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5-Hydroxytryptamine receptors mediating vasoconstriction in pulmonary arteries from control and pulmonary hypertensive rats

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1 We investigated 5-hydroxytryptamine (5-HT)-receptor mediated vasoconstriction in the main, first branch and resistance pulmonary arteries removed from control and pulmonary hypertensive rats. Contractile responses to 5-HT, 5-carboxamidotryptamine (5-CT, non-selective 5-HT_1 agonist), and sumatriptan (5-HT_{1D}-like receptor agonist) were studied. The effects of methiothepin (non-selective 5-HT_{1+2}-receptor antagonist) and ketanserin (5-HT_{2A} receptor antagonist) and GR55562 (a novel selective 5-HT_{1D} receptor antagonist) on 5-HT-mediated responses were also studied. Basal levels of adenosine 3':5'-cyclic monophosphate ([cyclic AMP]$_i$) and guanosine 3':5'-cyclic monophosphate ([cyclic GMP]$_i$) were determined and we assessed the degree of inherent tone in the vessels under study.

2 5-HT was most potent in the resistance arteries. pEC_{50} values were 5.6 ± 0.1, 5.3 ± 0.1, 5.0 ± 0.2 in the resistance arteries, pulmonary branch and main pulmonary artery, respectively (n=at least 5 from 5 animals). The sensitivity to, and maximum response of, 5-HT was increased in all the arteries removed from the chronic hypoxic (CH) rats. In CH rats the pEC_{50} values were 5.9 ± 0.2, 6.3 ± 0.2, 6.4 ± 0.2 and the increase in the maximum response was 35%, 51% and 41% in the resistance arteries, pulmonary branch and main pulmonary artery, respectively. Sumatriptan did not contract any vessel from the control rats whilst 5-CT did contract the resistance arteries. In the CH rats, however, they both contracted the resistance arteries (responses to sumatriptan were small) (pEC_{50}: 5-CT; 5.4 ± 0.2) and the pulmonary artery branches (pEC_{50}: sumatriptan, 5.4 ± 0.2; 5-CT, 5.4 ± 0.2). 5-CT also caused a contraction in the main pulmonary artery (pEC_{50}: 6.0 ± 0.3).

3 Ketanserin (1 nM–1 μM) caused a competitive antagonism of the 5-HT response in all vessels tested. In control rats, the estimated pK_b values for ketanserin in resistance arteries, pulmonary branches and main pulmonary artery were 8.3, 7.8 and 9.2, respectively. Methiothepin (1 nM–1 μM) inhibited responses to 5-HT in the first branch (estimated pK_b value: 7.8) and main pulmonary artery. In CH rats, the estimated pK_b values for ketanserin in resistance arteries, pulmonary branches and main pulmonary artery were 7.7, 8.3 and 9.6, respectively. Methiothepin also inhibited contractions to 5-HT in the pulmonary artery branch and main pulmonary artery with estimated pK_b values of 7 and 9.5, respectively. In control animals, GR55562 had no effect on responses to 5-HT in any of the vessels tested. In the CH rats the estimated pK_b values for GR55562 were 6.5, 7.8 and 7.0 in the pulmonary resistance arteries, first branch and main pulmonary artery, respectively.

4 Large pulmonary arteries from controls demonstrated inherent tone and this was increased three fold in the CH rats. The resistance arteries from controls demonstrated little inherent tone though this was enhanced in those from the CH rats.

5 [Cyclic AMP]$_i$ was 259 ± 23 pmol mg^{-1} protein in the pulmonary artery branches removed from control rats and decreased to 192 ± 11 pml mg^{-1} protein in the CH rats ($P<0.01$, $n=8$). [Cyclic GMP]$_i$ also decreased in the pulmonary artery branches (from 550 ± 15, control to 462 ± 31 pmol mg^{-1} protein in CH vessels, $n=8$, $P<0.01$) and in the main pulmonary arteries (from 566 ± 33, control to 370 ± 25 pmol mg^{-1} protein in CH vessels, $n=8$, $P<0.001$). No changes in either [cyclic AMP]$_i$ or [cyclic GMP]$_i$ were observed in the resistance arteries.

6 The results suggest that the increased vasoconstrictor response to 5-HT in CH rat pulmonary arteries is due to an increase in 5-HT_{2A}-receptor mediated contraction combined with an increase in r5-HT_{1B}-like receptor-mediated contraction. An increase in vascular tone and decreased levels of [cyclic GMP]$_i$ in the large pulmonary arteries may contribute to the observed increase in activity of r5-HT_{1B}-like receptors.

Keywords: Pulmonary artery; 5-HT receptors; vasoconstriction; sumatriptan; cyclic nucleotides; vascular tone; pulmonary hypertension; chronic hypoxia

Introduction

5-Hydroxytryptamine (5-HT) has been implicated in the aetiology of pulmonary hypertension (PHT), both clinical and experimental. It may be involved in PHT related hypoxia in newborns (Johnson & Georgieff, 1989). Platelet release of 5-HT may be implicated in PHT secondary to cardiac surgery and also in primary PHT (Johnson & Georgieff, 1989; Reneman & Starre, 1990; Hervé *et al.*, 1990; 1995). It may also be associated with the development of PHT secondary to ingestion of monocrotaline in rats (Wanstall & O'Donnell, 1990; Kanai *et al.*, 1993).

5-HT acts at a minimum of 10 different receptor types. These include receptors 5-HT_{1A-F}, 5-HT_{2A-C}, 5-HT_3 and 5-HT_4 (Hoyer *et al.*, 1994). Evidence indicates that the vasoconstrictor effect of 5-HT in systemic arteries is mediated mainly through the 5-HT_{2A} receptor type (Saxena & Villalon, 1990; Hoyer *et al.*, 1994). Sumatriptan (GR43175) is a 5-HT_1-like agonist having its greatest affinity at the 5-HT_{1D} subtype (Peroutka & McCarthy, 1989; Sumner & Humphrey, 1989). In systemic vessels it is typically 4–10 fold less potent than 5-HT,

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as a vasoconstrictor at 5-HT_1-like receptors (e.g. Humphrey *et al.*, 1988; Parsons & Whalley, 1989). We have shown, however, that it is equipotent to 5-HT in human pulmonary arteries suggesting 5-HT_{1D}-like activation in these vessels (Templeton *et al.*, 1993; 1994). In rat brain, small numbers of 5-HT_{1D}-like receptors may exist (Herrick-Davis & Titeler, 1988; Bruinvels *et al.*, 1993). These have been suggested to be the species homologue of the human 5-$HT_{1D\alpha}$ receptor (Bach *et al*, 1993) and have recently been reclassified the r5-HT_{1D} receptors (Hartig *et al.*, 1996). The r5-HT_{1B} receptor is the species homologue of the human 5-$HT_{1D\beta}$ receptor and is the 5-HT_1 receptor subtype prevalent in the rodent (Voigt *et al.*, 1991; Adham *et al.*, 1992; Hartig *et al.*, 1992; 1996).

Responses to 5-HT are potentiated in the rat monocrotaline-induced model of PHT (Wanstall & O'Donnell, 1990) and have been associated with the development of PHT in these animals (Kanai *et al.*, 1993). Here we investigate further the vasoconstrictor responses to 5-HT in pulmonary hypertensive rats. We studied pulmonary arteries removed from control rats and rats subjected to two weeks of chronic hypoxia (CH). We also investigated the possible contribution of 5-HT_1-like receptors to 5-HT-induced contraction. We have previously shown regional differences in the pharmacology of endothelin receptors in the rat and man (MacLean *et al.*, 1994b; McCulloch *et al.*, 1994). As it is the pulmonary resistance arteries which are the determinants of pulmonary vascular resistance (Staub, 1985), we compared responses to 5-HT in resistance arteries as well as in the larger main pulmonary artery and first pulmonary artery branches.

Intracellular concentrations of the cyclic nucleotides, adenosine 3':5'-cyclic monophosphate ([cyclic AMP]$_i$) and guanosine 3':5' cyclic monophosphate ([cyclic GMP]$_i$) markedly influence expression of 5-HT_1-like receptor-mediated responses in pulmonary arteries (Sweeney *et al.*, 1995). In the present study, we measured [cyclic AMP]$_i$ and [cyclic GMP]$_i$ in pulmonary arteries removed from control and CH rats to assess the possible influence of these on the 5-HT receptor-mediated responses observed.

Increased vascular tone can 'uncover' responses to sumatriptan in bovine pulmonary arteries (MacLean *et al.*, 1994a; Sweeney *et al.*, 1995). In order to investigate the possibility that inherent tone may influence responses to 5-HT receptor activation in the rat pulmonary arteries, we assessed the degree of inherent tone in the vessels under study.

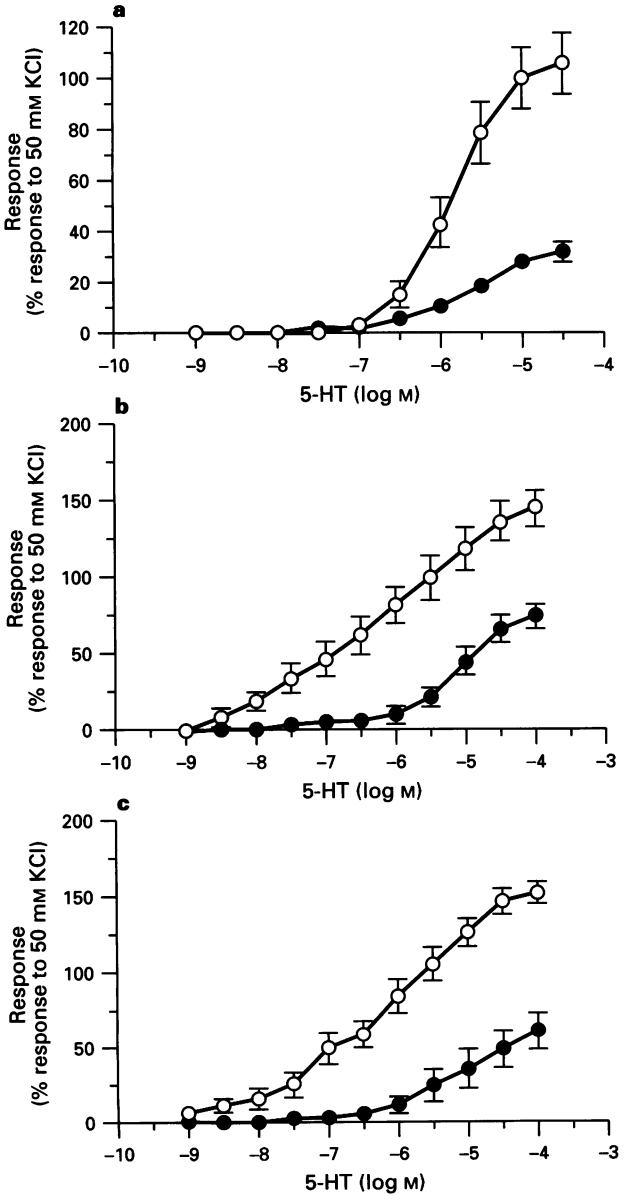

Figure 1 The effect of 2 weeks chronic hypoxia on contractile responses to 5-hydroxytryptamine (5-HT) in rat pulmonary resistance arteries (a), the first pulmonary artery branch (b) and the main pulmonary artery (c). (●). Control rats (a: $n=19$ rings in 12 animals; b: $n=38$ rings in 27 animals; c: $n=11$ rings in 11 animals). (○) Chronic hypoxic rats (a: $n=20$ rings in 13 animals; b: $n=22$ rings in 11 animals; c: $n=5$ rings in 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

Methods

Chronic hypoxic rats

Male Wistar rats of 28–30 days (at start of experiment) were placed in a specially designed perspex hypobaric chamber (Royal Hallamshire Hospital Sheffield). This was depressurised, over two days, to 550 mbar (oxygen concentration reduced to 10%). The temperature of the chamber was maintained at 21–22°C and the chamber was ventilated with air at approximately 45 l min^{-1}. They were maintained in these hypoxic/hypobaric conditions for two weeks. Aged-matched controls were held in room air.

