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5-Hydroxytryptamine receptors mediating vasoconstriction and vasodilation in perinatal and adult rabbit small pulmonary arteries

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1 Vasoconstrictor responses to 5-HT, 5-carboxamidotryptamine (5-CT, 5-HT_1 receptor agonist), α-methyl-5-HT (5-HT_2 receptor agonist) and sumatriptan (5-$HT_{1D/1B}$ receptor agonist) were studied in fetal, 0–24 h, 4 day, 7 day and adult rabbit pulmonary resistance arteries (PRAs), alone and in the presence of the NO synthase inhibitor N^{ω}-nitro-L-arginine methylester (L-NAME). The effect of the selective 5-HT receptor antagonists ketanserin (5-HT_{2A} receptor) and GR55562 (5-$HT_{1B/1D}$ receptor) on vasoconstrictor responses to 5-HT were studied in the presence of L-NAME. Vasodilator responses to 5-CT were also studied in pre-contracted PRAs.

3 5-HT and α-methyl-5-HT were equipotent in causing contraction in the PRAs at each age (e.g. pEC_{50}s for 5-HT and α-methyl-5-HT were 6.74 ± 0.13 and 6.63 ± 0.22 respectively in adult vessels). In the perinatal PRAs, sumatriptan and 5-CT produced negligible contractions, but in adult PRAs, 5-CT and sumatriptan were potent agonists with pEC_{50}s of 6.05 ± 0.3 and 5.70 ± 0.20 respectively.

4 L-NAME markedly increased the maximum response to 5-HT in the 0–24 h, 4 day and 7 day vessels and increased 5-HT potency in the 4-, 7-day-old and adult rabbit vessels.

5 In perinatal vessels, responses to 5-HT, with L-NAME present, were antagonized by ketanserin (30 nM and 0.1 μM) but not GR55562 (1 μM). A small ketanserin-resistant, GR55562-sensitive component was observed at 0–24 h. In adult vessels, both ketanserin and GR55562 inhibited 5-HT-induced responses.

7 Vasodilator responses to 5-CT were observed in pre-contracted PRAs from 4- and 7-day-old rabbits but not in the fetus, 0–24 h old or adult rabbit vessels. At 4 days the vasodilator response was inhibited both by L-NAME and GR55562. At 7 days the response was only partly blocked by L-NAME and resistant to GR55562. The L-NAME resistant component was antagonized by the 5-HT_7 receptor antagonist spiperone (1 μM).

8 The results suggest that 5-HT_{2A}-receptors mediate vasoconstriction in perinatal vessels whilst the 5-HT_{1D} or 5-HT_{1B} receptor contributes in adult rabbit vessels. The 5-HT_{1D} or 5-HT_{1B} receptor mediates NO-dependent vasodilation in vessels from rabbits at 4 days of age whilst 5-HT_7 receptors mediate NO-independent vasodilation by 7 days.

Keywords: 5-Hydroxytryptamine; perinatal; pulmonary arteries; vasoconstriction; vasodilation

Introduction

At birth, initiation of lung ventilation and concomitant increase in pulmonary arterial PO_2 results in a rapid fall in pulmonary vascular resistance (PVR). A 10 fold increase in pulmonary blood flow occurs in order to adapt to life outside the uterus and permit normal gas exchange, via the lungs, to begin. In a significant number of neonates, PVR does not decrease at birth resulting in persistent pulmonary hypertension of the newborn (PPHN) (Gersony, 1973; John *et al*., 1988). This condition results in a considerably high mortality rate.

5-hydroxtryptamine (5-HT) is a potent pulmonary vasoconstrictor *in vitro* in several species including man (Raffestin *et al*., 1985; MacLean *et al*., 1996a) and several studies have implicated a role for 5-HT in the aetiology of pulmonary hypertension (PHT). Elevated plasma levels of 5-HT have been reported in primary PHT (Herve *et al*., 1995) and isolated pulmonary arteries from PHT patients undergoing transplantation exhibit exaggerated vasoconstrictor responses to 5-HT (Brink *et al*., 1988). 5-HT has also been linked to PHT associated with hypoxia in the newborn infant (Johnson & Georgieff; 1989) and, recently, 5-HT turnover has been shown to be increased in children with PHT secondary to congenital heart disease (Breuer *et al*., 1996). Hence, the 5-HT receptors mediating pulmonary vasoconstriction in the perinatal period are of interest.

5-HT interacts with a multiplicity of receptors causing either vasoconstriction or vasodilation depending on receptor subtype, species, pulmonary vascular location and underlying vascular tone (Hoyer *et al*., 1994). The two variants of the 5-HT_{1D} receptor previously described as the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor (Weinshank *et al*., 1992) have recently been renamed the 5-HT_{1D} and 5-HT_{1B} receptor, respectively (Hartig *et al*., 1996). Where we cannot distinguish between the 5-HT_{1D} and 5-HT_{1B} receptor, we subsequently refer to the receptor involved as the 5-$HT_{1D/1B}$ receptor.

5-HT causes vasoconstriction in isolated bovine intrapulmonary arteries *via* a 5-$HT_{1D/1B}$ receptor (MacLean *et al*., 1994) but induces endothelium-dependent relaxation in isolated porcine pulmonary arteries *via* the '5-HT_{1C}' receptor (Glusa & Richter, 1993). The 5-HT_{1C} receptor has subsequently been renamed the 5-HT_{2C} receptor (Hoyer *et al*., 1994).

We have shown that in human pulmonary arteries, both large and small, 5-HT-mediated vasoconstriction is *via* stimulation of 5-$HT_{1D/1B}$ receptors (MacLean *et al*., 1996a; MacLean & Morecroft, 1998). In other vascular beds, 5-HT can induce endothelium-dependent vasodilation *via* the 5-HT_{2C}

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receptor (Bodelson *et al*., 1993), the 5-HT_{2B} receptor (Glusa & Roos, 1996; Ellis *et al*., 1995) and the 5-$HT_{1D/1B}$ receptor (Gupta, 1992). There is also evidence that 5-HT can mediate vasodilation directly through 5-HT_4 and 5-HT_7 receptors located on vascular smooth muscle (Cocks & Arnold, 1992; Leung *et al*., 1996; Terron, 1996).

The 5-HT receptors mediating vasoconstriction and vasodilation in perinatal pulmonary arteries have not previously been studied. The neonatal lung is still undergoing development and maturation at birth (Hislop & Reid, 1973; Meyrick & Reid, 1982), and very little is known at present about the actions of 5-HT on perinatal pulmonary resistance vasculature. We therefore compared the functional responses to 5-HT, the influence of endothelium-derived NO and the 5-HT receptor subtype(s) involved in mediating 5-HT-induced vasoconstriction and vasodilation, in PRAs from fetal, neonatal and adult rabbits.

