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Pulmonary hypertension secondary to left ventricular dysfunction: the role of nitric oxide and endothelin-1 in the control of pulmonary vascular tone

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1 Using an *in vivo* model of pulmonary hypertension (PHT) secondary to left ventricular dysfunction (LVD), the pulmonary arterial response to the nitric oxide synthase (NOS) blocker L-NAME (30 μmol.min^{-1} i.v.) and the subsequent responses to cumulatively administered endothelin-1 (ET-1) (0.001–4 nmol.kg^{-1} i.v.) or big ET-1 (0.1–2.0 nmol.kg^{-1} i.v.) were studied. Additionally, the effect of the non-selective ET-1 receptor antagonist, SB209670, was investigated.

2 Eight weeks after coronary artery ligation or sham operation, rabbits demonstrated increased mean pulmonary arterial pressure (PAP) accompanied by right ventricular hypertrophy.

3 Blockade of NOS caused a greater increase in basal PAP (increased by 7.7 ± 1.1 mmHg *c.f.* 3.8 ± 1.0 mmHg in controls, $P<0.05$) and uncovered a greater pulmonary pressor response to exogenous ET-1 in rabbits with PHT (increased by 10.2 ± 2.3 mmHg *c.f.* 4.9 ± 1.0 mmHg in controls, $P<0.05$).

4 Big ET-1 evoked a pulmonary pressor effect, in both groups of rabbits, that was increased following blockade of NOS and was more potent in rabbits with PHT.

5 The non-selective ET-1 receptor antagonist, SB209670, reduced basal PAP (from 16.9 mmHg to 15.9 mmHg, $P<0.05$) in rabbits with PHT and blocked the response to ET-1 in the presence of L-NAME.

6 In conclusion, the results demonstrate that basal NO activity masks a pulmonary pressor response to exogenously administered ET-1. An increased responsiveness to ET-1 was shown in the pulmonary arterial bed of rabbits with PHT secondary to LVD, implicating a pathophysiological role for ET-1 in this model.

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Abbreviations: AOP, aortic pressure; b.p.m, beats per minute; CI, cardiac index; ECE, endothelin converting enzyme; EF, ejection fraction; ET-1, endothelin-1; L-NAME, N^{w}-nitro-L-arginine methyl ester; LVD, left ventricular dysfunction; NO, nitric oxide; NOS, nitric oxide synthase; PAP, pulmonary arterial pressure; PHT, pulmonary hypertension; PVR, pulmonary vascular resistance; RVH, right ventricular hypertrophy

Introduction

Pulmonary hypertension (PHT) can occur secondary to various forms of heart disease where it is associated with a poor prognosis and an increased morbidity and mortality among patients (Abramson *et al*., 1992). One mechanism which may be related to the pathophysiology of PHT is endothelial dysfunction within the pulmonary vasculature (Loscalzo, 1992). Evidence suggests that production of substances within the endothelium may be altered in cases of PHT. Much of the work has focused on nitric oxide (NO) and endothelin-1 (ET-1) which have opposing haemodynamic effects, with NO being a vasodilator and ET-1 a vasoconstrictor. To date, little is known about the *in vivo* interaction between ET-1 and NO in the pulmonary arterial circulation following left ventricular failure.

In the pulmonary circulation, production of ET-1 occurs primarily in the endothelial cells from the precursor molecule prepro-ET-1. This is processed to big ET-1, which in turn is cleaved *via* the action of an endothelin converting enzyme (ECE) to produce the biologically active, 21 residue peptide, ET-1 (Yanagisawa *et al*., 1988). ET-1 is a potent vasoactive peptide with pro-mitogenic properties for pulmonary vascular smooth muscle cells (Janakidevi *et al*., 1992). It has been implicated as a potential mediator in many diseases including PHT, where both increased pressure and vascular remodelling are prevalent within the pulmonary circulation. ET-1 is both produced and cleared by the lungs and an increased production of ET-1 in the lungs correlates with pulmonary vascular resistance and the extent of PHT in patients with heart disease (Tsutamoto *et al*., 1994; Cody *et al*., 1992).