PHT was assessed by measuring the ratio of right ventricular (RV)/total ventricular (TV) weight. The right ventricle was carefully dissected free from the septum and left ventricle and both were blotted lightly and weighed. This is a reliable index of the degree of PHT in rats (Wanstall *et al.*, 1991).

Large isolated pulmonary arteries

Rats were killed by an overdose of sodium pentobarbitone and the lungs removed and placed in cold Krebs. The first pulmonary artery branch (2–3 mm i.d.) and main pulmonary arteries (4–5 mm i.d.) were dissected out and cleaned of the surrounding parenchyma. Arterial rings were mounted between two stainless steel hooks for tension recording. Initial tension was set at that giving optimum contraction (1.5 g) and tissues left to equilibrate in Krebs-bicarbonate solution (composition in mM) NaCl 119, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.1, for 1 h at 37°C. They were bubbled with 16% O_2/6% CO_2 balance N_2. This gave a final bath O_2 concentration (measured with an oxygen electrode and blood-gas analyser) of approximately 120 mmHg and CO_2 tensions of around 35–36 mmHg to give values equivalent to those found *in vivo* given that these vessels are well served by the vasa vasorum. As the walls of these pulmonary arteries typically have media only 200 μm thick, O_2 diffusional problems are not encountered with constant, rapid bubbling (Pittman & Duling, 1973; Fishman, 1976).

Intrapulmonary arteries

Intralobar resistance arteries (~150 μm i.d.) were dissected out and cleaned of the surrounding parenchyma. Pairs (one

control, one from a CH rat) were then mounted as ring preparations (2 mm long) in the same bath of a small vessel myograph. The vessels were bathed in Krebs solution at 37°C and bubbled with 16% O_2/5% CO_2 balance N_2. Vessels were subjected to tension to give transmural pressures equivalent to ~16 mmHg controls – approximately the pressure of pulmonary arteries and arterioles of control animals *in vivo*, and ~34 mmHg CH – approximately the pressure of pulmonary arteries and arterioles of CH animals *in vivo*.

We performed cumulative concentration-response curves (CCRCs) to the agonists 5-hydroxytryptamine (5-HT), 5-carboxamidotryptamine (5-CT, a non-selective 5-HT_1 receptor agonist) and sumatriptan (a selective 5-HT_{1D} receptor agonist) (1 nM–100 μM). Some CCRCs were repeated after 45 min incubation with either ketanserin (1 nM–1 μM, a selective 5-HT_{2A} receptor antagonist), methiothepin (1 nM–1 μM, a non-selective 5-HT_1 and 5-HT_2 receptor antagonist) or the novel 5-HT_{1D} receptor antagonist, GR55562 (0.3 μM–3 μM) (Connor *et al.*, 1995) (See Hoyer *et al.*, 1994 for details of selectivity of other agonists and antagonists).

Assessment of inherent tone

In the large vessels, the vascular endothelium was removed by gentle rubbing with forceps before they were set up. The vascular endothelium of the resistance arteries was left intact as experience dictates that attempts to remove it frequently damage the vascular smooth muscle. Sodium nitroprusside 1 μM was administered before administration of any other agent and

Table 1 Responses to 5-hydroxytryptamine (5-HT), 5-carboximidotryptamine (5-CT) and sumatriptan in pulmonary arteries from control and chronic hypoxic (CH) rats

	pEC_{50} values					
	5-HT		5-CT		Sumatriptan	
Artery	*Controls*	*CH*	*Controls*	*CH*	*Controls*	*CH*
Resistance	5.6±0.1 (19/12)	5.9±0.2 (20/13)***	R (5/5)	5.4±0.2 (5/5)	NR	SR (5/5)
First branch	5.3±0.1 (38/27)†††	6.3±0.2 (22/11)***†††	NR	5.4±0.2 (8/5)	NR	5.7±0.2 (7/5)*
Main	5.0±0.2 (11/11)†††	6.4±0.2 (5/5)***†††	NR	6.0±0.3 (10/10)	NR	NR

Statistical significance of differences determined by ANOVA. CH groups vs. control group: *$P<0.05$, ***$P<0.001$ vs. resistance artery: †††$P<0.001$. NR: no response; SR: small response, EC_{50} not calculated; R: response but EC_{50} not calculated as maximum response not achieved.

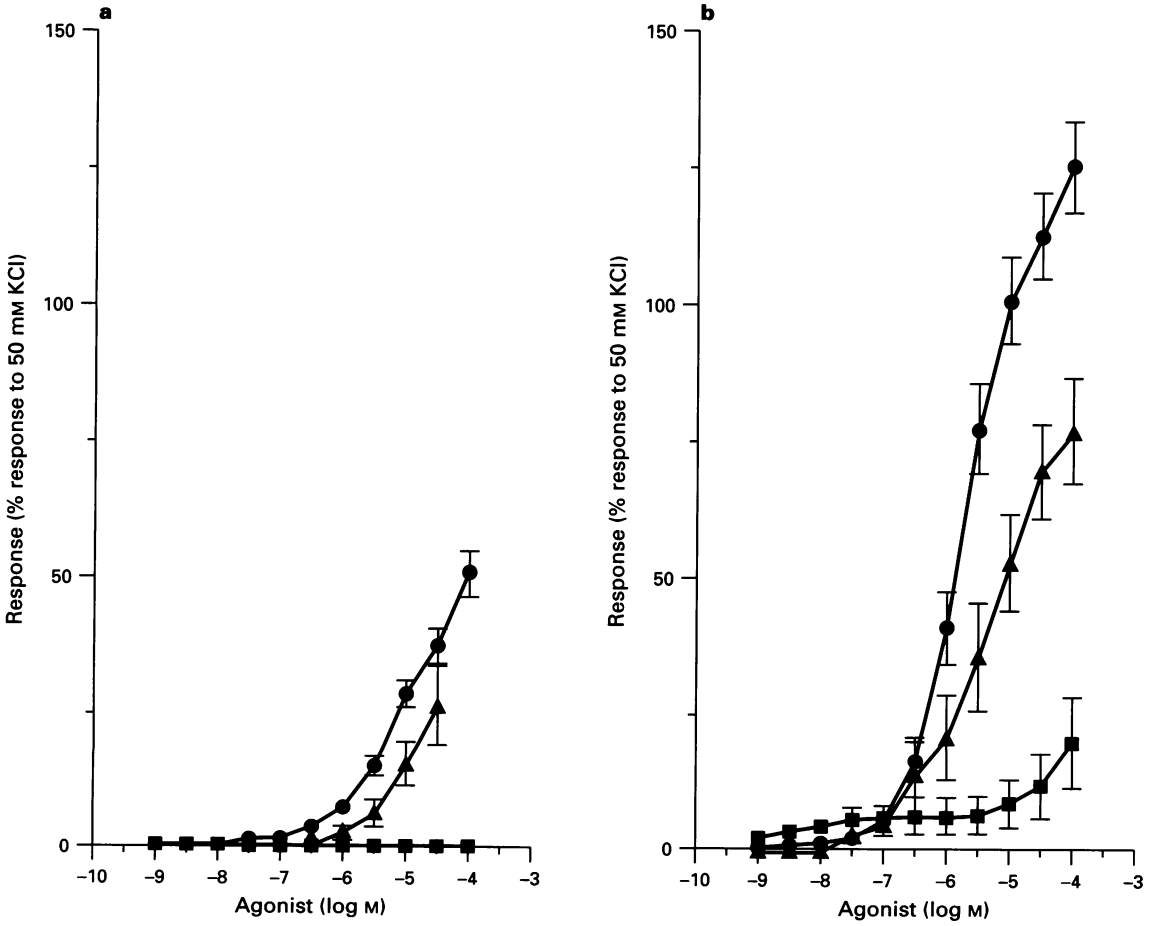

Figure 2 Responses to 5-hydroxytryptamine (●), 5-carboxamidotryptamine (▲) and sumatriptan (■) in rat pulmonary resistance arteries. (a) Control rats (●: $n=5$ rings in 5 animals; ▲: $n=5$ rings in 5 animals; ■: $n=5$ rings in 5 animals). (b) Chronic hypoxic rats (●: $n=5$ rings in 5 animals; ▲: $n=8$ rings in 5 animals; ■: $n=7$ rings in 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

the degree of relaxation assessed as a percentage of a subsequent response to 50 mM KCl after washing out the sodium nitroprusside.

Assay of intracellular cyclic nucleotide concentrations

Rat pulmonary artery branches (2–3 mm i.d.), main pulmonary arteries (4–5 mm i.d.) and intrapulmonary arteries (0.2–2 mm i.d.) were dissected free of parenchymal tissue, weighed then gassed at 37°C with 16% O_2/5% CO_2 for 1 h before use. Tissues were rapidly frozen in liquid N_2 before being homogenised in 4% perchloric acid and then left for 1 h at 4°C. After they had been sonicated for 15 min, samples were centrifuged at 3000 r.p.m. for 10 min and the supernatant neutralised with KOH. Intracellular cyclic AMP concentration was determined by a modification of the procedure described by Brown *et al.* (1972). Briefly, samples were incubated with a fixed amount of [^{3}H]-cyclic AMP ($\sim$500,000 c.p.m. ml^{-1}) and cyclic AMP binding protein (partially purified regulatory subunit of A kinase) in 50 mM Tris.HCl (pH 7.4), 4 mM EDTA buffer. A standard curve was constructed with dilutions of unlabelled cyclic AMP ranging from 0–320 pmol ml^{-1}, allowing cyclic nucleotide concentrations within this range to be accurately assessed. After a two hour incubation to allow the reaction mixture to reach equilibrium, unbound cyclic AMP was precipitated using a solution of 2% (w/v) activated charcoal and 1% (w/v) BSA. [^{3}H]-cyclic AMP remaining in the supernatant was assessed by liquid scintillation counting. A similar technique was applied to cyclic GMP determination. Results for various samples are corrected according to the original wet weight of tissue used.