Methods

Rabbit pulmonary resistance arteries

New Zealand White, fetal (2 days pre-term) 0–24 h, 4 days, 7 days after birth and adult rabbits were studied. They were killed by sodium pentobarbitone (200 mg kg^{-1}) and the lungs removed. Under a dissecting microscope, intralobar pulmonary resistance arteries ([PRAs], ~250–300 μM i.d.) were carefully dissected out and cleaned of surrounding parenchyma. Two mm long segments of the arteries were threaded onto 40 μM stainless steel wires and mounted as ring preparations in isometric wire myographs (J.-P. Trading, Denmark) and bathed in Krebs-buffer solution (pH 7.4) (composition (mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 0.6, $CaCl_2$ 2.5, glucose 11.0, and EDTA 23.0) at 37°C. Fetal vessels were bubbled with 3%O_2/5%CO_2 balance N_2 in order to mimic *in utero* partial oxygen pressures, and the neonatal and adult vessels bubbled with 16%O_2/5%CO_2 balance N_2 to give values similar to those found *in vivo*. As these vessels have walls less than 1.5 μM thick, O_2 diffusion problems are not encountered with active bubbling. Tension was then applied to all vessels to give a transmural pressure equivalent of approximately 12–16 mmHg, similar to *in vivo* pressures of pulmonary arterioles after birth. Fetal vessels were also tensioned to this pressure equivalent as this was the minimum tension at which reproducible, consistent contractile responses could be observed.

Experimental protocol

Following a 45 min equilibration period, the response to 50 mM KCl was determined, followed by wash-out and further equilibration. 50 mM is the concentration at which KCl produces the greatest contraction. The vessels were then subjected to one of the following experiments.

Cumulative concentration-response curves (CCRCs) to 5-HT (1 nM–0.1 mM) were constructed either in the presence or absence of 100 μM L-NAME, a non-selective NO synthase inhibitor.

In order to evaluate the 5-HT receptor(s) involved in the pulmonary vasoconstrictor activity in the PRAs, CCRCs were also constructed to the 5-HT agonists 5-carboxamidotryptamine (5-CT, 1 nM–0.1 mM; 5-HT_1 receptor agonist), α-methyl-5-HT (5-HT_2-selective, 1 nM–0.1 mM) and the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan. Previous experience dictates that attempts at mechanical removal of the vascular endothelium in these arteries damages the thin and fragile underlying smooth muscle. These experiments were therefore, carried out in the presence of 100 μM L-NAME to prevent any inhibitory role of NO release on constrictor responses. CCRCs to 5-HT are not reproducible in each tissue as tachyphylaxis often occurs with a second CCRC, therefore, only one CCRC to 5-HT was constructed either in the absence or presence of antagonist in each vessel. The antagonists used were ketanserin (selective for 5-HT_{2A} receptors) and GR55562 (3-[3-(dimethylamino) propyl] - 4- hydroxy- N- [4- (4-pyridinyl) phenyl] benzamide) (selective 5-$HT_{1B/1D}$ antagonist). All antagonists studies were also carried out in the presence of L-NAME (100 μM).

The second protocol examined the 5-HT receptor(s) mediating vasodilation in perinatal and adult rabbit PRAs and these experiments were conducted in the presence of 0.1 μM ketanserin to exclude any 5-HT_{2A}-receptor mediated effects. Vessels were contracted with endothelin-1 (ET-1) (0.1–1 nM) as it reliably induces a maintained contraction in these vessels (Docherty & MacLean, 1998). Vessels were pre-contracted to give a contraction approximately half the maximal contractile response to this ET-1 as preliminary studies indicated that contraction above this level actually decreased the sensitivity of vasodilators including 5-CT. We studied vasodilation in responses to 5-CT as preliminary results showed that 5-HT gave a variable vasodilator response with a tendency to cause contraction at higher concentrations in preconstricted PRAs. A CCRC to 5-CT was constructed in each vessel either in the presence or absence of one of the following; GR55562 (1 μM), 100 μM L-NAME, 100 μM L-NAME + spiperone (1 μM, 5-HT_7 receptor antagonist). Antagonists were allowed a 45 min equilibrium period prior to constructing the CCRCs to 5-CT.

Drugs and solutions

The following drugs were used: 5-hydroxytryptamine creatinine sulphate and N^W-nitro-L-arginine methylester (L-NAME) (Sigma Chemical Co. Ltd., Poole, Dorset, U.K.); 5-carboxamidotryptamine (5-CT) and spiperone (Semat, St. Albans, U.K.); α-methyl-5-hydroxytryptamine and ketanserin bitartrate (Roth); sumatriptan and GR55562 (3-[3-(dimethylamino)propyl] - 4- hydroxy- N- [4- (4-pyridinyl) phenyl] benzamide) (GlaxoWellcome); ET-1 (Thistle Peptides, Glasgow, Scotland, UK). Drugs and dilutions were prepared in distilled water.

Data analysis

Contractile responses are expressed as a percentage of the contraction to 50 mM KCl determined at the start of the experiment in each preparation. The results are shown as the mean ± s.e.mean. Dilator responses are expressed as the % reduction of ET-1-induced tone. *p*EC50s were calculated by computer interpolation from individual CCRCs. Statistical comparison of the means of groups of data were made by Student's unpaired *t* test or one way analysis of variance where appropriate. $P<0.05$ was considered statistically significant. n = number of animals. In antagonist studies, estimated pK_B values for antagonists were calculated whenever possible (Schild, 1947).

Results

Responses to 5-HT and the influence of NO

pEC_{50} values and maximum contractile responses are summarized in Table 1. At all ages, 5-HT elicited concentra-

tion-dependent contractions in the PRAs (Figure 1A). The maximum contractile responses to 5-HT were significantly smaller at 4 days and 7 days than at other ages (Figure 1A, Table 1). The potencies of 5-HT at the various ages were in the following order: 0–24 h > fetus ⩾ 7 day > adult ⩾ 4 day. In the fetal PRAs, NOS inhibition did not significantly alter the contractile response to 5-HT either in terms of sensitivity or maximum contractile response (Figure 1B, Table 1). There was an increase in maximum contractile response to 5-HT in the presence of L-NAME in the 0–24 h PRAs but there was no change in 5-HT potency (Figure 1B, Table 1). In both the 4-day-old and 7-day PRAs, inhibition of NOS resulted in profound increases in the sensitivity and in the maximum contractile responses to 5-HT (Figure 1B, Table 1). In the adult PRAs NOS inhibition resulted in a significant increase in potency to 5-HT without any significant change in maximum contractile response. The order of 5-HT potency was altered in the presence of L-NAME: 7 day > > 0–24 h = fetus ⩾ adult > 4 day. In the 0–24 h and 4 day vessels, in the presence of L-NAME, the maximum responses to 5-HT were significantly greater than at other age points (Table 1).