ET-1 is a potent vasoconstrictor in isolated pulmonary arteries from various species, hence it was surprising to observe that i.v ET-1 had no effect on pulmonary arterial pressure (PAP) in an *in vivo* rabbit model of PHT secondary to left ventricular dysfunction (LVD) (Deuchar *et al*., 1998). The absence of a pulmonary pressor effect to exogenous ET-1

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has, however, also been reported in human patients with LVD (Cowburn *et al*., 1998). This may be explained by considering the location of the different ET receptor subtypes within the pulmonary circulation. ET-1 acts through both ET_A and ET_B receptor subtypes in the pulmonary arteries from a variety of species including man (MacLean *et al*., 1994; La Douceur *et al*., 1993; Haynes *et al*., 1995). The ET_A and ET_B receptors located on the vascular smooth muscle cells mediate vasoconstriction while there are also ET_B receptors located on the vascular endothelial cells, which mediate vasodilation, mainly *via* the production of NO (Warner *et al*., 1993). Therefore it is possible that i.v ET-1 *in vivo* results firstly in activation of ET_B receptors located on the pulmonary vascular endothelium, thereby masking the effects of the vasoconstrictor subtypes located on the underlying smooth muscle layer. Interaction between NO and ET-1 has been demonstrated in the systemic circulation of rats where inhibition of NO synthesis causes a pressor effect which can be blocked by bosentan, a mixed ET receptor antagonist (Richard *et al*., 1995).

In vitro work with the rabbit model used in the present study has shown that while inhibition of NOS has no effect on the contractile response to ET-1 in small pulmonary arteries from control rabbits it enhances the contractile reponse to ET-1 in rabbits with LVD (Docherty & MacLean, 1998). This indicates there may be a change in pulmonary NO activity secondary to PHT induced by LVD. However, the influence of NO on the responsiveness of the pulmonary circulation to ET-1 has, as yet, not been investigated in an *in vivo* model of PHT secondary to LVD. Several studies have showed potentially beneficial effects of both selective ET_A and mixed ET_A/ET_B receptor antagonists in animal models of PHT (Dicarlo *et al*., 1995; Chen *et al*., 1995) and in patients with chronic heart failure (Sutsch *et al*., 1998).

Using an *in vivo* model of PHT secondary to LVD, we have studied the effect of ET-1 in the presence of a NO synthase (NOS) inhibitor, L-NAME, thus removing the effect of the ET_B receptor-mediated NO release. This also allowed examination of the effect of L-NAME alone on basal pressures in this model. In an alternative approach we employed big-ET-1 which has been shown to result in little or no activation of the endothelial ET_B receptors (Haleen *et al*., 1993). Big ET-1 is largely converted to the biologically active ET-1 in endothelial cells before being released predominantly abluminally.

In addition the effects of a mixed ET_A/ET_B receptor antagonist SB209670 (Ohlstein *et al*., 1994) was also studied. Previous results have shown this compound to be the most effective antagonist against the contractile effects of ET-1 in rabbit, rat and human pulmonary resistance arteries (Docherty & MacLean, 1998; McCulloch *et al*., 1998; MacLean *et al*., 1998).

Methods

Fifty-one adult male New Zealand White rabbits weighing 2.5–3.5 kg were used in this study. All investigations 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 1996) and with the provisions of the UK Animals (Scientific procedures) Act 1986.

Coronary artery ligation

A previously described model of left ventricular dysfunction following coronary artery ligation was used with sham-operated rabbits acting as controls (Deuchar *et al*., 1998). Briefly, following pre-medication with an intra-muscular injection of fentanyl (0.315 $mg.kg^{-1}$)/fluanizone (10 $mg.kg^{-1}$), 0.3–0.4 $ml.kg^{-1}$ (Hypnorm, Jansen) rabbits were intubated following the administration of midazolam (0.15–0.3 $mg.kg^{-1}$), with anaesthesia being 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 $mg.kg^{-1}$ 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 base and apex of the left ventricular free wall, giving rise to a large homogeneous, full thickness area of myocardial infarction 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 joule epicardial shock. When an acceptable area of infarction (approx 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.