Drugs

5-Hydroxytryptamine creatinine sulphate (Sigma), ketanserin bitartrate (Roth), methiothepin maleate (Hoffman La Roche), 5-carboxamidotryptamine maleate (Semat), sumatriptan (GR 43175, Glaxo Group Research) and GR55562 (Glaxo Group Research, 3-[3-(dimethylamino)propyl]-4-hydroxy-N-[4-(4-pyridinyl)phenyl]benzamide) were all dissolved in distilled water.

Analysis of results

pEC_{50} values were calculated by BBC microcomputer graphical interpolation from individual CCRCs in which responses were assessed as a percentage of the maximum response. Responses were also expressed as a percentage of the response to 50 mM KCl to illustrate changes in the maximum responses achieved. Statistical comparisons of the means of groups of data were made by one-way analysis of variance (ANOVA) unless otherwise stated. Wherever possible, when maximum responses were achieved in the presence of antagonist, estimated pK_b values were calculated for single stated concentrations of the antagonists assuming $-\log K_b = pA_2$. Unless stated otherwise, dose-ratios were calculated by use of the pEC_{50} values.

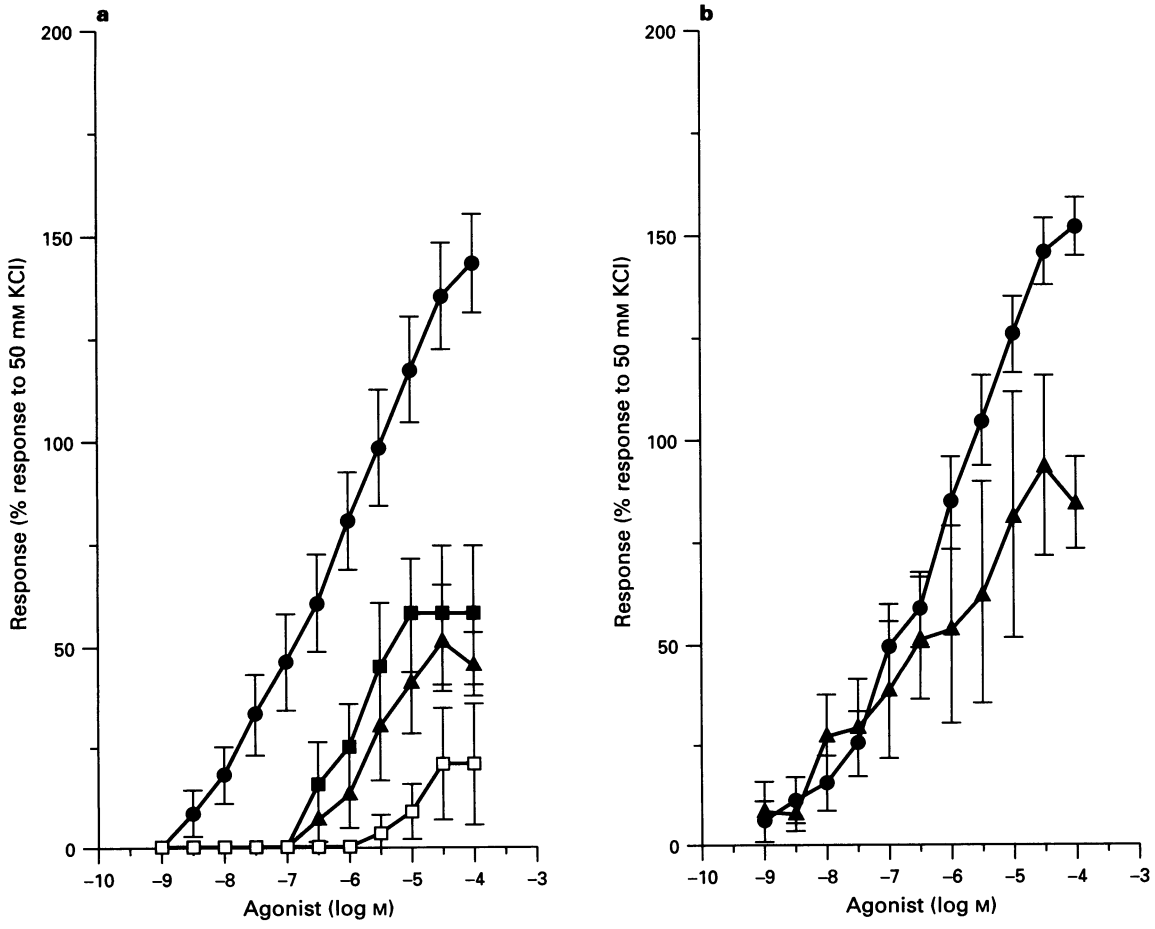

Figure 3 Responses to 5-hydroxytryptamine (●), 5-carboxamidotryptamine (▲) and sumatriptan (■) in chronic hypoxic rat pulmonary arteries. (□) Sumatriptan in the presence of 3 μM GR55562. (a) First pulmonary artery branch (●: $n=22$ rings in 11 animals; ▲: $n=8$ rings in 5 animals; ■: $n=13$ rings in 13 animals; □: $n=6$ rings in 6 animals). (b) Main pulmonary artery (●: $n=5$ rings in 5 animals; ▲: $n=10$ rings in 10 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

Results

Assessment of PHT

The RV/TV ratio of the control rats was 0.247 ± 0.006 and of the CH rats it was 0.393 ± 0.006 ($P > 0.001$; unpaired Student's t test, $n = 12$), indicating development of severe PHT.

Responses to KCl

In the main pulmonary arteries, 50 mM KCl-induced contractions were of the same magnitude in both the control and CH rats, being 748 ± 52 mg wt (24 rings in 24 animals) and 633 ± 55 mg wt (24 rings in 24 animals), respectively. Similarly, 50 mM KCl-induced contractions were of the same magnitude in the pulmonary artery branches from both the control and CH rats, being 461 ± 28 mg wt (12 rings from 11 animals) and 459 ± 29 mg wt (14 rings in 9 animals), respectively. In the pulmonary resistance arteries, 50 mM KCl-induced contractions were also of the same magnitude in both the control and CH rats, being 235 ± 29 mg wt (16 rings in 8 animals) and 264 ± 33 mg wt (16 rings in 8 animals), respectively.

Responses to 5-HT

Figure 1 compares responses to 5-HT in the pulmonary arteries studied and Table 1 summarises the pEC_{50} values. Figure 1a–c shows that exposure to chronic hypoxia markedly in-

Table 2 Effects of, and estimated pA_2 values for, ketanserin, methiothepin and GR55562 against 5-hydroxytryptamine (5-HT) in pulmonary arteries from control and chronic hypoxic (CH) rats

	Estimated pK_b values					
	Ketanserin		*Methiothepin*		*GR 55562*	
Artery	*Controls*	*CH*	*Controls*	*CH*	*Controls*	*CH*
Resistance	~8.3 (–7; 5/5)	7.7±0.1 (–7; 5/5)	–	–	NS	6.5±0.2 (–6; 5/5)
First branch	7.8±0.1 (–7; 7/5)	8.3±0.2 (–7; 9/5)	7.8±0.2 (–7; 8/5)	7±0.2 (–7; 5/4)	NS	7.8±0.4 (–6.5; 6/4)*
Main	9.2±0.2 (–9; 4/4)	9.6±0.1 (–7; 5/5)	TB	9.5 (–9; 5/5)	NS	7.0±0.1 (–6; 4/4)

Concentrations of antagonist used to estimate pK_b values; number of rings/number of rats are shown in parentheses. TB: total block of resopnse at all concentrations tested; NS: no significant change in response at EC_{50} value. *pK_b calculated by use of pEC_{10} value.

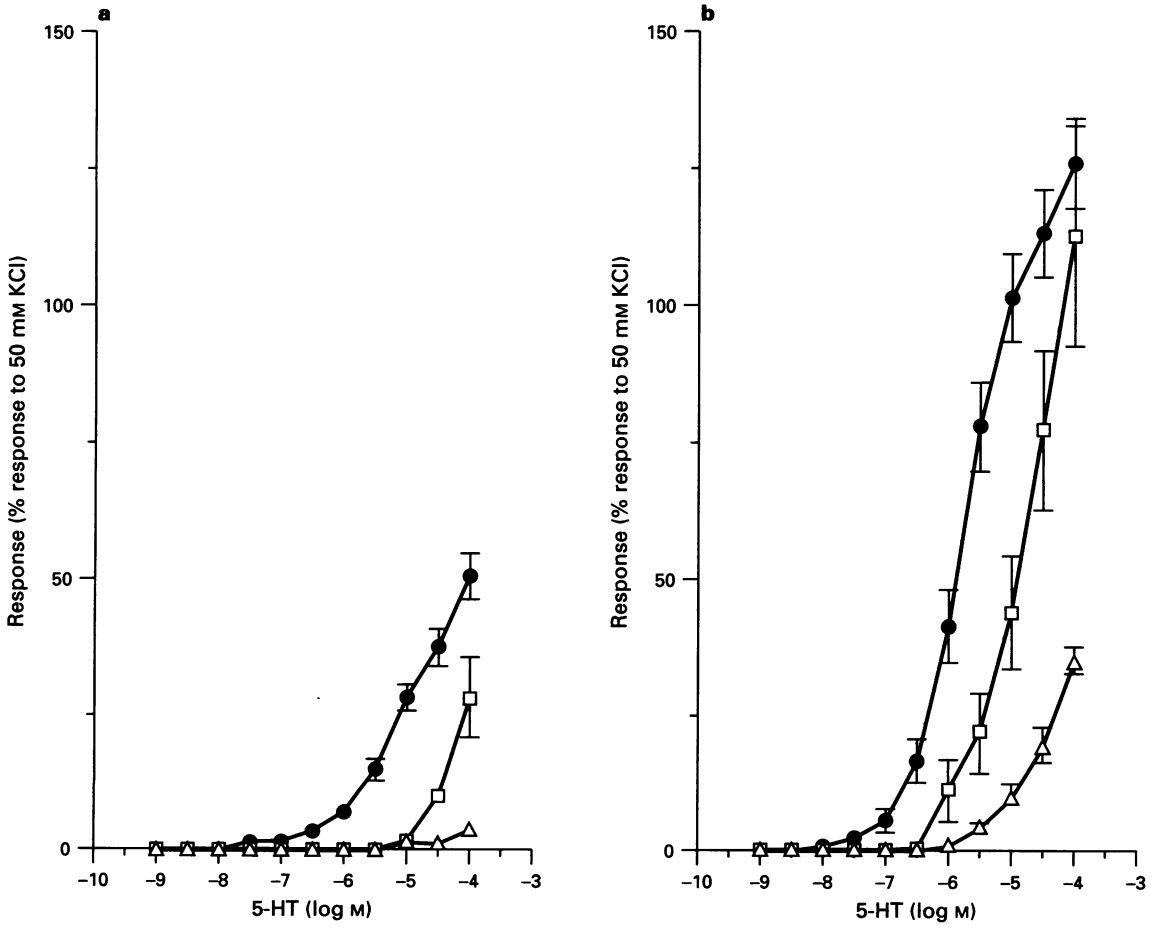

Figure 4 Effect of ketanserin on responses to 5-hydroxytryptamine (5-HT) in rat pulmonary resistance arteries from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (□) in the presence of 0.1 μM ketanserin (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (△) in the presence of 1 μM ketanserin (a: 5 rings from 5 animals; b: 5 rings from 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

creased the maximum response to 5-HT in all the vessels tested. Table 1 and Figure 1 show that, in control animals, sensitivity to 5-HT was in the following order: resistance arteries > first branch = main pulmonary artery and maximum responses were first branch = main pulmonary artery > resistance artery. In the CH rats the orders were, sensitivity: first branch = main pulmonary artery > resistance artery, maximum response: first branch = main pulmonary artery ≥ resistance artery.