5-HT receptor agonists studies

The selective 5-HT_{2A} receptor agonist α-methyl-5-HT (in the presence of L-NAME) elicited concentration-dependent contractions in rabbit PRAs at all ages (Table 1). In the adult rabbit vessels, the maximum response to α-methyl-5-HT was significantly smaller than the maximum response to 5-HT ($P<0.01$). There was no significant difference in sensitivity or maximum response to α-methyl-5-HT when compared to 5-HT at any other age. However, at 0–24 h and 4 days (as with 5-HT in the presence of L-NAME) the maximum responses to α-methyl-5-HT was significantly greater than at other age points (Table 1).

5-CT only caused contractions at very high (>10 μM) concentrations, in the fetal to 7 day period, and the size of these contractions were extremely variable and never greater than ~15% of the response to 50 mM KCl. Sumatriptan did not contract vessels from the fetal-7 day period. In direct contrast, 5-CT elicited a contractile response in the adult vessels and the CCRC to 5-CT was biphasic in nature (Figure 2). The pEC_{50} of 6.05±0.3 ($n=6$) was not significantly different ($P=0.058$) from 5-HT. The maximum contractile response to 5-CT (Emax. = 56±7%) was not significantly different to that of 5-HT. Sumatriptan also elicited a concentration dependent contractile response (Figure 2) with a significantly lower potency ($pEC_{50}=5.70\pm0.20$; $n=6$) compared to 5-HT ($P<0.001$). The maximum contractile response (39±6%) was also significantly lower ($P<0.01$) than that to 5-HT. 5-CT and sumatriptan, however, were equipotent.

5-HT receptor antagonist studies: perinatal rabbit vessels

The 5-$HT_{1B/1D}$ receptor antagonist GR55562 did not inhibit the 5-HT-evoked vasoconstriction in PRAs from fetal to 7 day

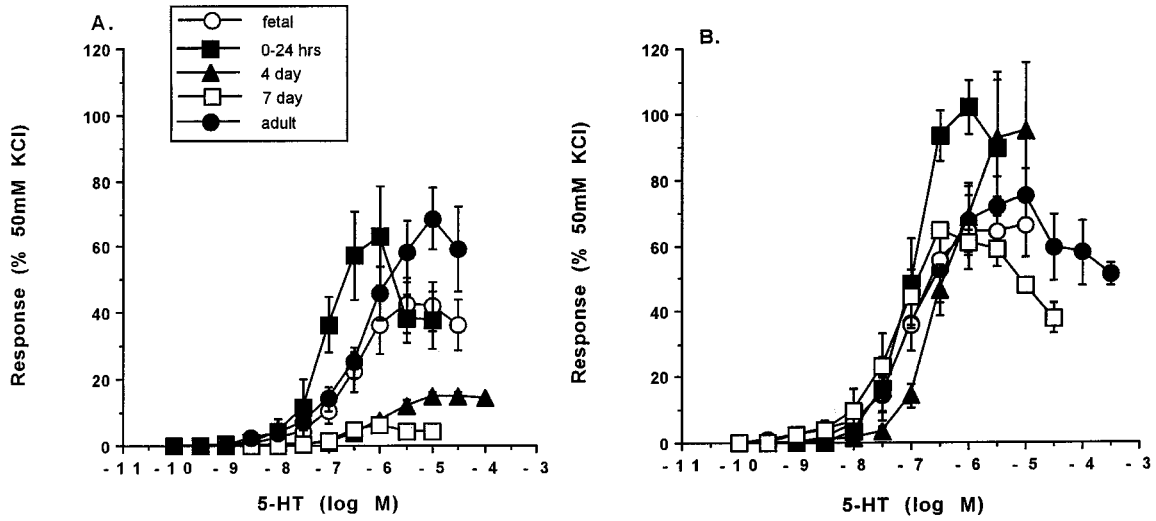

Figure 1 Vasoconstrictor responses to 5-hydroxytryptamine in fetal ($n=6$), 0–24 h ($n=7$), 4 day ($n=6$), 7 day ($n=6$), and adult ($n=12$) rabbit PRAs in (A) absence and (B) presence of 100 μM L-NAME. Data are expressed as a percentage of the response to 50 mM KCl in each preparation and shown as mean±s.e.mean. n = number of lungs.

Table 1 pEC_{50} and E_{max} values for 5-hydroxytryptamine (5-HT) and α-methyl-5-HT in rabbit small pulmonary arteries

	5-HT Control			*5-HT + 0.1 mM L-NAME*			*α-methyl-5-HT + 0.1 mM L-NAME*		
Age	pEC_{50}	E_{max}	n	pEC_{50}	E_{max}	n	pEC_{50}	E_{max}	n
Fetus	6.47±0.11	42±8	6	6.79±0.17	66±9§	6	6.80±0.19	65±7§	6
0–24 h	6.93±0.08†	63±15	6	6.98±0.15	102±8*	7	6.79±0.16	100±9	6
4 days	5.99±0.03†	15±1	6	6.38±0.12*	95±20**	6	6.48±0.19	95±9	6
7 days	6.32±0.04	7±1§§	6	7.73±0.07***	64±2***§§§	6	7.57±0.05	66±4§§	6
Adult	6.08±0.12	69±9§§	8	6.75±0.13	75±8§	8	6.63±0.22	43±2§§§	6

Mean data±s.e.mean. E_{max}: maximum contractile resonse (% of response to 50 mM KCl); L-NAME: N^{ω}-nitro-L-arginine methylester. Statistical comparison using ANOVA: compared with 5-HT controls *$P<0.05$, **$P<0.01$; ***$P<0.001$; compared with fetal response, †$P<0.05$; compared with 0–24 h data. §$P<0.05$, §§$P<0.01$, †††$P<0.001$ (ANOVA). n = number of rabbits.

old rabbits (Figure 3A–D, Table 2). The exception to this was in the 0–24 h PRAs (Figure 3B). In these vessels, GR55562 (1 μM) significantly inhibited the 5-HT-evoked contraction at the level of the pEC_{10} value ($pEC_{10}=7.26\pm0.03$ in the presence of GR55562, compared to $pEC_{10}=7.52\pm0.10$ without GR55562; $P<0.05$) and at the pEC_{20} level ($pEC20=7.07\pm0.05$ and 7.33 ± 0.10, with and without 1 μM GR55562 respectively; $P<0.05$).

In the fetal to 7 day vessels, ketanserin (30 nM) inhibited the 5-HT-induced response (Figure 3A–D), with estimated pK_B values of 9.06 ± 0.2 and 8.5 ± 0.2 for fetal and 4-day-old vessels respectively, where maximum responses to 5-HT were achieved in the presence of this antagonist. In 0–24 h PRAs (Figure 3B), ketanserin caused a non-parallel rightward shift of the CCRC for 5-HT and only inhibited the contractile response to 5-HT at concentrations $\geqslant0.1$ μM although this ketanserin resistant component formed only $\sim20\%$ of the total contractile response to 5-HT. Although this concentration of ketanserin did not significantly affect the pEC_{50} in these vessels (Table 2), the pEC_{75} and pEC_{80} values (5.64 ± 0.2 and 5.45 ± 0.21 respectively) were significantly less ($P<0.01$) than their respective control values of 6.76 ± 0.12 and 6.72 ± 0.12). In these 0–24 h vessels, 100 nM ketanserin did inhibit responses to 5-HT in a competitive fashion with an estimated pK_B of 8.9 ± 0.2. At 7 days (Figure 3D), 30 nM ketanserin inhibited responses to 5-HT with an estimated $pK_B=9.6\pm0.2$.

