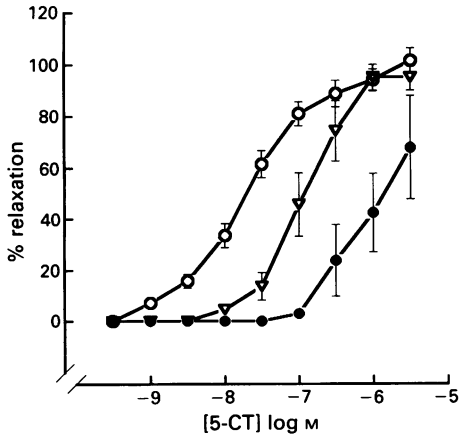

Figure 4 Effect of 5-HT_1-like receptor antagonists on the relaxation-response curve to 5-carboxamidotryptamine (5-CT). 5-CT alone (○; $n = 13$) and in the presence of methiothepin 10 nM (●; $n = 6$) or methysergide 100 nM (▽; $n = 3$). Data points are means ± s.e.mean (vertical bars).

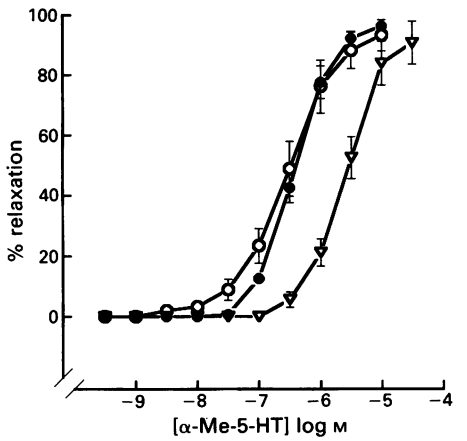

Figure 5 Effect of 5-hydroxytryptamine (5-HT) receptor antagonists on the relaxation response curve to α-methyl-5-HT: α-methyl-5-HT alone (○; $n = 4$), and in the presence of methiothepin 10 nM (●; $n = 3$) or ICS 205-930 3 μM (▽; $n = 6$). All curves were carried out in the presence of ketanserin 1 μM. Data points are means ± s.e.mean (vertical bars).

Effect of cocaine

Preincubation with cocaine (6 μM) had no effect on either 5-HT- or BIMU8-mediated relaxation. Respective pEC_{50} values for 5-HT and BIMU 8 were 8.5 ± 0.1 and 8.7 ± 0.1 in the presence of cocaine, which were not significantly different from control values of 8.4 ± 0.1 and 8.5 ± 0.1 respectively ($n = 4$ in each group).

Discussion

The results presented here strongly suggest that the potent relaxation to 5-HT in the sheep isolated pulmonary vein is due to activation of 5-HT_4 receptors. Thus the response was not blocked by 5-HT_1-like, 5-HT_2 or 5-HT_3 receptor antagonists whereas it was competitively antagonized by the 5-HT_4 receptor antagonist, ICS 205-930. The pA_2 estimate of 6.7 obtained here for ICS 205-930 was similar to that reported in the rodent brain ($pK_B = 6.2$; Dumuis *et al.*,

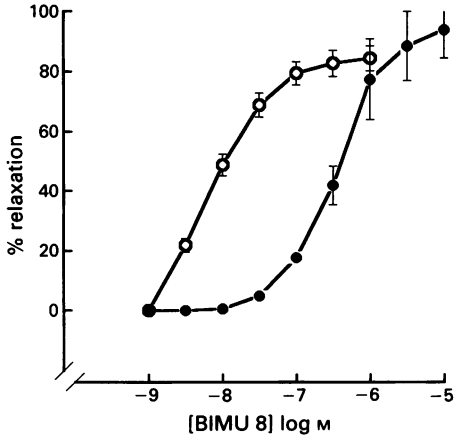

Figure 6 Effect of ICS 205-930 on the relaxation curve to BIMU 8. BIMU 8 alone (○; $n = 4$), and in the presence of ICS 205-930 (10 μM) (●; $n = 4$). Data points are means ± s.e.mean (vertical bars).