Post-operative analgesia (buprenorphine 0.3 $mg.kg^{-1}$) was administered every 8–12 h for the first 24–48 h.

Echocardiography

Left ventricular function was assessed using echocardiograpy, 8 weeks after surgery as previously described (Deuchar *et al*., 1998).

Briefly, under light sedation (Hypnorm 0.3 $ml.kg^{-1}$) using a right parasternal transducer position 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. The measurement of ejection fraction was taken in a plane with the tip of the papillary muscles. Therefore positioning was such that in the coronary artery ligated animals the short axis view rarely included an area of infarct and if anything is likely to underestimate the severity of left ventricular dysfunction observed in these animals. The ejection fraction (EF) was calculated as the (End diastolic area – End systolic area)/End diastolic area.

Haemodynamic measurements

The rabbits were anaesthetized and the pulmonary artery and ascending aorta were catheterized in the closed chest animal as previously described (Deuchar *et al*., 1998). Briefly, animals were anaesthetized as described above and ventilated *via* a tracheal cannula. A custom made J-shaped catheter (Portex, 1.65 mm OD) was positioned at the right ventricular outflow tract *via* the right external jugular vein with the aid

of X-ray image intensification and a guide wire. A smaller cannula (Portex, 0.75 mm OD) was then passed through the J-shaped cannula into the pulmonary artery. The catheter position was confirmed by the morphology of the pressure trace and by injection of radio opaque dye.

Cardiac output was measured using a technique of thermodilution, as previously described in detail (Pye *et al*., 1996). Cardiac output values were converted to a cardiac index (CI) by dividing by the final body weight in kg.

Experimental protocols

Basal pulmonary and systemic blood pressures, cardiac output and heart rate measurements were made following a period of stabilization using an Elcomatic E751A pressure transducer connected to an MP100 data acquisition system (BIOPAC Systems Inc, Santa Barbara, CA, U.S.A.). Results were analysed using the built-in software package (Acq*Knowledge* 3.5).

The following experimental protocols were carried out. All drugs were administered through femoral vein cannulae.

(1) L-NAME (30 μmol.min^{-1}) was infused throughout the protocol. Following stabilization of the responses to L-NAME cardiac output was measured and ET-1 (0.001–4 nmol.kg^{-1}) was given cumulatively i.v ($n=7$ in each group). Cardiac output was measured following a maximal effect to the final dose. (2) Following measurement of basal pressures, big ET-1 (0.1–2.0 nmol.kg^{-1}) was given cumulatively i.v either alone ($n=6$ in each group) or following L-NAME (30 μmol.min^{-1}) infusion ($n=6$ in each group). Cardiac output was measured following the final dose. (3) The mixed ET receptor antagonist, SB209670 (10 mg.kg^{-1}, i.v.) was given under basal conditions and left for 20 min ($n=6$ for ligated and 7 for controls). Cardiac output was measured, and L-NAME (30 μmol.min^{-1}) was infused for the remainder of the protocol. Once the responses to L-NAME had stabilized, cardiac output was measured and ET-1 (0.001–4 nmol.kg^{-1}) was administered as before. Cardiac output was measured following the final dose.

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/final body weight was used as a measure of right ventricular hypertrophy (RVH), a reliable index of the degree of PHT present in experimental animals (Hunter *et al*., 1974; Wanstall & O'Donnell, 1990).

Statistical methods

All data is expressed as mean$\pm$s.e.mean. All statistical comparisons were performed using GraphPad Prism analysis package. Analysis of the changes in pressure, within a group, compared to basal values was by repeated measures ANOVA followed by Dunnett's *post hoc* test. The magnitude of response between groups was compared by either student's unpaired *t*-test or one-way ANOVA followed by Tukey's *post hoc* test when appropriate. A value of $P < 0.05$ was taken as statistically significant.