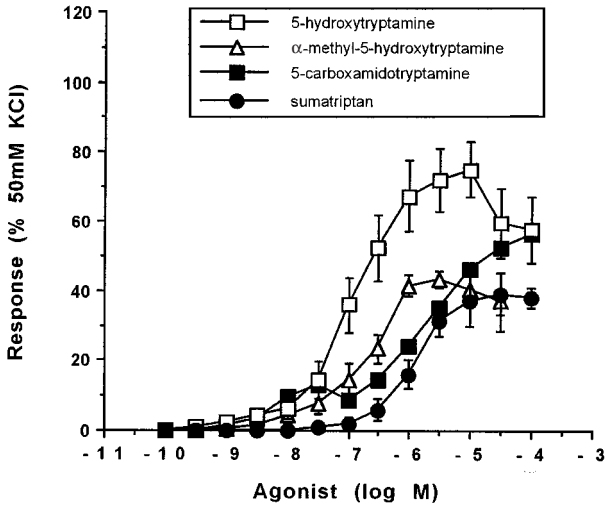

Figure 2 Vasoconstrictor response to 5-hydroxytryptamine ($n=8$), α-methyl-5-hydroxytryptamine ($n=6$), 5-carboxamidotryptamine ($n=6$) and sumatriptan ($n=6$) in adult rabbit pulmonary resistance arteries in the presence of 100 μM L-NAME. Data are expressed as a percentage of the response to 50 mM KCl in each preparation and shown as mean±s.e.mean. n=number of lungs.

5-HT receptor antagonist studies: adult rabbit vessels

In adult PRAs ketanserin (30 nM) significantly inhibited 5-HT-evoked vasoconstriction only at 5-HT concentrations $\geqslant0.1$ μM (Figure 4A, Table 2). Responses to lower concentrations of 5-HT were resistant to ketanserin (Figure 4A). A higher concentration of ketanserin (0.1 μM) did not significantly inhibit the 5-HT responses any further and the 'ketanserin-resistant' component observed at low concentrations of 5-HT was still present. GR55562 (1 μM) inhibited responses to all concentrations of 5-HT in the adult PRAs, including the 'ketanserin-resistant' component giving an estimated $pK_B=6.6\pm0.2$ (Figure 4B, Table 2). Higher concentrations of GR55562 (10 μM), further inhibited responses to 5-HT (pEC_{50}: 5.54 ± 0.18) but also significantly reduced the maximum response to 5-HT from $75\pm8\%$ to $41\pm7\%$ (% of response to 50 mM KCl, $P<0.005$).

5-CT-mediated vasodilation

5-CT evoked concentration-dependent vasodilation in the vessels of both the 4- and 7-day-old animals (Figure 5, Table 3). 5-CT failed to elicit relaxation of any significance in the foetal and 0–24 h rabbit vessels (Figure 5) and totally failed to vasodilate vessels from adult rabbits. In the 4-day-old rabbit PRAs, L-NAME virtually abolished vasodilation induced by 5-CT, suggesting an indirect, NO-dependent dilation at this age (Figure 6A, Table 3). In these 4-day vessels, GR55562 (1 μM) inhibited 5-CT-induced vasodilation (apparent pK_B value $=7.36\pm0.3$; Figure 6A, Table 3). In the 7-day vessels, L-NAME significantly reduced the maximum dilation to 5-CT compared to control responses to 5-CT but there was no significant change in sensitivity suggesting L-NAME only partly blocked the 5-CT-induced dilation at this age (Figure 6B; Table 3). In these 7-day vessels, GR55562 (1 μM) only inhibited the dilation evoked by high concentrations of 5-CT (Figure 6B, Table 3). In the presence of L-NAME and ketanserin, spiperone (1 μM) was a potent inhibitor of the vasodilator responses to 5-CT, in these vessels (Figure 7; Table 3). The estimated pK_B for spiperone was 7.64 ± 0.3.

Discussion

This study demonstrates that, in rabbit perinatal pulmonary resistance arteries, both the contractile and vasodilator responses to 5-HT alter significantly with developmental age

Table 2 pEC_{50} values for 5-hydroxytryptamine (5-HT, in the presence of 0.1 mM L-NAME) in perinatal rabbit pulmonary resistance arteries: effects of ketanserin and GR55562

	5-HT Control		*+30 nM ketanserin*		*+0.1 μM ketanserin*		*+1 μM* GR55562	
Age	*pEC_{50}*	n	*pEC_{50}*	n	*pEC_{50}*	n	*pEC_{50}*	n
Fetus	6.79 ± 0.17	6	5.22 ± 0.14**	6	<4.0	6	6.71 ± 0.1	6
0–24 h	6.98 ± 0.15	7	6.56 ± 0.13	6	5.07 ± 0.02*	6	6.64 ± 0.06	6
4 days	6.38 ± 0.12	6	5.34 ± 0.1**	6	<4.0	6	6.40 ± 0.18	6
7 days	7.73 ± 0.07	6	5.63 ± 0.22**	6	<4.0	6	7.62 ± 0.06	6
Adult	6.75 ± 0.13	8	5.95 ± 0.12**	7	6.04 ± 0.32**	7	6.05 ± 0.08**	6

Mean data±s.e.mean. L-NAME: N^{ω}-nitro-L-arginine methylester. Statitical comparisons using ANOVA: Compared with 5-HT controls *$P<0.05$, **$P<0.01$. n=number of rabbits.