Comparison of responses to 5-HT agonists

Resistance arteries Figure 2 compares responses to 5-HT, sumatriptan and 5-CT in the pulmonary resistance arteries from control and CH rats. The pEC_{50} values are summarised in Table 1. The results show that 5-CT alone contracted the control vessels and responses to 5-CT were markedly potentiated in the CH rats. Small responses to sumatriptan were also apparent in the CH rat vessels. The maximum response achieved to 5-CT was only 60% of that to 5-HT although the 5-HT and 5-CT were equipotent.

Large pulmonary arteries Neither 5-CT nor sumatriptan contracted the first branch or main pulmonary artery from control rats. Figure 3 compares responses to 5-HT, sumatriptan and 5-CT in the first branch and main pulmonary artery from CH rats. The pEC_{50} values are summarised in Table 1. Both sumatriptan and 5-CT contracted the first branch although maximum responses achieved were only ~30% those to 5-HT. The responses to sumatriptan were sensitive to GR55562 (Figure 3a). 5-CT also contracted the main pulmonary artery from the CH rats with a maximum response some 65% of that to 5-HT itself.

Effects of 5-HT receptor antagonists

Table 2 summarises the estimated pK_b values of the antagonists where these could be estimated (when a maximum response to 5-HT was obtained in the presence of antagonist).

Resistance artery Ketanserin antagonised responses to 5-HT in both control and CH rats resistance arteries with estimated pK_b values of ~8.3 and 7.7, respectively (see Figure 4). Figure 5 shows that GR55562 increased the threshold concentration for contraction of 5-HT in the control arteries, i.e. in the absence of GR55562, 0.1–1 μM 5-HT induced contractions, whilst in the presence of GR55562, significant contractions were not observed until >1 μM. GR55562 did not have a significant effect at the EC_{50} level in the control vessels but did inhibit responses in the CH rats with an estimated pK_b value of 6.5.

Pulmonary artery first branch Figure 6 shows that ketanserin antagonised responses to 5-HT in controls and CH rat

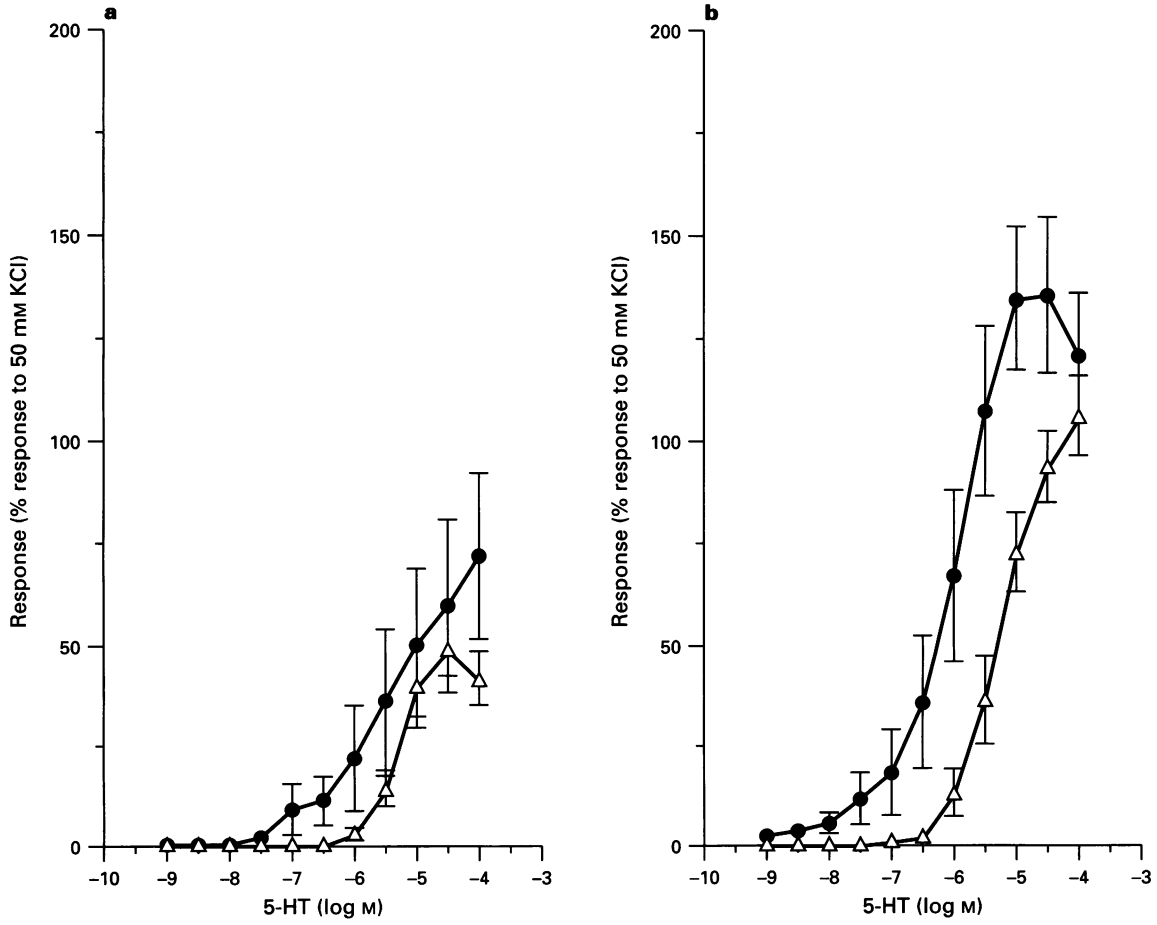

Figure 5 Effect of GR55562 on responses to 5-hydroxytryptamine (5-HT) in rat pulmonary resistance arteries from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (△) in the presence of 1 μM GR55562 (a: 5 rings from 5 animals; b: 5 rings from 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

pulmonary artery branches. Table 2 shows that the estimated pK_b values were 7.8 and 8.3, respectively. Figure 7 and Table 2 show that methiothepin also antagonized responses in the control and CH rats with estimated pK_b values of 7.5 and 7, respectively. Methiothepin 1 μM can be seen to have a non-competitive effect on responses to 5-HT in the vessels from the CH rats reflecting the non-selective nature of this compound at high concentrations. Figure 8 shows that GR55562 did not inhibit responses to 5-HT in control rats. In the CH rats, it inhibited responses only at the EC_{10} level where the estimated pK_b value was 7.8.

Main pulmonary artery Figure 9 shows that ketanserin antagonised responses to 5-HT in the controls and CH rat and Table 2 shows that the estimated pK_b values were 9.2 and 9.6, respectively. Figure 10 and Table 2 show that methiothepin (1–100 nM) totally blocked responses to 5-HT in controls and antagonized responses in the CH rats with an estimated pK_b value of 9.5. Figure 11 and Table 2 show that 0.3 μM GR55562 had no effect on responses to 5-HT in control rats (higher concentrations had no effect) whilst GR55562 antagonized responses in the CH rats with an estimated pK_b value of 7. Figure 11 shows that the effect of GR55562 was again greatest at the lowest concentrations of 5-HT. We were unable, however, to assess accurately the competitive nature of this antagonist on higher concentrations of 5-HT due to the high experimental concentrations of 5-HT that would be required.

Inherent tone

Figure 12 shows that there is a degree of inherent tone present in all the vessels from control rats although this is negligible in the resistance arteries. It also shows that the degree of inherent tone significantly increases in the vessels removed from the CH rats. The degree of inherent tone is considerable in the pulmonary artery first branch and main pulmonary artery whilst it is still small in the resistance arteries.

Intracellular cyclic nucleotide concentrations

Figure 13 illustrates changes in [cyclic AMP]$_i$ and [cyclic GMP]$_i$ in pulmonary arteries removed from control and CH rats. It shows that neither [cyclic AMP]$_i$ nor [cyclic GMP]$_i$ was changed by chronic hypoxia in the resistance arteries. There was, however, a decrease in both [cyclic AMP]$_i$ and [cyclic GMP]$_i$ in the first branch and main pulmonary arteries.

Discussion

There was a 60% increase in the RV/TV ratio of the CH rats indicating development of severe PHT in the rats used in this study (see MacLean *et al.*, 1995 for a complete characterization of this model).

