Pulmonary responses to 5-hydroxytryptamine and endothelin-1 in a rabbit model of left ventricular dysfunction

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Abstract

Objective: To determine whether pulmonary hypertension developed in a coronary artery-ligated rabbit model of left ventricular dysfunction (LVD) and to examine the effects of i.v. 5-hydroxytryptamine (5-HT) and endothelin-1 (ET-1) on pulmonary arterial pressure (PAP).

Methods: Eight weeks after experimental coronary artery ligation or sham operation, ejection fractions were assessed by echocardiography. The rabbits were later anaesthetised and pulmonary arterial pressure was measured via a catheter inserted into the pulmonary artery via the right external jugular vein. 5-HT (1–400 μg/kg) and ET-1 (0.001–4 nmol/kg) were administered i.v.

Results: Ejection fraction was significantly decreased from 76.6 ± 1.4% in sham-operated to 42.2 ± 1.3% in coronary artery-ligated rabbits (n = 9 in each group; P < 0.001), consistent with LVD. Baseline mean pulmonary arterial pressure was significantly increased in the coronary artery-ligated group compared to the shams, 16.5 ± 0.5 vs. 11.5 ± 0.8 mmHg; P < 0.001. A significant degree of right ventricular hypertrophy was found in the coronary artery-ligated rabbits 0.70 ± 0.04 g/kg f.b.wt. in coronary artery-ligated rabbits (n = 8 cf. 0.48 ± 0.02 g/kg f.b.wt. in sham-operated controls, n = 8; P < 0.001). There was a significant increase in the percentage of muscularised pulmonary vessels adjacent to alveolar ducts and alveoli < 60 μm i.d. in the LVD rabbits compared with their sham-operated controls (8.5 ± 0.4 cf. 20 ± 0.5%; P < 0.0005). 5-HT produced a greater response in the coronary artery-ligated rabbits (a maximum increase of 8.7 ± 1.0 mmHg in mean pulmonary arterial pressure vs. 4.6 ± 1.5 mmHg for sham-operated controls; P < 0.05). ET-1 did not have any effect on pulmonary arterial pressure in either group.

Conclusion: In the rabbit, LVD secondary to coronary artery ligation, causes right ventricular hypertrophy, pulmonary vascular remodelling, and an increased PAP consistent with the onset of pulmonary hypertension (PHT). The greater PAP response to i.v. 5-HT in the PHT group supports the hypothesis that this substance could be involved in the development of PHT. A role for ET-1 cannot be excluded, despite its lack of effect on PAP when intravenously administered in either group. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Pulmonary Hypertension; 5-hydroxytryptamine; Endothelin; Left ventricular dysfunction; Rabbit

1. Introduction

Pulmonary hypertension (PHT) can occur as a primary or secondary phenomenon and is a consequence of many cardiopulmonary diseases where it often remains unidentified and therefore untreated. Once irreversible changes in pulmonary vasculature have occurred, pulmonary hypertension is associated with a poor prognosis, high mortality and is a contraindication to cardiac transplantation [1,2].

The possibility that heart failure may be the commonest cause of PHT is often overlooked. Heart failure following
various forms of left ventricular dysfunction (LVD) affects 1–2% of the entire population and associated PHT is common [3,4].

The pulmonary circulation is normally a high-flow, low-pressure circuit, but persistent increases in left atrial pressure cause an elevation in pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR). However, the possibility of therapeutic intervention is limited as many standard vasodilators have a greater effect on the systemic circulation, causing hypotension, than on the pulmonary vasculature [5,6]. In advanced PHT of any systemic circulation, causing hypotension, than on the pulmonary circulation [7,8]. Right ventricular hypertrophy is an early and common consequence of PHT and an accurate marker of the development of PHT [9,10]. 5-hydroxytryptamine (5-HT) and endothelin-1 (ET-1) have been implicated in the aetiology of PHT. 5-HT is a pulmonary vasoconstrictor released from pulmonary neuroendocrine cells and platelets and has been associated with the progression of monocrotaline induced PHT in rats [11]. It has been demonstrated that isolated pulmonary arterial responses to 5-HT are potentiated in patients with primary PHT and in the chronic hypoxic rat model of PHT [12,13]. 5-HT has also been implicated in secondary PHT related to hypoxia in newborns and following cardiac surgery [14].