Drugs

The following drugs were prepared in distilled water on experimental days: ET-1 (Thistle Peptides, Glasgow, Scotland). Big Endothelin 38 (human), N^{W}-nitro-L-arginine methyl ester (L-NAME) and Quinidine hydrochloride monohydrate (Sigma Chemical Co. Ltd., Poole, Dorset, U.K.). SB209670 ([(+)-(1S, 2R, 3S)-3-()1-(3,4-methylenedoxyphenyl)-5-(prop-1-yloxy)indene-2-carboxylic acid]) (SmithKline Beecham Pharmaceuticals, King of Prussia, PA, U.S.A.). The following drugs were used in anaesthesia, analgesia and euthanasia. Hypnorm (Jansen animal health), hypnovel (midazolam) (Roche), Fluothane (halothane) (Zeneca Ltd, U.K.), buprenorphine (Alstoe animal health) and pentobarbitone sodium B.P (euthatal) (Rhone Merieux).

Results

Echocardiographic and post mortem data

Depressed left ventricular ejection fraction was evident in the coronary ligated rabbits compared to sham operated controls (0.43 ± 0.01, $n=25$ *c.f.* 0.74 ± 0.01, $n=26$, $P<0.001$). In addition, both left atrial (15.0 ± 0.3 mm *c.f.* 10.5 ± 0.3 mm, $P<0.001$) and left ventricular end diastolic (22.7 ± 0.2 mm *c.f.* 17.6 ± 0.2 mm, $P<0.001$) diameters were significantly dilated in the coronary ligated rabbits. These findings are consistent with previous data in this model (Deuchar *et al*., 1998) and provide evidence of LVD in the coronary ligated rabbits. Post mortem examination showed evidence for hypertrophy of the surviving left ventricle in the ligated rabbits as left ventricular weight corrected for final body weight was greater in this group (1.56 ± 0.03 g.kg^{-1} *c.f.* 1.49 ± 0.03 g.kg^{-1} in controls, $P<0.05$). RVH was also evident with right ventricular weight corrected for final body weight being greater in the ligations (0.72 ± 0.02 g.kg^{-1} *c.f.* 0.49 ± 0.01 g.kg^{-1} in controls, $P<0.001$). There was also evidence of lung and liver congestion with greater lung weights (4.96 ± 0.23 g.kg^{-1} *c.f.* 4.06 ± 0.10 g.kg^{-1} in controls, $P<0.001$) and a trend towards greater liver weights (23.4 ± 0.6 g.kg^{-1} *c.f.* 22.2 ± 0.5 g.kg^{-1} in controls, $P=0.07$). These findings together with an elevated PAP provide evidence for the onset of PHT secondary to LVD in the coronary ligated rabbits.

Baseline haemodynamic characteristics

There was no difference in basal mean aortic pressure (AOP) (62.3 ± 0.8 mmHg in ligated *c.f.* 60.4 ± 0.6 mmHg in controls), heart rate (237 ± 6 b.p.m. in ligated *c.f.* 250 ± 4 b.p.m. in controls) or CI (142.4 ± 3.9 ml.min^{-1} kg^{-1} in ligated *c.f.* 137.1 ± 4.6 ml.min^{-1} kg^{-1} in controls) between the coronary ligated rabbits ($n=25$) and sham-operated controls ($n=26$). PAP was significantly elevated in the coronary ligated rabbits (mean PAP was 17.2 ± 0.4 mmHg *c.f.* 13.0 ± 0.2 mmHg in controls, $P<0.001$).

Effect of L-NAME on baseline pressures and the response to ET-1

L-NAME caused an increase in PAP in both groups of rabbits with the magnitude of this effect being greater in the rabbits

with PHT (Figure 1a, c). L-NAME induced a significant and comparable, increase in AOP in both experimental groups (Figure 1b) and reduced cardiac index to a similar extent in both groups of rabbits (-33 ± 6 ml.min^{-1} kg^{-1} in rabbits with PHT *c.f.* -18 ± 6 ml.min^{-1} kg^{-1} in controls). Heart rate was unaffected by L-NAME in both groups of rabbits.