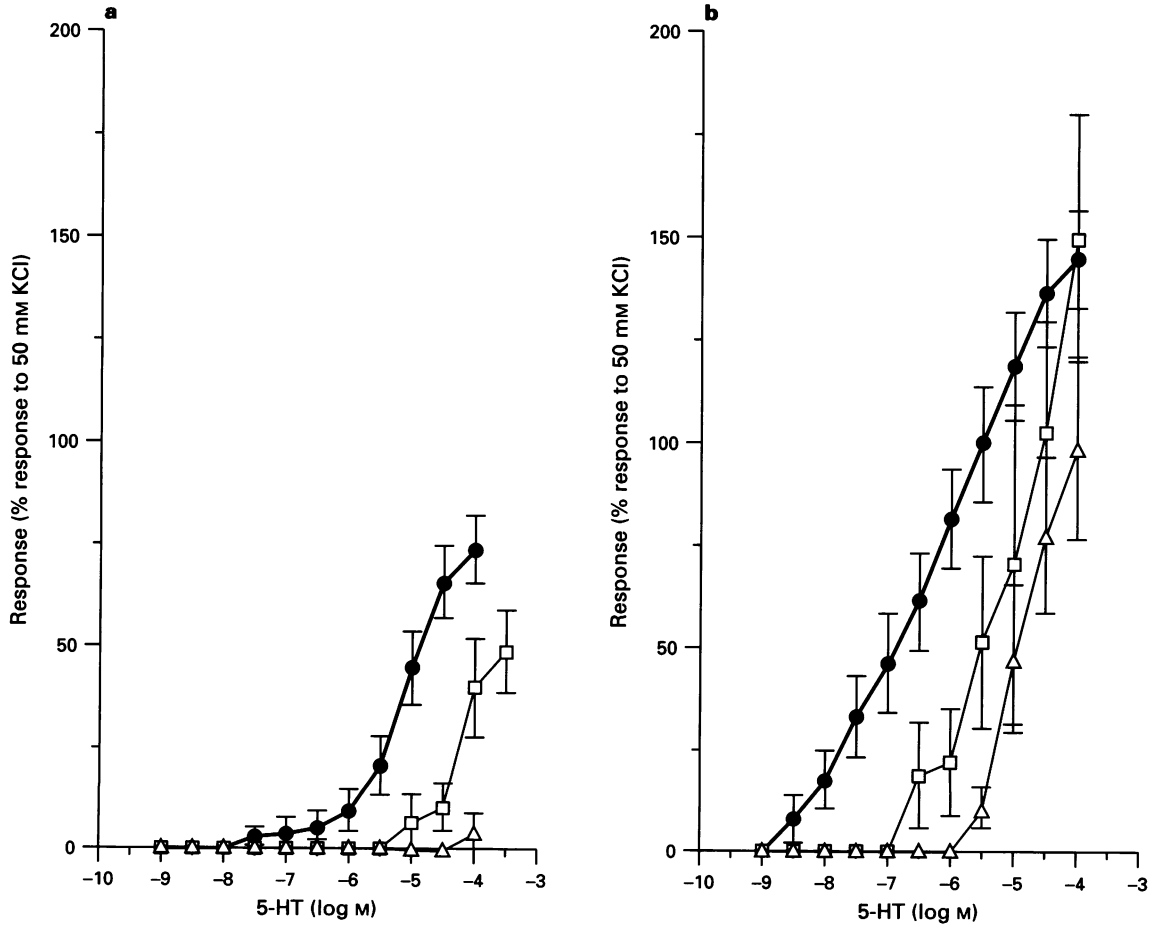

Figure 6 Effect of ketanserin on responses to 5-hydroxytryptamine (5-HT) in rat first pulmonary artery branches from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (□) in the presence of 0.1 μM ketanserin (a: 7 rings from 5 animals; b: 9 rings from 5 animals); (△) in the presence of 1 μM ketanserin (a: 5 rings from 4 animals; b: 6 rings from 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

Effect of chronic hypoxia on responses to 5-HT

The results show that responses to 5-HT were observed in all the vessels tested from the control animals, 5-HT being most potent in the pulmonary resistance arteries. Chronic hypoxia enhanced the maximum responses to, and sensitivity of, 5-HT at all levels of the circulation. The effect on sensitivity was greatest in the first pulmonary branch and the main pulmonary artery so that, in the CH rat, sensitivity to 5-HT was now least potent in the pulmonary resistance arteries. Pulmonary arteries from pulmonary hypertensive rats demonstrate media hypertrophy (Heath *et al.*, 1973; Hunter *et al.*, 1974). However, we do not believe that this can be responsible alone for the increased responses to 5-HT. Responses to 50 mM KCl were not increased in any of the vessels studied and we have shown that not all agonist responses are increased in these vessels. For example, maximum responses to endothelin-1 (ET-1) are not increased in CH rat capacitance pulmonary arteries (MacLean *et al.*, 1995).

Similar pulmonary arterial hyperreactivity to 5-HT has been observed in rats with PHT secondary to monocrotaline injection (Kanai *et al.*, 1993), secondary to a platelet 5-HT storage disorder (Ashmore *et al*, 1991) and in isolated pulmonary arteries from patients with primary PHT (Brink *et al.*, 1988). Such hypersensitivity is associated with elevations in plasma 5-HT and is thought to be associated with abnormalities in platelet 5-HT storage (Hervé *et al.*, 1995). There is no evidence for an elevation of plasma [5-HT] in the CH model of pulmonary hypertension (Oka *et al.*, 1993) and these rats are not known to exhibit abnormalities in platelet 5-HT storage. The increased response to 5-HT may be due to receptor up-regulation. Unfortunately it is not possible to demonstrate specific binding of 5-HT ligands in these arteries and we are currently investigating this possibility further at the molecular level.

Characterization of 5-HT receptors

Resistance arteries 5-CT contracted both the control and CH vessels, whilst sumatriptan only contracted the vessels removed from CH rats. In the control rats, GR55562 increased the 5-HT threshold for contraction without having a significant effect on the pEC_{50} values of 5-HT. In the CH rats, the order of potency was 5-HT = 5-CT > > sumatriptan. In the rat, two 5-HT_1 receptors have been identified, the r5-HT_{1D} receptor and the r5-HT_{1B} receptor (Hartig *et al.*, 1996). Hoyer *et al.* (1994) describe how 5-CT does have a greater potency at the r5-HT_{1B} receptor than sumatriptan which suggests that a population of r5-HT_{1B} receptors may be present in the pulmonary resistance artery. This is confirmed by the observed effect of the selective 5-HT_{1D} receptor antagonist GR55562. In the CH rats GR55562 antagonised responses to 5-HT. GR55562 has a pA_2 value of 7.9 against sumatriptan-induced contractions of the dog and monkey isolated basilar artery (Connor *et al.*, 1995). In the dog saphenous vein it has a pA_2 of 7.6 and radioligand binding studies have shown pK_i values of 5.3, 5.9, 7.6 for 5-HT_{1A}, r5-HT_{1B} (brain) and 5-HT_{1D} receptors (H.Connor,

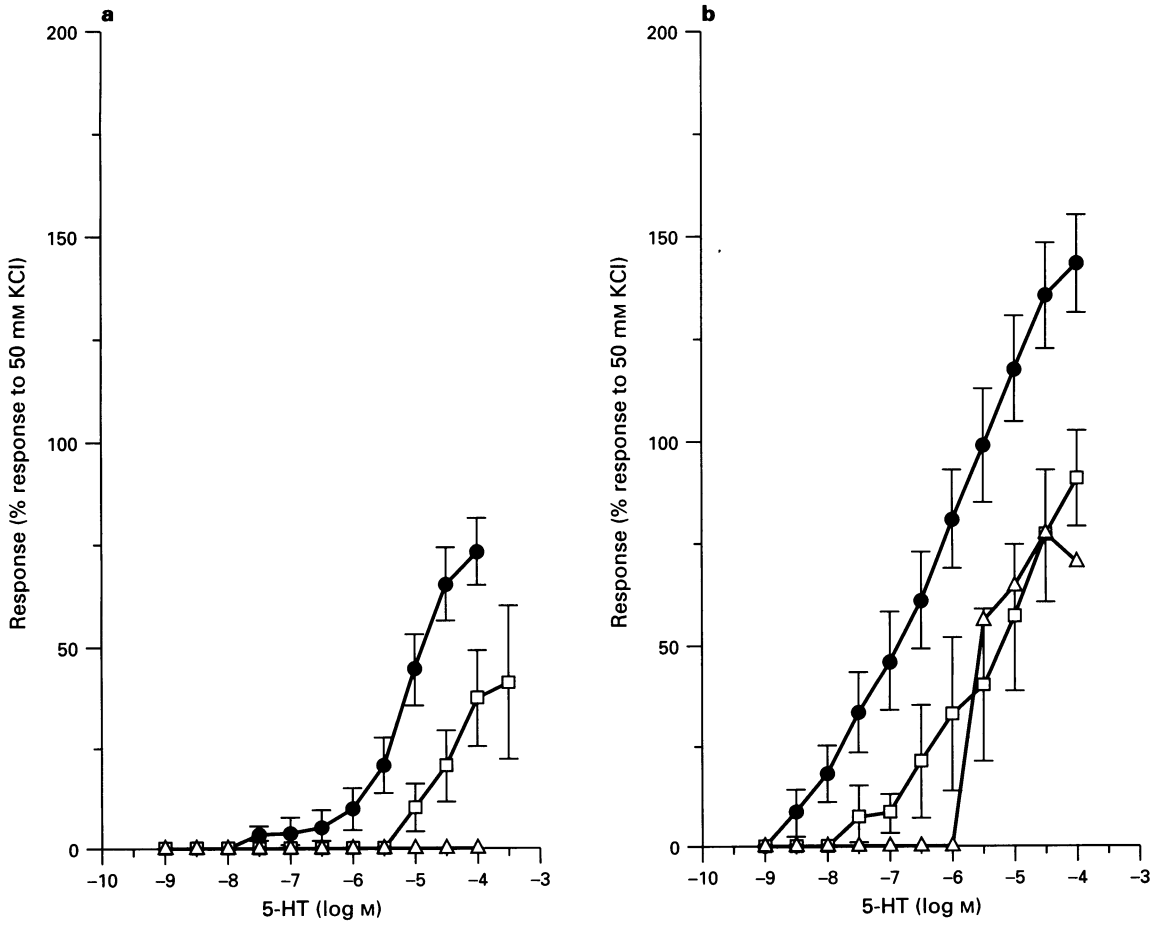

Figure 7 Effect of methiothepin on responses to 5-hydroxytryptamine (5-HT) in rat first branch pulmonary arteries from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 11 rings from 11 animals; b: 5 rings from 5 animals); (□) in the presence of 0.1 μM methiothepin (a: 8 rings from 5 animals; b: 5 rings from 4 animals); (△) in the presence of 1 μM methiothepin (a: 5 rings from 5 animals; b: 5 rings from 4 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

personal communication). We have recently demonstrated that GR55562 is a competitive antagonist against the 5-HT_{1D}-like receptor in human pulmonary arteries with a pA_2 of 8.88 (MacLean *et al.*, 1996). Here we show that GR55562 has a pK_b of 6.5 against the r5-HT_{1B}-like receptor present in the rat pulmonary resistance artery of CH rats. The 5-HT_{2A} receptor antagonist, ketanserin also caused a competitive shift of the 5-HT response in both control and CH animals (pK_b: 7.7–8.3). This suggests the presence of the 5-HT_{2A} receptor in this preparation (Hoyer *et al.*, 1994). These results therefore suggest that, in control pulmonary resistance arteries, the 5-HT response is mediated mainly by the 5-HT_{2A} receptor although the presence of a relatively small population of 5-HT_{1B}-like receptors cannot be ruled out as 5-CT did contract these vessels and this may explain the relatively shallow slopes of the 5-HT CCRCs and the effect of GR55562 on low concentrations of 5-HT. As discussed, GR55562 did cause an increase in the threshold concentration of 5-HT, suggesting an influence of 5-HT_{1B}-like receptors at low concentrations of 5-HT. Sumatriptan, however, did not cause a contraction in the control rat vessels whilst 5-CT did. A similar observation was made in the main pulmonary artery which is discussed in detail below. These observations may be explained by the relatively low potency of sumatriptan at the r5-HT_{1B} receptor compared with 5-CT (Hoyer *et al.*, 1994). We have previously shown that increased agonist-induced vascular tone can 'uncover' responses to 5-HT_{1D}-like receptors (MacLean *et al.*, 1994a; Sweeney *et al.*, 1995). Therefore, an alternative explanation for an effect of GR55562 on 5-HT-induced vasoconstriction is that 5-HT_{1B}-like receptor-induced responses are facilitated by synergistic vasoconstriction induced by simultaneous 5-HT_{2A} receptor activation.