ET-1 is a potent vasoconstrictor which has been implicated as a possible pathological mediator in diseases of most physiological systems [15]. Increased plasma levels of ET-1 have been observed in patients with secondary PHT due to chronic congestive heart failure and these increased levels were found to correlate with the extent of PHT and prognosis [16]. Increased levels of ET-1 production have also been shown in cases of PHT associated with congenital heart defects and mitral valve disease [17,18]. Endothelin antagonists have been shown to reduce the PHT associated with coronary artery ligation in the rat [19].

There is substantial evidence to suggest a possible role for either of these vasoconstrictors in the development and progression of PHT secondary to heart failure. However, their precise role remains unknown.

This study was undertaken to compare the effects of left ventricular dysfunction (LVD) on pulmonary and systemic pressures and to determine the effect of artificially increasing circulating 5-HT and ET-1 on pulmonary arterial pressure.

2. Methods

Adult male New Zealand White rabbits (n = 18, weighing 2.5–3.5 kg) were used in this study.

2.1. Coronary ligation model

A well-characterised model of chronic left ventricular dysfunction secondary to coronary ligation was utilised [20]. Rabbits received premedication using intramuscular fentanyl/fluanizone, 0.3–0.4 ml/kg (Hynnorm, Jansen). Anaesthesia was induced with midazolam (0.2–0.4 ml/kg) and, following intubation was maintained with a mixture of nitrous oxide, oxygen (1:1 ratio) and 1% halothane.

A left thoracotomy was performed through the 4th intercostal space to expose the heart. Quinidine hydrochloride (3–5 mg/kg i.v.) was administered prior to coronary artery ligation to reduce the incidence of ventricular fibrillation. The major branch of the left coronary artery was occluded approximately midway between the left atrial appendage and the cardiac apex. This gives rise to a large homogeneous infarct due to the poor collateral circulation of the rabbit coronary system. In cases where ventricular fibrillation occurred (usually 8–12 min following occlusion), defibrillation was undertaken with an 8-J epicardial shock. When an acceptable area of infarction (approximately 20% of the left ventricle) had been produced and the animal was haemodynamically and electrically stable, the thoracotomy was closed. In sham-operated controls, hearts were manipulated as in the coronary artery-ligated animals, but the artery was left unoccluded.

Postoperative analgesia (buprenorphine 0.3 mg/kg) was administered every 8 h for the first 24–48 h, together with broad-spectrum antibiotics. The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985) and with the provisions of the Animals (Scientific procedures) Act 1986.

2.2. Echocardiography

Echocardiographic examination of the animals was performed 8 weeks after coronary artery ligation or sham operation as previously described in detail [20]. This was performed under light sedation (Hynnorm 0.3 ml/kg) using a Toshiba SSH160A echocardiograph and a 5-MHz short focused paediatric transducer. A right parasternal transducer position was used which gives the best acoustic window and provides views of the left ventricle equivalent to the left parasternal window in man. M mode long axis measurements of left ventricular end diastolic dimension at the level just below the mitral valve and left atrial internal diastolic diameter at the level of the aortic root were made. By rotating the transducer 90°, a short axis image enabled end-diastolic and end-systolic frames to be captured and traced onto the screen via an on-line cineloop computer analysis facility. This positioning was such that in the coronary artery-ligated animals, the short axis view rarely included an area of infarct and therefore, if anything, is likely to underestimate the severity of left ventricular dysfunction.
Following measurement of baseline pressures, 5-HT 1±\textit{y} ion EF was calculated as the end diastolic area dysfunction observed in these animals. The ejection fraction (EF) was calculated as the (end diastolic area – end systolic area)/end diastolic area.

All the measurements were made by a single operator. A repeat measures study was also performed to determine the reproducibility of image acquisition and analysis. Twelve animals were examined twice within 24–72 h. The coefficient of variation and the largest difference between consecutive measurements on the same animal were 2.3% and 1.3 mm for the dimension measurements and 3.9 and 5% for ejection fraction.

2.3. Measurement of pulmonary arterial pressure

Animals were anaesthetised as previously described and ventilated via a tracheal cannula. A specially designed J-shaped catheter (Portex, 1.65 mm o.d.) was manoeuvred into the right ventricle via the right external jugular vein and right atrium with the aid of X-ray image intensification and a guide wire. The guide wire was removed and the catheter positioned at the right ventricular outflow tract using radio opaque dye. A smaller cannula (Portex, 0.75 mm o.d.) was then passed through the wide bore cannula into the pulmonary artery. The catheter position was confirmed by the morphology of the pressure trace and by injection of radio opaque dye. The ascending aorta was cannulated via the right carotid artery. Following catheter placement, steady state with respect to systemic and pulmonary pressure was obtained before recordings were made. Baseline PAP and systemic arterial pressure (SAP) were measured using an Elcomatic E751A pressure transducer and recorded on a Siemens Elena Mingograph 803. Following measurement of baseline pressures, 5-HT (1–400 \(\mu\)g/kg) or ET-1 (0.001–4 nmol/kg) was infused cumulatively into a cannula placed in the left femoral vein. Pulmonary arterial and aortic pressure recordings were recorded onto tape (Racal Store-7 DS) and results were analysed off-line.