1988b) and guinea-pig ileum ($pK_B = 6.4$; Craig & Clarke, 1989), and identical to that reported in human atria ($pK_B = 6.7$; Kaumann *et al.*, 1990). We have also shown that the selective 5-HT$_4$ receptor agonist, BIMU 8, is an agonist in this tissue with a similar order of potency to 5-HT itself. The potencies of both 5-HT and BIMU 8 were unaffected by the neuronal uptake blocker, cocaine. Also, α-methyl-5-HT acts as an agonist at this receptor although less potent by approximately 2 orders of magnitude than 5-HT, which supports the findings of Villalon *et al.* (1991). Taken together our data indicate that the 5-HT receptors in the sheep pulmonary vein resemble 5-HT$_4$ receptors found in rat and guinea-pig brain, guinea-pig ileum, porcine heart and human atria.

We have also shown that relaxant 5-HT$_1$-like receptors are present in this tissue. Thus the 5-CT-mediated relaxation with a pEC_{50} value of 7.7 was blocked by the 5-HT$_1$-like antagonists, methysergide and methiothepin at concentrations that had no effect on the 5-HT-mediated response. The 5-CT-mediated relaxation was unaffected, however, by high concentrations (30 μM) of ICS 205-930. Also, failure to antagonize further the relaxation to 5-HT when the concentration of ICS 205-930 was increased from 10 to 30 μM was completely overcome by first treating the tissue with methiothepin (see Figure 2a). Under these conditions 30 μM ICS 205-930 gave a further 3 fold shift of the relaxation curve, and the value of the log (CR − 1) lay exactly on the Schild regression line (not shown). This implies that the pEC_{50} of 6.5 for 5-HT in the presence of 30 μM ICS 205-930 (without methiothepin) can be taken as a good estimate for the pEC_{50} of 5-HT at 5-HT$_1$-like receptors, and shows in accordance with convention, that 5-CT has a higher affinity for 5-HT$_1$-like receptors than 5-HT. Therefore, both 5-HT$_1$-like and 5-HT$_4$ receptors are present in sheep pulmonary vein, 5-HT being more potent at 5-HT$_4$ receptors.

Although it was relatively difficult to remove the endothelium completely in this tissue without causing damage to the underlying smooth muscle, both the 5-HT$_1$-like and 5-HT$_4$-mediated responses to 5-HT appeared to be endothelium-independent. The pulmonary vein relaxed only poorly to the endothelium-dependent agent, acetylcholine (ACh). As such there was often little or no relaxation, but a small further contraction in vessels optimally contracted with endothelin. In preparations that did relax to ACh, both mechanical damage to the intima (removal of endothelium) and treatment with the NO-synthase inhibitor L-NNA (see Moncada *et al.*, 1991) completely abolished this relaxation to reveal the direct contractile response. Both luminal damage and L-NNA, however, had no effect on the relaxation curve to 5-HT. Therefore, there appeared to be no role for the endothelium in mediating the relaxation response to 5-HT in this tissue as reported for other vessels (Cocks & Angus, 1983; Leff *et al.*, 1987; Sumner, 1991; for review see Angus & Cocks, 1989). Further evidence against 5-HT endothelial receptors in the sheep pulmonary vein was the resistance of the relaxation to methiothepin which blocked 5-HT-mediated relaxations in the rabbit jugular vein (Leff *et al.*, 1987), and the slow time-course of the relaxation compared to the responses to 5-HT in pig vena cava (Sumner, 1991).

The presence of 5-HT$_4$ receptors in the pulmonary vein appears to be species-specific, since in similar experiments on dog, pig and human pulmonary vein we have found no evidence for 5-HT$_4$-mediated relaxations (unpublished data). Preliminary experiments on bovine pulmonary vein, however, suggest that the relaxation to 5-HT (observed in the presence of ketanserin (1 μM) and methiothepin (10 nM)), could be mediated via 5-HT$_4$ receptors. The 5-HT-induced relaxation reported in the goat isolated pulmonary vein (Chand, 1981) might also be mediated via 5-HT$_4$ receptors since responses were similar in time course and occurred over a similar concentration-range to the response in the sheep. Therefore it appears that ruminants possess pulmonary vein 5-HT$_4$ receptors, but for what physiological reason remains unknown.

In conclusion, the receptors responsible for 5-HT-mediated relaxation of the sheep pulmonary vein appear identical to the 5-HT$_4$ receptors recently described in other tissues. Therefore, this is the first demonstration of vascular 5-HT$_4$ receptors. Whilst their physiological role(s) is unknown, given the availability of abattoir material, this robust and sensitive bioassay may thus prove particularly useful in the development of new selective 5-HT$_4$ agonists and antagonists provided that care is taken to eliminate possible complications of the effects of activation of 5-HT$_1$-like and 5-HT$_2$ receptors present in this tissue.

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