Responses to 5-HT were markedly increased in the CH rat resistance arteries and this was due to the enhanced, and combined, effect of 5-HT_{2A} receptors and r5-HT_{1B}-like receptor stimulation.

Pulmonary artery first branch In control rats, ketanserin and methiothepin antagonised responses to 5-HT in these vessels with a pK_b value of 7.8. Ketanserin and methiothepin typically exhibit a pA_2 of ~8.5–9 against 5-HT_{2A} receptors (Hoyer *et al.*, 1994). Ketanserin is inactive against r5-HT_{1B} receptors whilst methiothepin typically exhibits a pA_2 of ~8.1 against the 5-HT_{1B} receptor. The effects of both antagonists observed here provide evidence for a 5-HT_{2A} receptor but do not exclude the possibility that methiothepin may be antagonizing a 5-HT_{1B}-like receptor. GR55562, however, had no effect on responses to 5-HT and neither 5-CT nor sumatriptan contracted these vessels. This clearly suggests that, in control rats, in the first pulmonary artery branch, responses to 5-HT are mediated by 5-HT_{2A} receptors. In contrast to the control rats, pulmonary artery branches removed from CH rats contracted in response to both 5-CT and sumatriptan. These were equipotent with a maximum contraction ~30% of that to 5-HT. The response to sumatriptan was sensitive to GR55562. This suggests the presence of a small population of r5-HT_{1B}-like receptors in these vessels. 5-HT-induced contraction was sensitive to both ketanserin and methiothepin (estimated pK_b

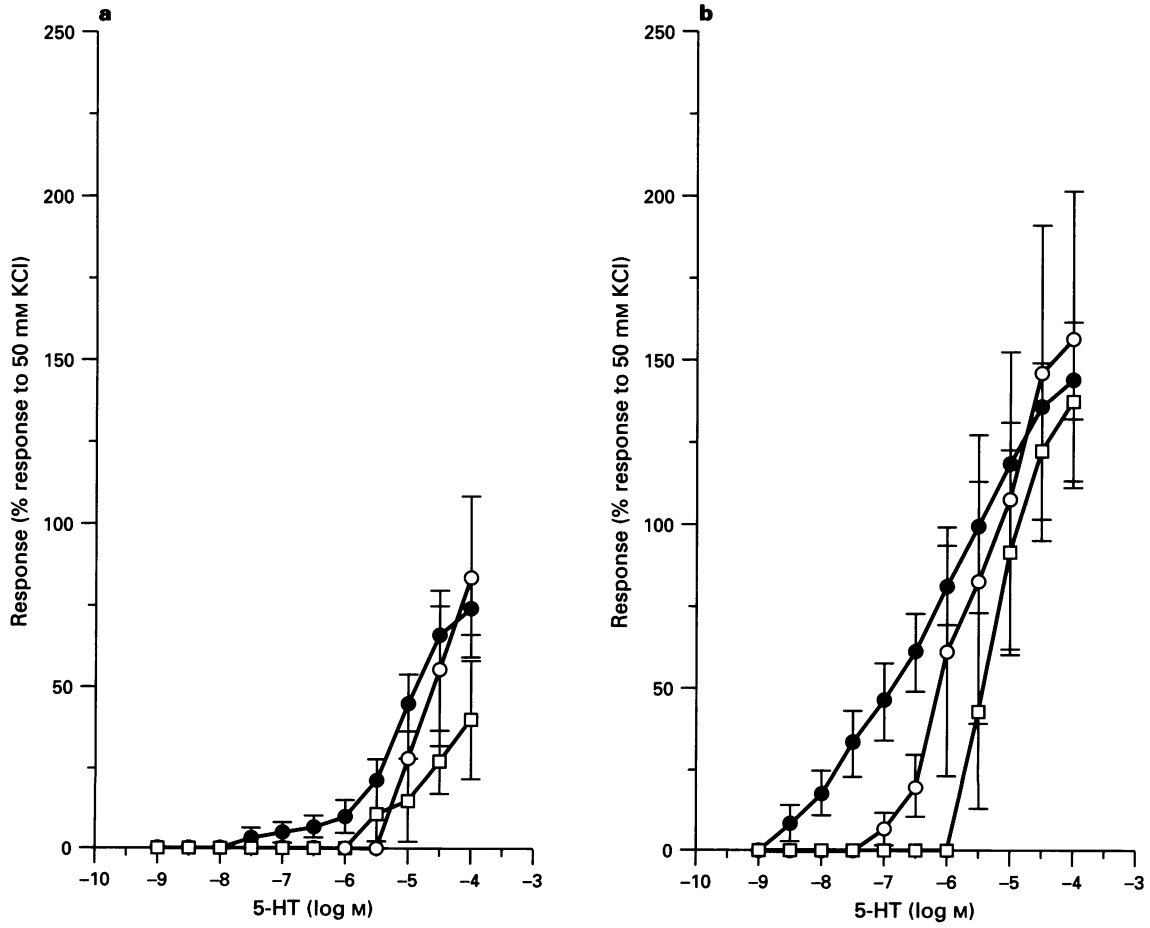

Figure 8 Effect of GR55562 on responses to 5-hydroxytryptamine (5-HT) in rat first pulmonary artery branches from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (○) in the presence of 0.3 μM GR55562 (a: 5 rings from 4 animals; b: 6 rings from 4 animals); (□) in the presence of 3 μM GR55562 (a: 5 rings from 4 animals; b: 6 rings from 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

values: 8.3 and 7.0, respectively). GR55562 inhibited responses to 5-HT but only at the pEC_{10} level, showing an estimated pK_b of 7.8. These results suggest that the increased response to 5-HT observed in these vessels is due to an increase in a 5-HT_{2A}-mediated response and also contributed to by activation of a small population of r5-HT_{1B}-like receptors at lower concentrations of 5-HT.

Main pulmonary artery In the control rats, both ketanserin and methiothepin were potent antagonists against the 5-HT response in these vessels (pK_b for ketanserin: 9.2). GR55562 has no significant effect and neither sumatriptan nor 5-CT contracted these vessels. This suggests that the response to 5-HT is mediated via the 5-HT_{2A} receptor in the main pulmonary artery of control rats. In the CH rats, ketanserin and methiothepin both antagonized responses to 5-HT with estimated pK_b values of 9.6 and 9.5, respectively. GR55562 did, however, inhibit responses to 5-HT with an estimated pK_b (1 μM GR55562) of $\sim$7 and 5-CT (but not sumatriptan) also contracted these vessels. This suggests the presence of a r5-HT_{1B}-like receptor in this preparation. The increased response to 5-HT in the main pulmonary artery from CH rats is therefore a consequence of an increase in 5-HT_{2A} and r5-HT_{1B}-like receptor stimulation.

From Figure 1 it can be seen that the CCRCs to 5-HT in the CH rat pulmonary first branch and main pulmonary artery are extremely shallow. The presence of more than one receptor subtype in these vessels may explain this observation.

It can be seen from Table 2 that there is a variation in the pA_2 values of GR55562 in the different arterial segments. This may indicate that it is not acting at a single receptor population and more than one 5-HT_{1B}-like receptor may be active and affected by GR55562 in this preparation. Evidence for a vascular 5-HT_{1B} receptor has been comparatively recent (eg. Craig & Martin, 1993). The effectiveness of GR55562 against the vascular r5-HT_{1B} receptor has, therefore, not yet been studied extensively. Future studies will be required to characterize the selectivity of this antagonist against vascular r5-HT_{1B}-like receptors. A possible synergistic effect of 5-HT_{2A} receptor stimulation has been discussed. An alternative explanation, therefore, is that different degrees of synergy with 5-HT_{2A} receptor stimulation may exist in the different arterial segments which would influence the potency of the antagonist. In addition, the actual phenotype of the pulmonary vascular smooth muscle is different throughout the pulmonary arterial tree and therefore ligand-receptor interactions may be affected (eg. Meyrick & Reid, 1978; Frid *et al.*, 1994; Sasaki *et al.*, 1995).

Effects of pulmonary hypertension on pulmonary arterial vascular tone and cyclic nucleotides

We have previously shown that raised vascular tone 'uncovers' 5-HT_{1D}-like responses in bovine pulmonary arteries (MacLean *et al.*, 1994a; Sweeney *et al.*, 1995). r5-HT_{1B} receptors have