The pressures reported concerning 5-HT represent the maximum changes in the pressures observed following each dose. The values presented for ET-1 are those seen 3 min after each dose. ET-1 caused changes in pressure which reached a new steady state after approximately 2 min.

### Table 1

<table>
<thead>
<tr>
<th>Echocardiographic measurements</th>
<th>Sham ((n = 9))</th>
<th>Ligated ((n = 9))</th>
<th>(P &lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD (mm) 11.7±0.3 (9.7–13.1)</td>
<td>16.6±0.6 (14.7–19.8)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>LV (mm) 16.9±0.4 (15.1–17.9)</td>
<td>20.3±0.4 (18.6–22.5)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>EF (%) 76.6±1.4 (73–86)</td>
<td>42.2±2.3 (35–47)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as mean±s.e.m.; ranges are given in parentheses. LAD, left atrial diameter; LV, left ventricular end diastolic diameter; EF, ejection fraction. Comparison between ligated and control groups using Student’s unpaired \(t\)-test.

2.4. Post mortem

At the end of each experiment, the animal was sacrificed and the location of the pulmonary artery catheter confirmed. The heart, lungs and liver were removed and weighed. Care was taken to dissect the right and left ventricles free of the septum as the ratio of right ventricle weight/total body weight was used as a measure of right ventricular hypertrophy (RVH). This has been shown to be a reliable index of the degree of PHT present in experimental animals [9,10]. A second group of sham and ligated animals were used for histological examination to determine the percentage of pulmonary vessels with thickened walls. Generally, pulmonary vessel muscularisation in models of PHT involves a peripheral extension of the muscular coat to vessels with a diameter of < 60 \(\mu\)m i.d. Details of these methods and the validation of the vessel counting method have been described previously [21] and this is now considered to be an accurate method for the assessment of pulmonary vascular remodelling in PHT. The word ‘vessel’ is used rather than arteriole as small venules cannot readily be distinguished from arterioles in this method. Lungs from both groups of animals were paraffin embedded in a vacuum and 4–\(\mu\)m thick sections cut and stained with haemotoxylin/eosin stains. For each lung, 100 small pulmonary vessels < 60 \(\mu\)m i.d. next to alveoli and alveolar ducts were counted and examined for muscularisation from 20 fields at \(\times 40\) magnification. A vessel was considered muscularised if the medial layer had at least three layers of smooth muscle i.e. was 15–25 \(\mu\)m thick. Normally, these vessels have virtually no detectable smooth muscle in the medial layer. The percentage of muscularised vessels was used as a measure of vascular remodelling.

2.5. Statistical methods

Student’s two-sampled unpaired \(t\)-tests were applied for comparisons between the ligated and sham-operated groups. For continuous comparison between grouped data, correlation coefficients \((r)\) were estimated for the degree of association between variables. If they were found to be

### Table 2

<table>
<thead>
<tr>
<th>Baseline haemodynamic measurements</th>
<th>Sham ((n = 9))</th>
<th>Ligated ((n = 9))</th>
<th>(P &lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean AOP (mmHg) 66±2 (54–75)</td>
<td>66±2 (58–76)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HR (b.p.m.) 263±9 (225–306)</td>
<td>252±7 (228–285)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Systolic PAP (mmHg) 15.8±1.2 (8–19)</td>
<td>21.4±0.9 (18–27)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Mean PAP (mmHg) 11.5±0.8 (6–14)</td>
<td>16.5±0.5 (14–20)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Diastolic PAP (mmHg) 9.4±0.7 (5–12)</td>
<td>14.2±0.5 (13–16)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as mean±s.e.m.; ranges are given in parentheses. AOP, mean aortic pressure; HR, heart rate; PAP, pulmonary arterial pressure. Comparison between ligated and control groups using a Student’s unpaired \(t\)-test.
significant, a scatter plot of the two sets of data were made and simple regression analysis was performed. A value of $P < 0.05$ was taken as statistically significant.