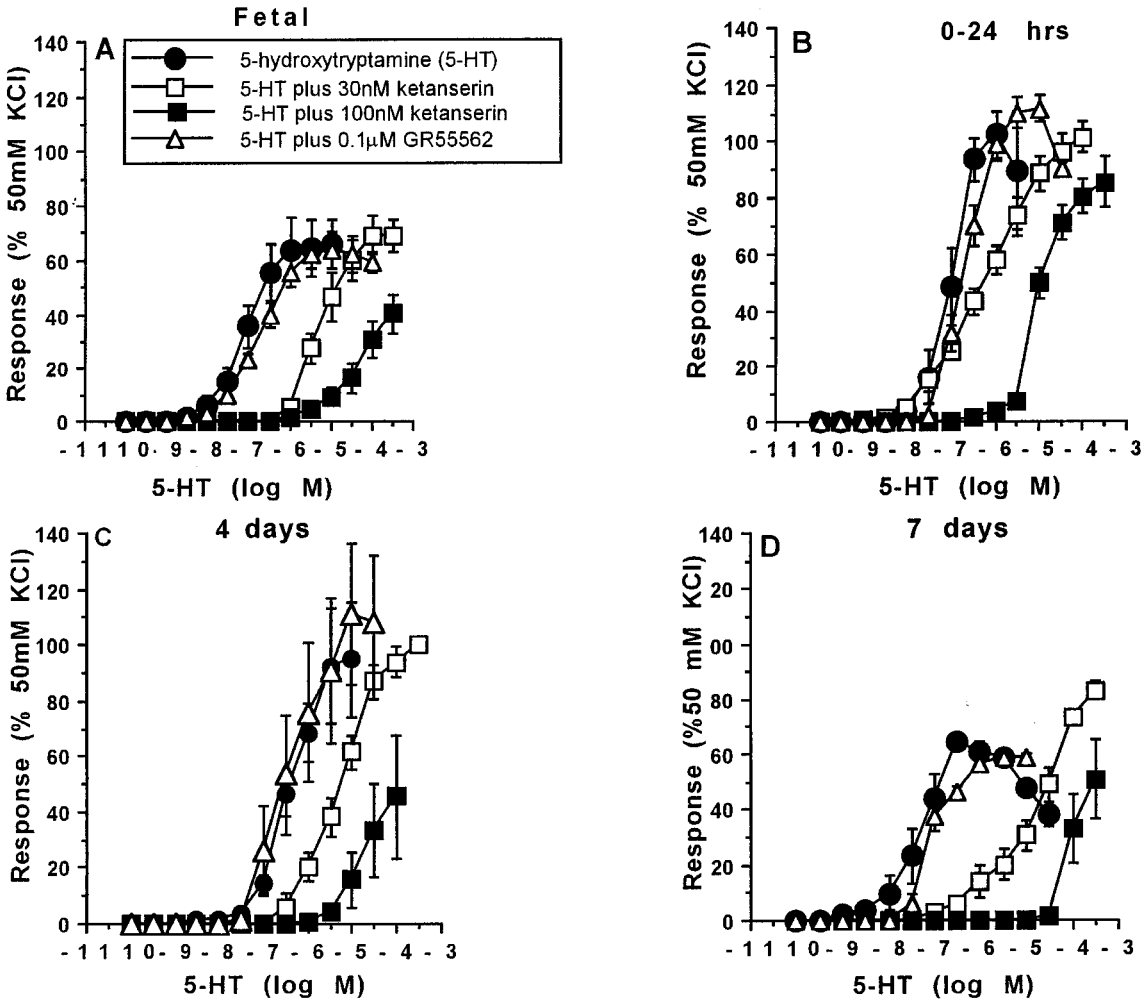

Figure 3 The effect of ketanserin and GR55562 on vasoconstrictor responses to 5-hydroxytryptamine in (A) fetal, (B) 0–24 h, (C) 4-day and (D) 7-day rabbit pulmonary resistance arteries. All in the presence of 100 μM L-NAME, control vasoconstrictor responses to 5-hydroxytryptamine in the presence of 30 nM ketanserin, 100 nM ketanserin and 0.1 μM GR55562. $n=7$ animals in each case. Data are expressed as a percentage of the response to 50 mM KCl in each preparation and shown as mean$\pm$s.e.mean.

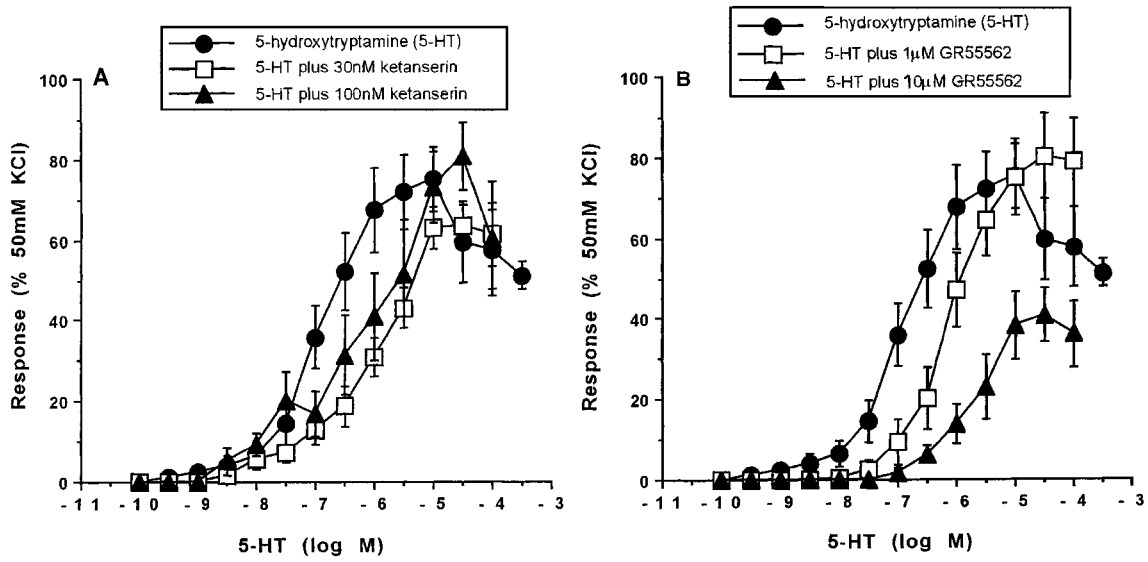

Figure 4 The effect of ketanserin and GR55562 on vasoconstrictor responses to 5-hydroxytryptamine in adult rabbit pulmonary resistance arteries. Vasoconstrictor responses to 5-hydroxytryptamine in the presence (A) 30 nM and 100 nM ketanserin and (B) 1 μM and 10 μM GR55562 All in the presence of 100 μM L-NAME, $n=8$ animals in each case. Data are expressed as a percentage of the response to 50 mM KCl in each preparation and shown as mean$\pm$s.e.mean.

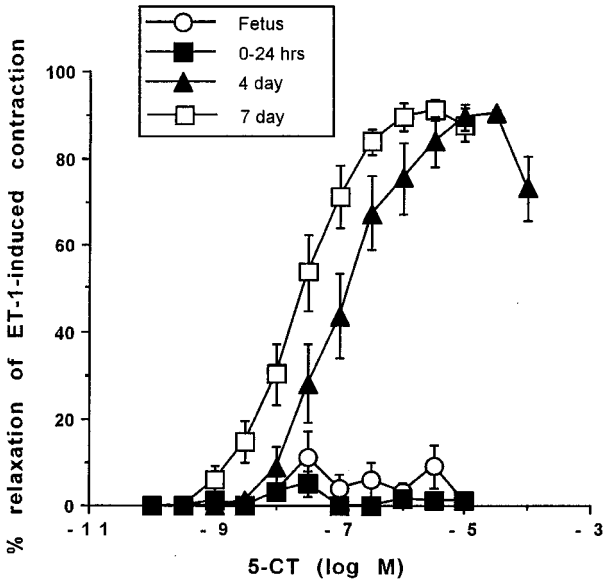

Figure 5 Relaxation, by 5-carboxamidotryptamine, of ET-1 pre-contracted fetal ($n=6$), 0–24 h ($n=6$), 4 day ($n=7$) and 7 day ($n=6$) rabbit pulmonary resistance arteries in the presence of 0.1 μM Ketanserin. Data are expressed as % relaxation of the ET-1-induced pre-contraction ± s.e.mean. n = number of lungs.

and that endothelium-derived NO plays a significant role in modulating these responses.