ET-1, following L-NAME infusion, significantly increased mean PAP in both the rabbits with PHT and the controls (Figure 2a) with the magnitude of this increase being greater in rabbits with PHT (Figure 2b). As in our previous study (Deuchar *et al*., 1998), ET-1 resulted in a pressor effect in the systemic circulation of both groups of rabbits (magnitude of peak response 18.8 ± 3.6 mmHg in rabbits with PHT *c.f.* 31.1 ± 2.8 mmHg in controls). Higher doses of ET-1 (above 0.5 nmol.kg^{-1}), often caused arrhythmias in both groups and associated falls in AOP. In animals in which it was still possible to obtain cardiac output data there was a further decrease in CI from the values obtained following L-NAME ($-\ 63\pm7$ ml.min^{-1} kg^{-1} in rabbits with PHT, $n=4$ *c.f.* -47 ± 14 ml.min^{-1} kg^{-1} in controls, $n=3$).

Effect of big ET-1 in the presence and absence of L-NAME

Big ET-1 alone resulted in an increase in PAP in both rabbits with and without PHT (Figure 3a) but the magnitude of the response at the maximum dose was not different between groups (Figure 3b). The systemic pressor response to big ET-1 was comparable in both groups (magnitude of response at 2.0 nmol.kg^{-1} was 27.7 ± 3.0 mmHg in rabbits with PHT *c.f.* 18.9 ± 5.4 mmHg in controls) as was the decrease in cardiac index (-25 ± 8 ml.min^{-1} kg^{-1} in rabbits with PHT *c.f.*

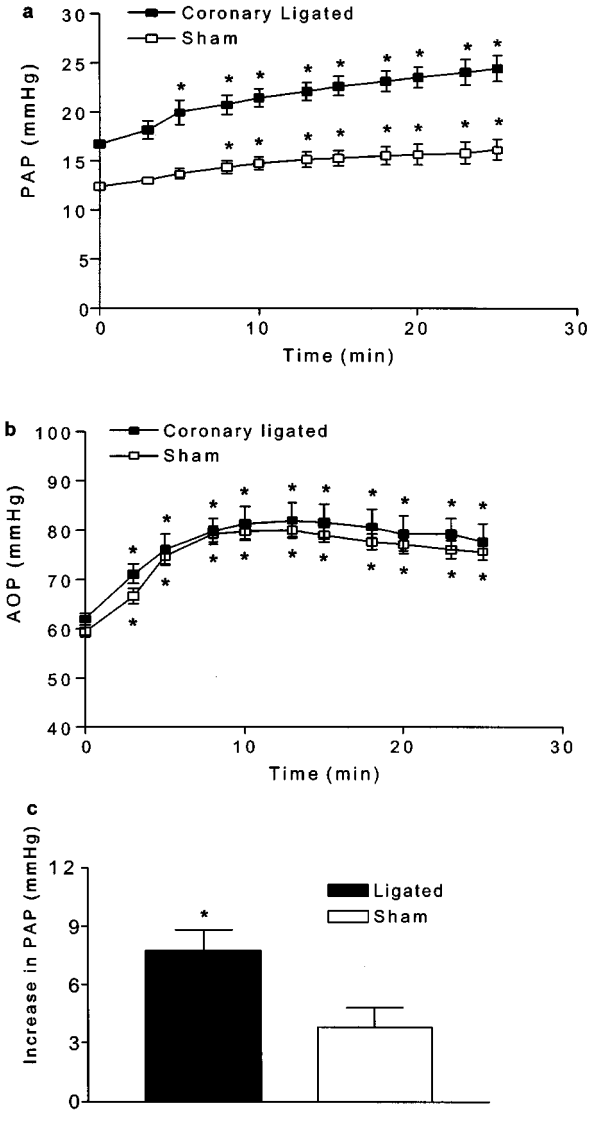

Figure 1 Effect of L-NAME infusion (30 μmol.min^{-1}) on (a) mean pulmonary arterial pressure (PAP) and (b) mean aortic pressure (AOP) in coronary ligated rabbits and sham-operated controls ($n=13$ in each group). Data shown as mean$\pm$s.e.mean. Statistical analysis of the changes in pressure, within a group, compared to basal values was by repeated measures ANOVA followed by Dunnett's *post hoc* test., $^*P<0.05$. Comparison of the magnitude of PAP response to L-NAME following 25 min infusion (c) is greater in coronary ligated rabbits, $^*P<0.05$, Student's unpaired *t*-test.