3. Results

3.1. Echocardiographic data

Table 1 shows that ejection fraction was significantly lower in the coronary artery ligation group (42.2 ± 1.3%, $n = 9$) compared to 76.6 ± 1.4% in the shams, $n = 9$) and there is no overlap in the ranges. In addition, both left atrial and left ventricular end diastolic diameters were significantly increased in the heart failure group. These values are similar to those reported previously [20] and show that in the coronary artery ligation group, there is evidence of left ventricular dysfunction.

3.2. Baseline haemodynamic characteristics of experimental groups

The baseline haemodynamic characteristics of the sham-operated controls and coronary artery-ligated animals are shown in Table 2. These measurements were taken immediately following the stabilisation period and prior to any drug administration. The baseline values for mean aortic pressure and heart rate were not different between the groups. There was an increase in systolic, mean and diastolic pulmonary arterial pressure in the coronary artery-ligated rabbits of 5.6 ± 5% , 4 ± 43% and 4.8 mmHg ± 1% respectively.

A significant correlation was found between ejection fraction and mean PAP for the population of animals used in this study, showing that poorer left ventricular function was associated with higher pulmonary arterial pressure.

Fig. 1. Scatter plot of correlation between echocardiographic ejection fraction and mean PAP in rabbits. Regression equation $y = -0.13x + 21.8$, $r = -0.73$; $P < 0.001$. Closed squares, coronary artery-ligated rabbits, $n = 9$; open squares, sham-operated rabbits, $n = 9$.

Fig. 2. Scatter plot of correlation between echocardiographic ejection fraction and degree of RVH in rabbits. Regression equation $y = -0.007x + 1.008$, $r = -0.84$; $P < 0.001$. Closed squares, coronary artery-ligated rabbits, $n = 8$; open squares, sham-operated rabbits, $n = 8$.

Table 3

<table>
<thead>
<tr>
<th>Tissue weights of experimental groups</th>
<th>Sham</th>
<th>Ligated</th>
<th>$P &lt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td>f.b.wt. (kg)</td>
<td>3.54 ± 0.1 (2.9–3.9)</td>
<td>3.6 ± 0.1 (2.7–4.2)</td>
<td>–</td>
</tr>
<tr>
<td>RV/f.b.wt. (g/kg)</td>
<td>0.48 ± 0.02 (0.42–0.62)</td>
<td>0.70 ± 0.04 (0.60–0.85)</td>
<td>0.001</td>
</tr>
<tr>
<td>LV/f.b.wt. (g/kg)</td>
<td>0.98 ± 0.05 (0.76–1.10)</td>
<td>1.05 ± 0.04 (0.82–1.21)</td>
<td>–</td>
</tr>
<tr>
<td>Lung (g)</td>
<td>13.6 ± 1.3 (11.1–22.6)</td>
<td>17.8 ± 2.1 (11.7–29.0)</td>
<td>–</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>79 ± 4 (53–90)</td>
<td>80 ± 5 (54–108)</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are shown as mean ± s.e.m.; ranges are given in parentheses. $n = 9$ with the exception of RV and LV data, where $n = 8$ in each group. RV/f.b.wt. and LV/f.b.wt., right and left ventricular mass as a ratio of final body weight, respectively. Comparison between ligated and control groups using a Student’s unpaired $t$-test.
Fig. 3. Graphs showing the effect of exogenous 5-HT on (A) mean pulmonary arterial pressure and (B) mean aortic pressure. Closed squares, coronary artery-ligated rabbits, n = 6; open squares, sham-operated rabbits, n = 7. Data are shown as mean ± s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001, for changes in pressure compared to baseline value, Student’s paired t-test.

3.3. Effect of 5-HT and ET-1

During these experiments there were no alterations in the rate and depth of respiration during the course of any of the experiments. Any changes in respiration could have affected right atrial and therefore right ventricular filling pressure leading to indirect changes in the contractile state of the right ventricle so we can therefore be confident that any changes in arterial pressure represent effects on the circulation of the drugs administered.

3.3.1. 5-Hydroxytryptamine

5-HT induced an increase in PAP in both the sham-operated controls and the coronary artery-ligated rabbits at concentrations as low as 1 µg/kg which reached a maximum at a concentration of 200 µg/kg (Fig. 3A). The changes in pressure were rapid, and occurred within 30 s of the administration of 5-HT. The increase in mean PAP observed at concentrations above 200 µg/kg was significantly greater in the coronary artery-ligated rabbits compared to the controls (P < 0.05 in each case). In contrast, 5-HT caused a gradual decrease in aortic pressure in both groups of animals which reached statistical significance at a concentration of 50 and 100 µg/kg in the sham and coronary ligation groups, respectively (Fig. 3B).