Vasoconstrictor response to 5-HT

Perinatal vessels An agonist potency order of 5-HT = α-me-5-HT > 5-CT satisfies one of the criteria for classifying 5-HT_2 receptors (Hoyer *et al.*, 1994). In the present study, the rank order of agonist potency in the vessels from fetal rabbits to 7 days of age was 5-HT = α-me-5-HT > > 5-CT. Sumatriptan did not induce contraction in these vessels. This suggests that the predominant receptor mediating 5-HT-induced vasoconstriction in these vessels, at the perinatal ages studied, is of the 5-HT_2 class. The maximum responses to both 5-HT and α-me-5-HT were significantly higher in the 0–24 h and 4-day rabbit vessels suggesting developmental differences in 5-HT_2 receptor density. Ketanserin, at submicromolar concentrations, shows selectivity for 5-HT_{2A} receptors (Hoyer *et al.*, 1994). 30 nM Ketanserin inhibited responses to 5-HT in a competitive manner in the fetal, 4- and 7-day-old rabbit vessels confirming that 5-HT_{2A} receptors mediate the contractile response to 5-HT in these vessels. The estimated pK_B values for ketanserin in the present study were 8.6–9.6 and are similar to the affinity values obtained for ketanserin in other systems where a 5-HT_{2A} subtype is described (Hoyer *et al.*, 1994). In the vessels from the 0–24 h old rabbits, 30 nM ketanserin caused a non-

Table 3 Vasodilation induced by 5-carboxamidotryptamine (5-CT, in the presence of 0.1 μM ketanserin) in neonatal rabbit pulmonary resistance arteries: pIC_{50} and E_{max} values for 5-CT and effects of L-NAME, GR55562 and spiperone

	4 days			*7 days*		
Age	pIC_{50}	E_{max}	n	pIC_{50}	E_{max}	n
Group						
Control (5-CT)	7.1 ± 0.2	91 ± 5	7	7.5 ± 0.2	91 ± 2	6
+ 0.1 mM L-NAME	–	21 ± 6**	5	7.3 ± 0.3	43 ± 5*	6
+ 1 μM GR55562	5.7 ± 0.2**	98 ± 5	6	7.7 ± 0.1	70 ± 7	5
+ 0.1 mM L-NAME + 1 μM spiperone				5.6 ± 0.3†	44 ± 3	5

Mean data ± s.e.mean. E_{max} = maximum response (% relaxation of ET-1 induced tone), L-NAME: N^{ω}-nitro-L-arginine methylester. Statistical comparison using ANOVA: with relevant control response in absence of antagonist/L-NAME. *8$P<0.01$; **$P<0.001$; with 7 day in presence of L-NAME, †$P<0.001$.

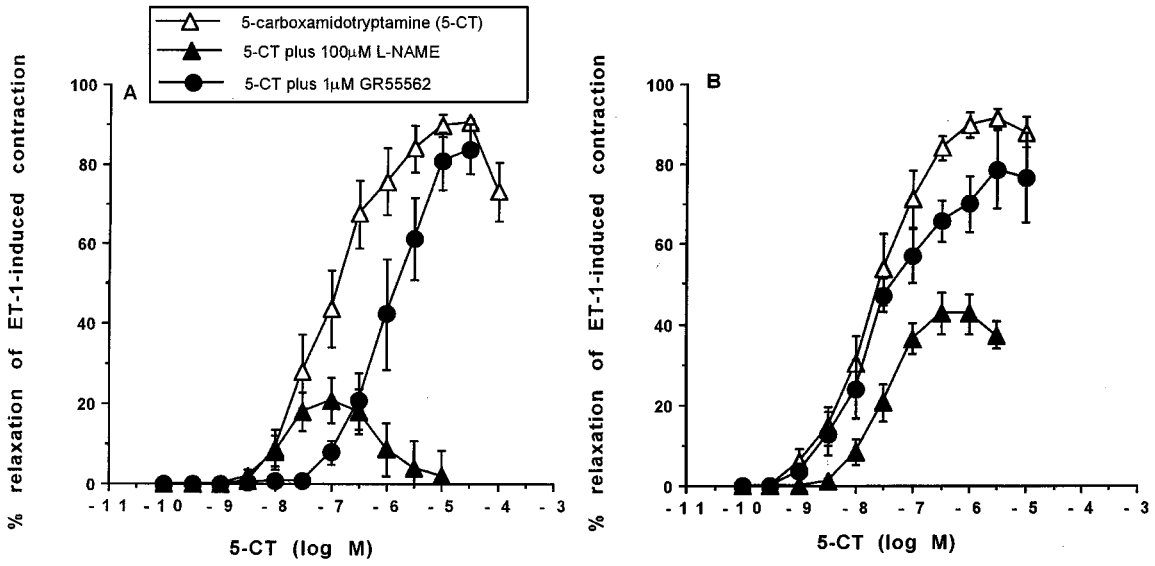

Figure 6 Effect of L-NAME (100 μM) and GR55562 (1 μM) on 5-carboxamidotryptamine-induced relaxation in ET-1 pre-contracted (A) 4-day-old and (B) 7-day-old rabbit pulmonary resistance arteries in the presence of 0.1 μM ketanserin. Data are expressed as % relaxation of the ET-1-induced pre-contraction ± s.e.mean. $n=5$ animals in each case.