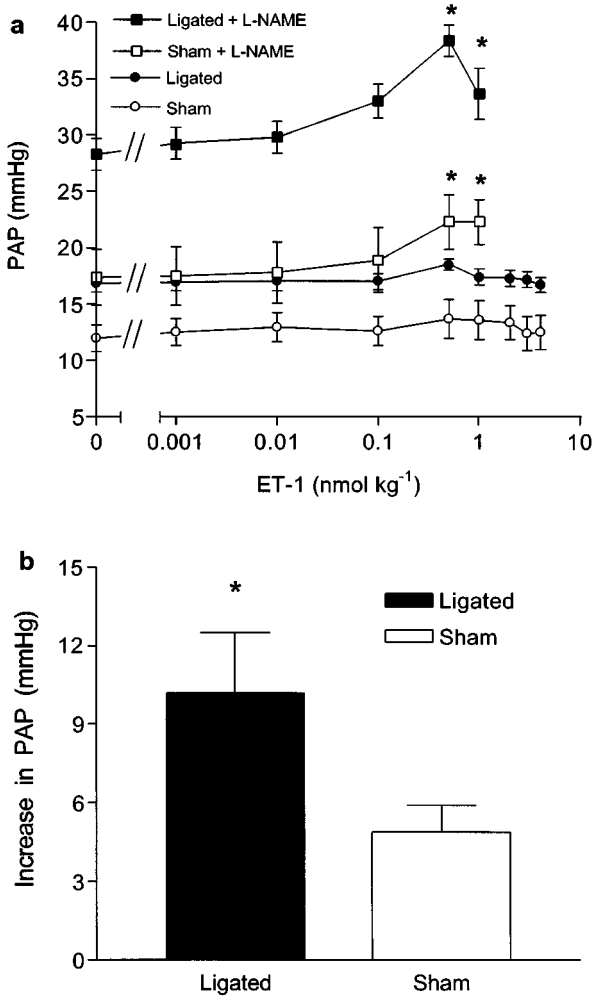

Figure 2 (a) Effect of ET-1 on mean PAP in the presence and absence of L-NAME in coronary ligated rabbits and sham-operated controls ($n=7$ in each group). Data shown as mean$\pm$s.e.mean. Statistical analysis was by repeated measures ANOVA followed by Dunnett's *post hoc* test. Changes in pressure compared to basal values, $^*P<0.05$. (b) Magnitude of PAP response to ET-1 (0.5 nmol.kg^{-1}) in the presence of L-NAME, $^*P<0.05$.

-26 ± 12 ml.min^{-1} kg^{-1} in controls). Heart rate was unaffected by big ET-1 in both groups.

Following L-NAME, big ET-1 produced a greater pulmonary pressor response in both groups of rabbits compared to the response to big ET-1 alone (Figure 3a). At a dose of 0.5 nmol.kg^{-1} in the presence of L-NAME, rabbits with PHT had a greater pulmonary pressor response compared to sham operated controls (Figure 3b). Therefore, in the presence of L-NAME, there is evidence of an increased potency to big ET-1 in the pulmonary circulation of rabbits with PHT (Figure 3a). This is indicated by a 1.8 fold leftwards shift in the response curve in rabbits with PHT, the