3.3.2. Endothelin 1

No significant effects of ET-1 on pulmonary arterial pressure were seen in either sham-operated or ligated animals (Fig. 4A). In contrast, ET-1 resulted in significant
increases in aortic pressure at concentrations of 1–4 nmol/kg which were not different between the groups (Fig. 4B). The systemic pressor response at each concentration generally reached a new steady state after ~2 min and was extremely long lasting in that we did not see any recovery in pressure after the final dose of ET-1 within the 20–30 min we continued to observe pressures in some of the preliminary experiments. In 5 experiments, following the completion of the dose–response curve for ET-1, either a bolus of 5-HT (400 μg/kg; n = 3) or a further maximal dose of ET-1 (4 nmol/kg; n = 2) was given and pulmonary arterial pressures monitored for 30 min. 5-HT always resulted in a pulmonary arterial pressor response confirming the viability of the pulmonary circulation. No effects of ET-1 were ever seen, even at this high final concentration, suggesting that we had not inadvertently missed a response with a much longer time course than that seen for the systemic circulation.

3.4. Histology

Lungs from 6 sham-operated and 7 ligated animals were examined. In the coronary artery-ligated animals, 20.9 ± 0.4% (range 19–22%) of the vessels were considered to be muscularised, which was significantly greater than the 8.5 ± 0.4% (range 7–10) seen in the control animals (P < 0.0005). A significant correlation between the number of muscularised vessels and the ejection fraction was found indicating that LVD is associated with pulmonary vascular remodelling (Fig. 5).

4. Discussion

As in previous studies, coronary artery ligation in these rabbits produced LVD, confirmed by significant left ventricular dilatation and a depressed ejection fraction [20,22]. In this study, there was also a trend towards heavier left ventricles in coronary artery-ligated rabbits. This is indicative of hypertrophy in the surviving left ventricle as this reflects the weight of only ~80% of the original left ventricle, the other 20% being infarcted and generally consisting of thin fibrous scar tissue.

The results presented here demonstrate that LVD was associated with the onset of PHT as indicated by right ventricular hypertrophy accompanied by elevated pulmonary arterial pressures and increased small pulmonary vessel muscularisation. There was also a trend towards lung congestion. It was not possible to measure PVR directly as the measurement of pulmonary wedge pressure or left atrial pressure was not possible. This difficulty is well recognised by workers using in vivo preparations and many studies use increased right ventricular hypertrophy, vessel muscularisation, combined with pulmonary pressures as markers to confirm PHT in animal models [10,19,21,23]. Indeed, Sakai et al. assessed the degree of pulmonary hypertension in coronary artery-ligated rats indirectly by measuring an increase in right ventricular systolic pressure [19].

Various studies have reported an increased pulmonary artery sensitivity to 5-HT in models of PHT. For example, in a monocrotaline-induced model of PHT in rats, responses of isolated pulmonary arteries to 5-HT have been shown to be potentiated following the onset of PHT. In addition increased plasma levels of 5-HT have been shown to be associated with abnormalities of platelet 5-HT storage [24,25]. Here, we have shown that there is an increased pulmonary pressor response to 5-HT in the ligated rabbits when compared to sham-operated controls. However, there is no evidence for abnormalities in platelet 5-HT storage in animal models or in humans with heart failure. In the chronic hypoxic rat model, the increase in vascular tone and decreased levels of [cyclic GMP] may contribute to the increased response to 5-HT seen in pulmonary hypertensive states via increased stimulation of an r5-HT1b-like receptor [13]. Thus, a possible explanation for the pulmonary hyper-responsive-ness of 5-HT seen in our studies could be an upregulation of a 5-HT receptor type or intracellular signalling pathway associated with the elevation in pulmonary vascular tone.