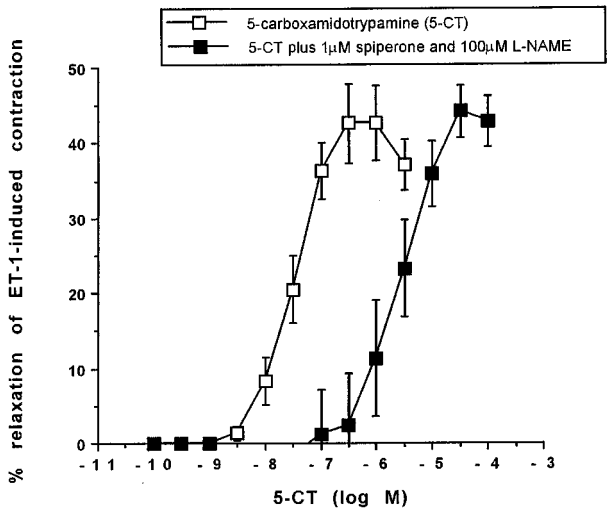

Figure 7 Effect of spiperone (1 μM) on 5-carboxamidotryptamine-induced relaxation in ET-1 pre-contracted 7 day pulmonary resistance arteries, in the presence of 100 μM L-NAME and 0.1 μM ketanserin. Data are expressed as % relaxation of the ET-1-induced pre-contraction $\pm$ s.e.mean. $n=5$ animals in each case.

parallel rightward shift of the 5-HT CCRC revealing small, ketanserin-resistant contractions to low concentrations of 5-HT. These were inhibited by the 5-$HT_{1B/D}$ receptor antagonist GR55562 and by higher concentrations of ketanserin. This suggests that, immediately after birth, there is transitory expression of a small population of contractile 5-$HT_{1D/1B}$ receptors. GR55562 has recently been shown to be selective for the 5-HT_{1B} and 5-HT_{1D} receptors (Connor *et al*., 1995). We have used this compound to identify a functional population of 5-$HT_{1B/1D}$ receptors in human pulmonary conduit and resistance arteries (MacLean *et al*., 1996a; MacLean & Morecroft, 1998). However, in the rabbit, GR55562 did not inhibit the 5-HT-mediated vasoconstriction at the fetal, 4- and 7-day age points and only inhibited responses to 5-HT in the 0–24 h vessels at the pEC_{10} and pEC_{20} levels. We can, therefore, rule out any major involvement of 5-$HT_{1B/1D}$ receptor subtype in mediating 5-HT-induced vasoconstriction in the rabbit perinatal small pulmonary artery. The dominant receptor is the 5-HT_{2A} receptor.

Adult vessels In the adult PRAs, the rank order of agonist potency was 5-HT = α-methyl-5-HT = 5-CT $\geqslant$ sumatriptan which does not strictly fit with the potency order for a single population of 5-HT receptor subtype (Hoyer *et al*., 1994). It is possible that the adult rabbit PRAs express a heterogeneous population of 5-HT receptors contributing to 5-HT-evoked vasoconstriction. Evidence for this co-existence of different vasoconstrictor 5-HT receptor subtypes exists in other rabbit vascular tissues including the cerebral arteries (Deckert *et al*., 1994; Ellwood & Curtis, 1997). In the adult rabbit PRAs, ketanserin inhibited responses to concentrations of 5-HT greater than 30 nM. Responses elicited by lower concentrations of 5-HT were resistant to ketanserin. This does suggests the involvement of two 5-HT receptor populations evoking vasoconstriction in these arteries; non-5-HT_{2A} receptors activated by low concentrations of 5-HT and 5-HT_{2A} receptors activated by higher concentrations. Furthermore, GR55562 antagonized responses to all concentrations of 5-HT, including those which were ketanserin resistant, suggesting that a significant population of 5-$HT_{1B/1D}$ receptors contributes towards 5-HT-induced vasoconstriction. Hence, in the adult rabbit PRA, both the 5-HT_{2A} receptor and the 5-$HT_{1B/1D}$ receptor mediate vasoconstriction. In this study, we were unable to determine if the receptor was involved was the 5-HT_{1D} or 5-HT_{1B} receptor. It has recently been shown that methiothepin can be used to discriminate between rabbit 5-HT_{1D} or 5-HT_{1B} receptors (Bard *et al*., 1996) and future studies using this antagonist and molecular determination of receptor expression should clarify the receptor subtype involved. In human pulmonary arteries, large and small, we have demonstrated that it is the 5-$HT_{1B/1D}$ receptor which mediates vasoconstriction using GR 55562 (MacLean *et al*., 1996a; MacLean & Morecroft, 1998) and we have recently demonstrated that 5-HT_{1B} receptor expression is significantly greater than 5-HT_{1D} receptor expression in these vessels (unpublished observation). We have previously shown, in the adult rat PRA, that both the 5-HT_{2A} receptor and the 5-$HT_{1B/1D}$ receptor mediate vasoconstriction but that it is the 5-HT_{2A} receptor which dominates (MacLean *et al*., 1996b). Hence, there is species variation in the 5-HT receptor/s mediating vasoconstriction in small muscular pulmonary arteries. In this study, vessels of 250–300 μM i.d. were used. These were 2nd order pulmonary arteries in the fetal and neonatal lungs but 4th order vessels in the adult rabbit lungs. This may explain any differences observed in receptors mediating vasoconstrictor responses as there is great heterogeneity of smooth muscle phenotypes throughout the pulmonary vascular tree (Frid *et al*., 1997). However, in the normal lung, pulmonary vascular resistance is equally distributed throughout the arterial circulation and therefore 2nd and 4th order vessels will normally contribute equally to pulmonary vascular resistance.

Previous studies have shown that in rat conduit pulmonary arteries, 5-HT may activate α_1-adrenoceptors (Ogawa *et al*., 1995). However, we have previously shown that noradrenaline itself is a very poor vasoconstrictor of perinatal rabbit pulmonary resistance arteries and actually fails to contract pulmonary resistance arteries from adult rabbits (Docherty & MacLean, 1998). In addition, preliminary experiments in adult vessels failed to demonstrate any effect of α_1-adrenoceptor antagonists on responses to 5-HT in rabbit pulmonary arteries (unpublished observations). Hence we do not believe that α_1 adrenoceptors contribute to the observed responses to 5-HT in this study.

The influence of NO on responses to 5-HT

Several studies have suggested that NO is released under basal conditions to maintain the pulmonary vasculature under a low state of tone in both adults (Wiklund *et al*., 1990; Steeds *et al*., 1997) and in neonatal guinea pigs (Davidson & Eldern 1991). We investigated the possibility that NO was having an inhibitory effect on the 5-HT-induced contractile response as this has been demonstrated in rabbit conduit pulmonary arteries and saphenous vein (Morecroft *et al*., 1995; Valentin *et al*., 1996). Inhibition of NO synthesis with L-NAME significantly potentiated the maximum contractile response to 5-HT at all ages, except the fetus and adult. In the absence of endogenous NO, in the presence of L-NAME, the potency of 5-HT at the various ages was: 7-day $>>$ 0–24 h = fetus $\geqslant$ adult $>$ 4-day. This suggests variable developmental changes in 5-HT receptor sensitivity or changes in the coupling of the second messenger system with the contractile apparatus. We have recently reported a similar phenomenon for ET-1 induced contraction in the same preparations (Docherty & MacLean, 1998).

The effect of L-NAME was most marked in the vessels removed from the 4- and 7-day-old rabbits where the response to 5-HT, in the absence of L-NAME, was extremely small. This suggests that significant responses to 5-HT in these vessels are normally masked by endogenous NO production in these vessels at this age. One interpretation of this data is that 5-HT can cause release of NO in 4- and 7-day-old vessels but is less able, or unable, to do this in the vessels from the fetus, 0–24 h old and adult rabbits. This is confirmed by our studies on 5-HT receptor-induced, NO-dependent vasodilation, which was only present in the vessels from the 4- and 7-day-old rabbits.