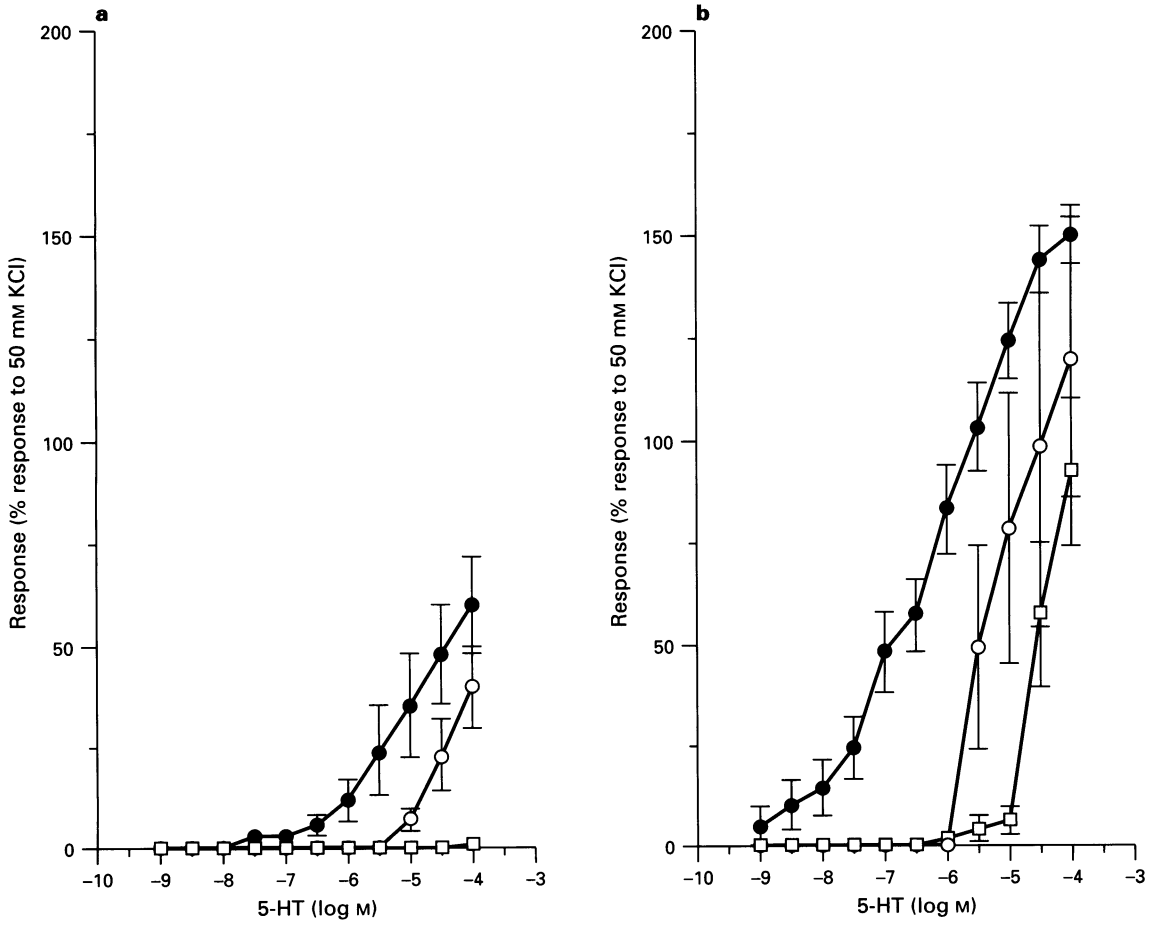

Figure 9 Effect of ketanserin on responses to 5-hydroxytryptamine (5-HT) in rat main pulmonary arteries from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (○) in the presence of 1 nM ketanserin (a: 4 rings from 4 animals; b: 7 rings from 5 animals); (□) in the presence of 0.1 μM ketanserin (a: 4 rings from 4 animals; b: 5 rings from 5 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

been shown to mediate contractile responses to 5-HT in the rat caudal artery but also only in partially contracted arteries (Craig & Martin, 1993). Given the increased contribution of the r5-HT_{1B}-mediated responses we observed in the CH rats in this study, we were interested in determining if an increase in endogenous vascular tone may have contributed to this effect. The results did indeed show that there was evidence for endogenous vascular tone in the pulmonary branches and main pulmonary arteries from control rats and that this was markedly potentiated in the CH rats. There was only a very small degree of endogenous tone in the pulmonary resistance arteries from control rats but this was also potentiated in the CH rats. The increased inherent tone is probably related to the vascular remodelling processes and related intracellular homeostatic changes that occur (Heath *et al.*, 1973; Hunter *et al.*, 1974). The observed increase in tone, combined with the increased contribution of r5-HT_{1B} receptors is in line with the theory that we and others have suggested, that increased vascular tone enhances the ability of 5-HT_1-like receptors to mediate vasoconstriction (Craig & Martin, 1993; MacLean *et al.*, 1994a; Sweeney *et al.*, 1995).

5-HT_1 receptors induce contraction through negative coupling with adenylate cyclase and a decrease in cyclic AMP (Sumner & Humphrey, 1990). In bovine pulmonary arteries, we have demonstrated that cyclic nucleotide levels influence the expression of 5-HT_{1D}-like receptors in that lowering [cyclic GMP]$_i$ (by nitric oxide synthase (NOS) inhibition) potentiates contraction to sumatriptan whilst elevating [cyclic GMP]$_i$ (with sodium nitroprusside) inhibits responses to sumatriptan (MacLean 1994a; Sweeney *et al.*, 1995). Elevation of [cyclic AMP]$_i$ can facilitate responses to sumatriptan although the effect of an increase in [cyclic GMP]$_i$ can override any effects of decreased [cyclic AMP]$_i$ (Sweeney *et al.*, 1995). We were interested, therefore, to determine if a combination of increased vascular tone and decreased [cyclic GMP]$_i$ may have contributed to the 5-HT_1-receptor mediated response we observed in the CH rat pulmonary arteries. The results demonstrate that [cyclic GMP]$_i$ was decreased in the pulmonary artery branch and the main pulmonary artery. This, combined with the increased endogenous tone, may well, therefore have contributed to the increased contribution of the 5-HT response by 5-HT_1 receptor stimulation in these vessels. Whilst [cyclic AMP]$_i$ was also decreased the effect of the decrease in [cyclic GMP]$_i$ would have overridden the possible effects this might have had independently (Sweeney *et al.*, 1995). The origin of the decreased [cyclic GMP]$_i$ in the large vessels may be due to overall changes in NOS activity in these vessels. A decrease in [cyclic GMP]$_i$ has been shown to occur in the main pulmonary arteries removed from rats subjected to prolonged hypoxia for seven days and this was assumed to indicate decreased endothelial NO production (Shaul *et al.*, 1993). However, pulmonary NO production has been shown to increase from the lungs of chronically hypoxic rats (Isaacson *et al.*, 1994) whilst Xue *et al.* (1994) demonstrated an increase in NOS activity in large pulmonary arteries from CH rats. In the latter study, however, it was the inducible, soluble NOS that was upregulated and so it is possible that

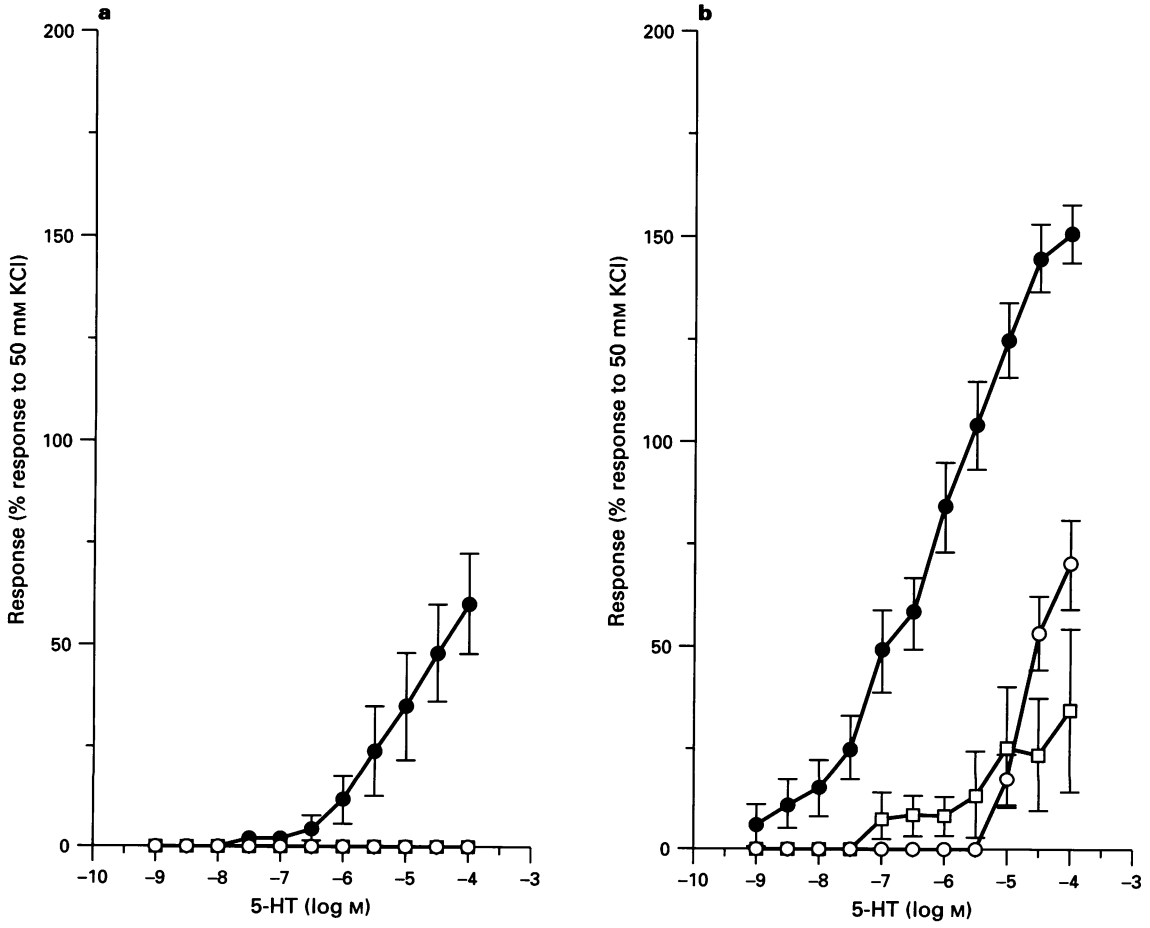

Figure 10 Effect of methiothepin on responses to 5-hydroxytryptamine (5-HT) in rat main pulmonary arteries from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (○) in the presence of 1 nM methiothepin (a: 5 rings from 5 animals; b: 5 rings from 5 animals); (□) in the presence of 0.1 μM methiothepin (a: 7 rings from 5 animals; b: 4 rings from 4 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