In pulmonary arteries and systemic arteries, 5-HT is known to cause endothelium-dependent and endothelium-independent vasodilation via 5-HT1B-like and 5-HT1D-like receptors, respectively [26,27]. Hence the effect of i.v. 5-HT on systemic and pulmonary pressures will be determined by the balance between direct smooth muscle vasoconstriction and vasorelaxation. In these rabbits, we have

![Graph](image)

Fig. 5. Scatter plot of correlation between echocardiographic ejection fraction and the percentage of thick-walled pulmonary vessels (TWPV). Regression equation \( y = -0.37x + 37.7, r = -0.88, P < 0.0001 \). Closed squares, coronary artery-ligated rabbits, \( n = 7 \); open squares, sham-operated rabbits, \( n = 6 \).
demonstrated that the direct contractile effect over-rides any vasodilator effect in the pulmonary circulation. However, the net effect of i.v. 5-HT was systemic vasodilation. Hence, the vasodilator effect of 5-HT over-rides the vasoconstrictor effect in the systemic circulation. This suggests that the distribution of and/or contribution from 5-HT receptor subtypes is different in the systemic and pulmonary circulations. The increased response to 5-HT in the pulmonary circulation secondary to left ventricular dys-function could, therefore, be due to increased vasoconstriction or decreased vasodilation via the 5-HT receptors. We propose that a decrease in production of endothelial-derived nitric oxide is unlikely as recent studies in isolated pulmonary resistance arteries from these animals indicate that endogenous nitric oxide synthase activity is enhanced in the coronary artery-ligated animals [28]. Immunocytochemical determination of endothelial nitric oxide synthase also suggests an upregulation of this enzyme (Dockerty and MacLean, unpublished). The possibility that vasodilation was decreased by a down regulation of endothelial or smooth muscle vasodilator 5-HT receptors cannot be excluded.

In contrast to the effects of 5-HT, we found that exogenous ET-1, which has been shown to be a potent vasoconstrictor of isolated human and rabbit pulmonary resistance vessels [28,29] had no effects on PAP. A number of possibilities for this lack of effect can be excluded. Following administration of the maximum ET-1 dose, a marked pulmonary arterial response could still be obtained with 5-HT demonstrating that the pulmonary circulation was still vasoactive. We also ruled out the possibility that ET-1 may exhibit a late response by demonstrating that a bolus injection of the highest dose of ET-1 used (4 nmol/kg), given following the usual range of concentrations, did not have any further effect on PAP after 30 min. ET-1 did, however, cause a profound increase in aortic pressure. Consistent with this selective pressor effect on the systemic circulation, we have recently shown that i.v. injection of ET-1 into patients with left ventricular dysfunction had little effect on their pulmonary pressure or resistance, whilst there was a marked increase in systemic pressure [30].

The reason that i.v. ET-1 did not have any pulmonary pressor effects may be due to ET-1 being a strong stimulus for endothelium-derived nitric oxide release through the ETB1 receptor [31]. As circulating ET-1 is in primary contact with the endothelial lining of the pulmonary arteries, this vasodilator effect may override the direct effect of ET-1 on the pulmonary vascular smooth muscle. Whilst these results may suggest that plasma ET-1 is a marker, rather than a mediator of PHT, they do not exclude the possibility that locally, abuminally released ET-1 plays a role in increasing pulmonary vascular tone. ET-1 levels and ET-1 mRNA are increased in various tissues in heart failure models, but particularly in the endothelium of the pulmonary arteries and lungs [19]. ET-1 is thought to be more paracrine than endocrine in nature. As 75% of ET-1 is thought to be released abuminally [32] then plasma levels do not reflect the endothelin concentration to which the vascular smooth muscle is exposed, which must be considerably higher. Given that ET-1 spillover in the lungs is uniquely correlated with PVR [33], this must relate to exposure of the pulmonary vascular smooth muscle to abuminally released ET-1. Indeed, endothelin receptor antagonists have been shown to inhibit the progression of PHT in coronary artery-ligated rats and chronic hypoxic rats which suggests that ET-1 is involved in this disease process [19,34].

In conclusion, we have provided evidence that the coronary artery-ligated rabbit model of LVD develops changes often associated with pulmonary hypertension before changes in systemic arterial pressures are observed. Impaired left ventricular function is associated with an increase in PAP accompanied by the development of RVH and small pulmonary vessel muscularisation. The findings of a greater response to 5-HT in the coronary artery-ligated group confirm the observations of studies performed on isolated vessels from this model and strengthen the argument for 5-HT being involved in the development of PHT. The lack of effect of ET-1 when administered intravenously, despite being a potent vasoconstrictor in vitro, cannot exclude a role for this peptide when abuminally released. It is hoped that the development of this model will lead to an improved understanding of the mechanisms responsible for the development and progression of PHT secondary to heart failure.

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