The response of the fetal PRAs to NO synthase inhibition differs from that of the rabbit fetal pulmonary conduit arteries where we have shown that L-NAME markedly potentiates the size of the 5-HT vasoconstrictor response (Morecroft *et al*., 1995). This comparison suggests heterogeneity of NO activity throughout the pulmonary arterial bed in the fetal rabbit, with 5-HT-sensitive, endogenous NO activity being present in the large conduit arteries but not in the small muscular pulmonary arteries. Such heterogeneity has been observed in the adult rat where eNOS can be detected in large conduit arteries but not small muscular vessels (Le Cras *et al*., 1996). In our previous study on fetal rabbit conduit pulmonary arteries, these were exposed to relatively low oxygen tensions, hence this excludes the possibility that these low oxygen tensions had inhibited NO production in the present study. We have observed no difference in endothelium-dependent vasodilation in fetal rabbit PRAs bubbled with both 3 and 16% oxygen (unpublished observations). We chose to maintain the vessels at the oxygen tension expected *in utero*.

5-HT receptor-induced vasodilation

5-HT has previously been shown to elicit endothelium-dependent relaxation in porcine pulmonary arteries (Glusa & Richter, 1993) as well as other, systemic preparations (Bodelson *et al*., 1993). Using 5-CT, we established that there is 5-HT-receptor-induced vasodilation present in the vessels from the 4- and 7-day-old rabbits. We have previously shown that pre-constriction of bovine pulmonary arteries can 'uncover' 5-HT_1-receptor-mediated vasoconstriction (MacLean *et al*., 1994; Sweeney *et al*., 1995). It is of interest, therefore, that no contractile responses to 5-CT were observed in the present study, indicating that under the conditions used, there was no uncovering of 5-HT_1-receptor-mediated vasoconstriction these rabbit pre-constricted vessels. Vasodilations to 5-CT were not observed in the fetal, 0–24 h or adult rabbit PRAs again demonstrating rapid developmental changes in the rabbit pulmonary circulation. It is possible that there is a maturational alteration in the ability of the pulmonary vascular smooth muscle to relax to NO as shown in porcine perinatal pulmonary arteries (Wilson *et al*., 1993). This explanation is, however, unlikely in our preparation as we have shown that fetal and 0–24 h rabbit PRAs relax to acetylcholine (Docherty & MacLean, 1995). 5-HT receptors are known to mediate vasodilation in blood vessels either directly *via* 5-HT receptors located on the vascular smooth muscle (Cocks & Arnold, 1992; Leung *et al*., 1996) or indirectly *via* endothelial released NO (Bodelson *et al*., 1993; Glusa & Richter, 1993; Glusa & Roos, 1996). We show that, in 4- and 7-day-old rabbit small pulmonary arteries, 5-CT-evoked vasodilation is inhibited by the NO synthase inhibitor L-NAME. This suggests that 5-HT-induced vasodilation in these vessels involves release of the endothelium-derived relaxing factor NO. The endothelial location of the receptor could not be confirmed by experiments in which the endothelium is removed as previous experience dictates that any attempt to remove the endothelium greatly damages the thin and fragile underlying smooth muscle in this preparation. Endothelial, vasodilator 5-HT receptors have previously been identified in arteries and veins as being similar to either the 5-HT_{2B} subtype or the 5-$HT_{1D/1B}$ subtype Gupta, 1992; Glusa Roos, 1996). In the present study, in the 4-day-old rabbit vessels, GR55562 inhibited 5-CT-induced vasodilation suggesting that the 5-$HT_{1B/1D}$ subtype mediated NO-dependent vasodilation in these vessels. In contrast, in the 7-day-old rabbit vessels, GR55562 only caused a small inhibition of 5-CT induced vasodilation at high concentrations of 5-CT. In these vessels L-NAME significantly reduced the maximum 5-CT-elicited relaxation and so NO does contribute to 5-CT-induced vasodilation in these vessels. Spiperone, however, potently inhibited 5-CT-induced vasodilation in these vessels. Recently, the novel 5-HT_7 receptor has been described and has been found on the vascular smooth muscle of several preparations including the dog coronary artery (Terron, 1996) and *Cynomolgus* monkey jugular vein (Leung *et al*., 1996). This receptor is thought to mediate smooth muscle relaxation directly. Several antipsychotic drugs, including spiperone have been shown to behave as antagonists at this receptor. Spiperone displays a high binding affinity for the cloned 5-HT_7 receptor (Roth *et al*., 1994) and 5-HT_7 receptors located on vascular smooth muscle stimulate adenylate cyclase and mediate vascular relaxation directly (Hoyer *et al*., 1994; Leung *et al*., 1996). The pK_B of spiperone against 5-CT mediated vasodilation was 7.6 which is comparable with its pK_B of 7.1–7.8 at 5-HT_7 receptors in the monkey jugular vein, rabbit femoral vein and dog coronary artery (Martin, 1994; Leung *et al*., 1996; Terron, 1996). Spiperone has a high affinity for 5-HT_{2A} receptors (Leff & Martin, 1986) but the presence of ketanserin in these studies rules out 5-HT_{2A} receptors effects. Hence it is likely that, in the vessels from the 7-day-old rabbits, 5-HT mediates NO-independent vasodilation through the 5-HT_7 receptor located on the vascular smooth muscle as well as NO-dependent vasodilation through the 5-$HT_{1D/1B}$ receptor.

The presence of 5-HT receptor-induced vasodilation between 4 and 7 days is consistent with previous studies in the pig where endothelium-dependent vasodilation is most marked 3–10 days after birth, declining with increasing age (Liu *et al*., 1992). After birth, there is a reduction in muscularisation of the pulmonary circulation between 24 h and 2 weeks (Haworth & Hislop., 1981). The presence of pulmonary vasodilator mechanisms at this age may, therefore, facilitate such changes.

In conclusion, we have demonstrated marked developmental changes in the 5-HT receptors involved in mediating vasoconstriction and vasodilation in rabbit small pulmonary arteries. The predominant receptor mediating vasoconstriction is the 5-HT_{2A} receptor in fetal to 7-day-old rabbits. In contrast, in adult vessels both 5-HT_{2A} and 5-$HT_{1D/1B}$ receptors contribute towards 5-HT-induced vasoconstriction. 5-HT receptor-mediated vasodilation is absent in the fetus, 0–24 h and adult rabbits. In the 4-day-old rabbit vessels 5-HT-mediated vasodilation is mediated indirectly *via* NO release following 5-$HT_{1D/1B}$ receptor activation. In 7-day rabbit vessels, however, vasodilation is also mediated directly by a 5-HT_7 receptor.

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