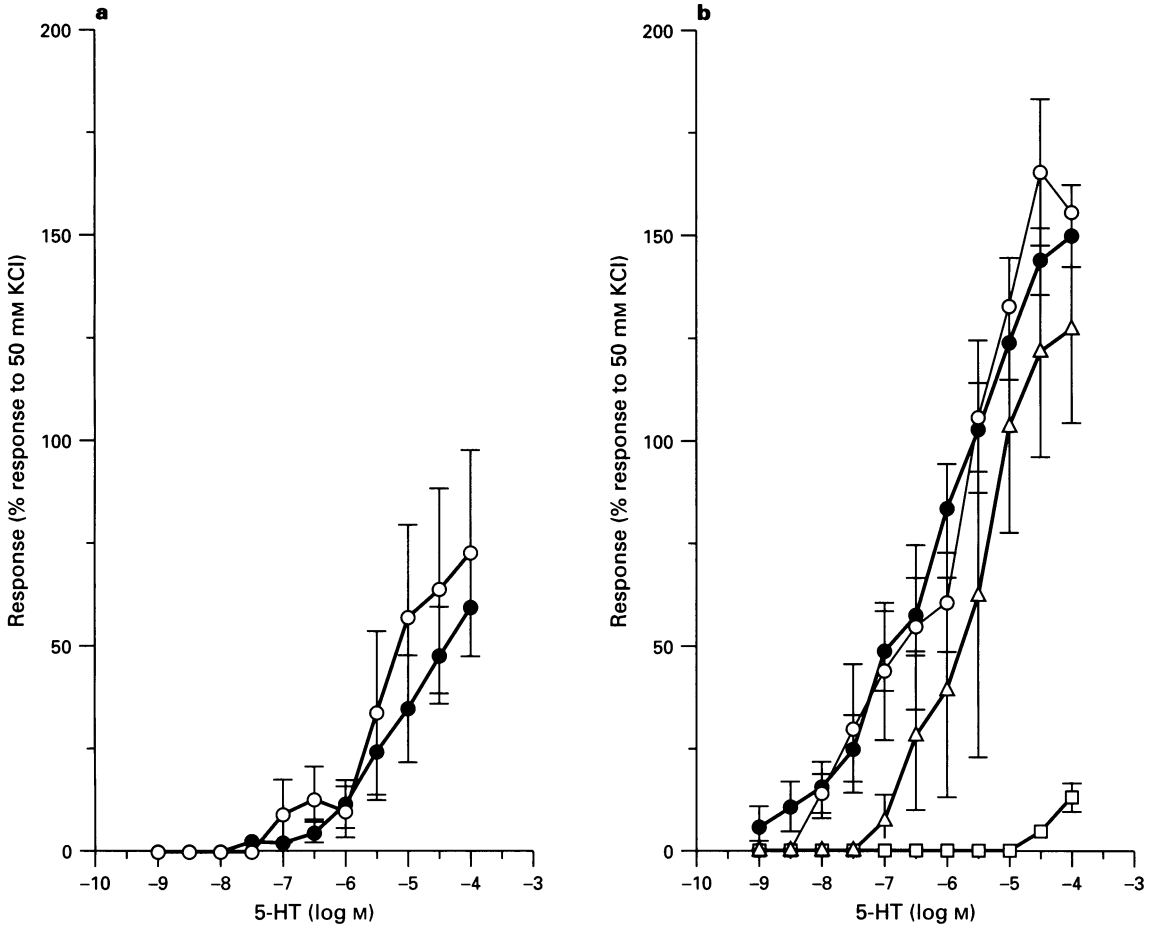

Figure 11 Effect of GR55562 on responses to 5-hydroxytryptamine (5-HT) in rat main pulmonary artery from control (a) and chronic hypoxic (b) rats. (●) Control responses (a: 7 rings from 6 animals; b: 7 rings from 6 animals); (○) in the presence of 0.3 μM GR55562 (a: 4 rings from 4 animals; b: 4 rings from 4 animals); (△) in the presence of 1 μM GR55562 (b: 4 rings from 4 animals); (□) in the presence of 3 μM GR55562 (b: 4 rings from 4 animals). Responses are shown as the percentage response to the contraction to 50 mM KCl. Data shown are means and vertical lines indicate s.e.mean.

whilst inducible NOS is upregulated, constitutive, endothelial NOS may be downregulated in these vessels. Indeed, we have presented evidence to support this theory (McCulloch *et al.*, 1995). What we have demonstrated here is that [cyclic GMP]$_i$ is decreased in these vessels and this may be the net effect of changes in activity of both isoforms of NOS at the time the vessels were harvested.

We have also recently suggested that changes in [cyclic GMP]$_i$ and [cyclic AMP]$_i$ may be linked at the level of phosphodiesterases in pulmonary arteries and we are currently investigating this at the cellular level (Sweeney *et al.*, 1995). Preliminary results do indeed suggest that both [cyclic GMP]$_i$ and [cyclic AMP]$_i$ phosphodiesterases are increased in the main and first branch pulmonary arteries of CH rats (Sweeney *et al.*, 1996). Hence the effect of chronic hypoxia on cyclic nucleotide levels described here could be due to changes in both NOS activity and phosphodiesterase activity.

There is evidence that inducible NOS is upregulated in the pulmonary resistance arteries from CH rats (Xue *et al.*, 1994) and we have shown that there may be increases in basal levels of NO in these vessels (McCulloch 1995). Results from the present study, however, indicate that there was no change in either [cyclic GMP]$_i$ or [cyclic AMP]$_i$ in the pulmonary resistance arteries. We have also demonstrated that there is no change in the ability of these vessels to relax in an endothelium-dependent fashion or in their [cyclic GMP]$_i$ and [cyclic AMP]$_i$ phosphodiesterase activities (McCulloch *et al.*, 1995; Sweeney *et al.*, 1996). As discussed above, the phenotype of the vascular smooth muscle cells in the pulmonary

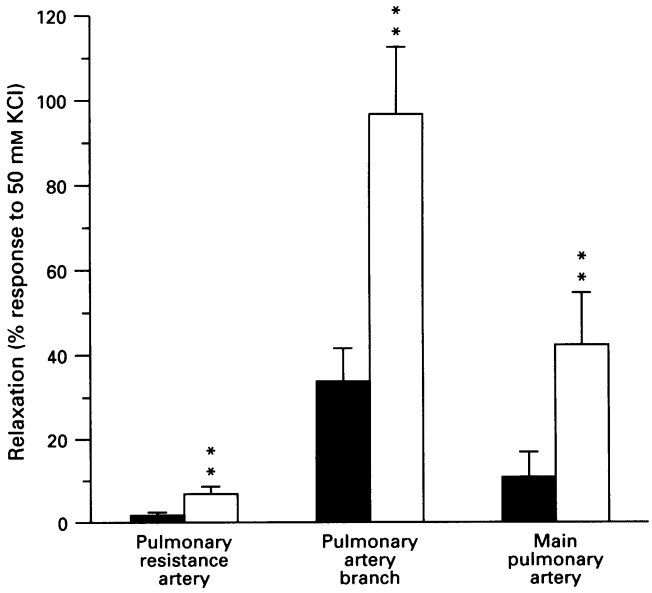

Figure 12 Endogenous tone in pulmonary resistance arteries, first branch pulmonary arteries and the main pulmonary artery from control rats (solid columns) and chronic hypoxic rats (open columns); $n=6$ rings from 6 animals for all groups. Data are shown as the effect of sodium nitroprusside on basal vascular tone and the relaxation is expressed as a percentage of the response to subsequent administration of 50 mM KCl. Means ± s.e. are present. Statistical comparisons between the control and chronic hypoxic groups were carried out by use of Student's unpaired t test; $^{**}P<0.01$.

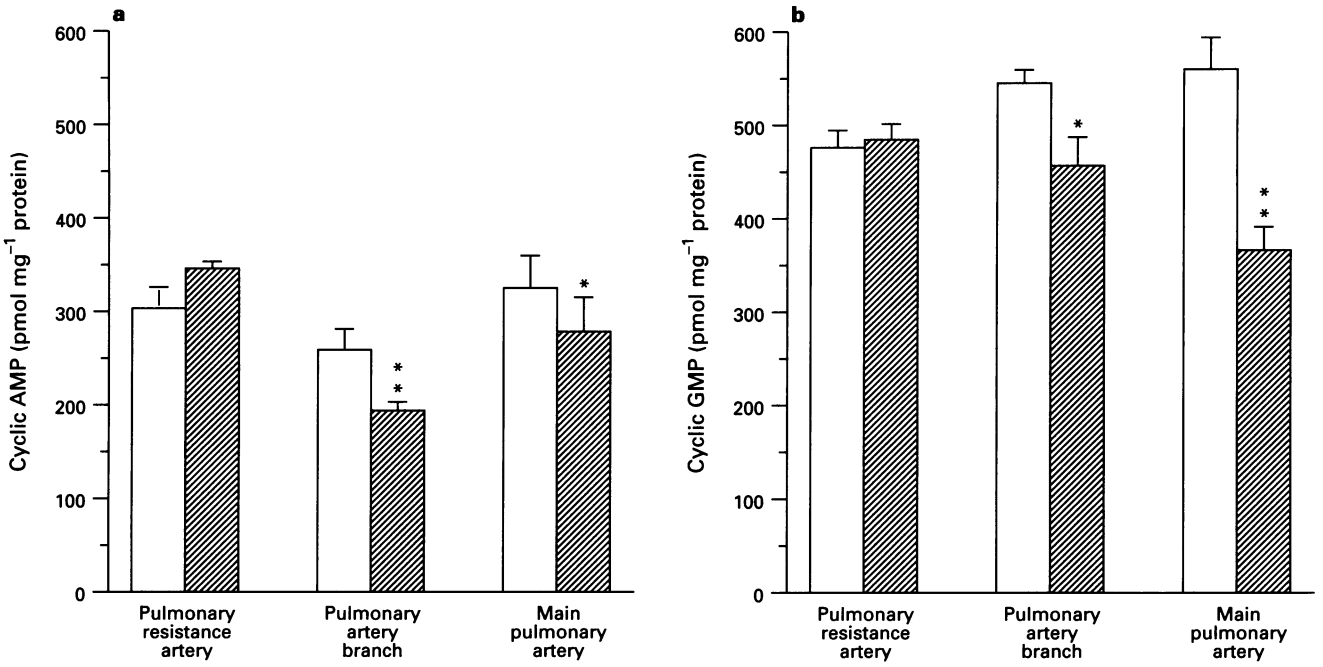

Figure 13 Cyclic nucleotide levels in pulmonary resistance arteries, first pulmonary artery branch and main pulmonary artery of control rats (open columns) and chronic hypoxic rats (hatched columns). (a) Cyclic AMP; (b) cyclic GMP; $n=8$ determinations from 8 animals for all groups. Data are presented as means ± s.e. Statistical comparisons between the control and chronic hypoxic groups were carried out by use of Student's unpaired t test; $^{*}P<0.05$; $^{**}P<0.01$.

resistance arteries is very different from those in the larger elastic arteries and there are further changes with the onset of PHT (eg. Meyrick & Reid, 1978; Sasaki *et al.*, 1995). This is reflected by the difference in their cyclic nucleotide handling observed in this study. Decreased levels of cyclic GMP cannot be the cause of the considerable increased responsiveness of these vessels to 5-HT and some other factor, along with the increase in endogenous tone, must be involved.

Conclusions

This study emphasizes the differences in both the pharmacology and biochemistry of pulmonary resistance arteries and larger pulmonary arteries. There are regional differences in 5-HT sensitivity with the resistance arteries normally being more sensitive to 5-HT in control rats but capacitance arteries being more sensitive in CH rats. There is evidence for a r5-HT_{1B}-like receptor mediating vasoconstriction in the control rat pulmonary resistance arteries but there is no evidence for these on the larger pulmonary arteries from the control rats. Responses to 5-HT were markedly increased in all the CH rat pulmonary arteries. This was due to the enhanced effect of 5-HT_{2A} receptor stimulation combined with the increased influence of r5-HT_{1B}-like receptor stimulation. In the pulmonary artery first branch and the main pulmonary artery, the increased response due to r5-HT_{1B}-like receptor stimulation may be related to the combination of increased vascular tone and decreased $[\text{cyclic GMP}]_i$ observed in these vessels.

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