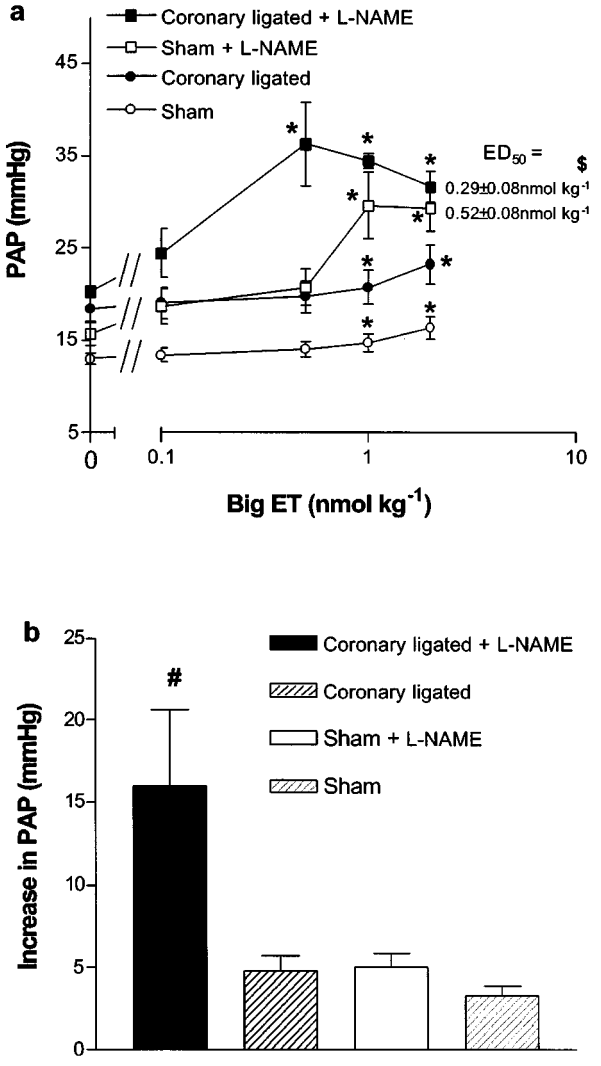

Figure 3 Effect of big ET-1 alone and in the presence of L-NAME on (a) mean PAP and (b) magnitude of PAP responses to big ET-1 alone and in the presence of L-NAME in coronary ligated rabbits and sham-operated controls. Data presented as mean$\pm$s.e.mean, $n=6$ in each group. Comparison of the ED_{50} values showed an increased potency to big ET-1 in the presence of L-NAME in the coronary ligated rabbits $\$P<0.05$ (Student's unpaired t-test). For changes in pressure compared to basal values, statistical analysis was by repeated measures ANOVA followed by Dunnett's *post hoc* test, $*P<0.05$. For between group analysis (b) one-way ANOVA followed by Tukey's *post hoc* test was used, $\#P<0.05$ indicates that in the presence of L-NAME the response to big ET-1 (0.5 nmol.kg^{-1}) is greater in coronary ligated rabbits.

ED_{50} value being significantly less in comparison to that for controls. Big ET-1 in the presence of L-NAME caused a pressor effect in the systemic circulation of both groups of rabbits (magnitude of peak response was 17.9 ± 5.1 mmHg in rabbits with PHT *c.f.* 27.0 ± 7.7 mmHg in controls).

Cardiac index was reduced in both groups of rabbits with this effect not being different between groups (-49 ± 10 ml.min^{-1} kg^{-1} in rabbits with PHT *c.f.* -40 ± 8 ml.min^{-1} kg^{-1} in controls). Heart rate was also reduced to a similar extent in both groups (-36 ± 6 b.p.m. in rabbits with PHT *c.f.* -30 ± 5 b.p.m. in controls).

Effects of SB209670

Following administration of SB209670 (10 mg.kg^{-1}) there was no effect on basal mean PAP in sham-operated control rabbits but a significant fall in mean PAP in rabbits with PHT (Figure 4a). SB209670 caused a decrease in mean AOP in both groups with no difference in this effect between groups (Figure 4b). There was no change in cardiac index or heart rate following SB209670, in either group.

L-NAME infusion, following the administration of SB209670, resulted in a pulmonary pressor response in both rabbits with PHT and sham-operated controls (Figure 5a). However, in comparison to the responses obtained previously, the magnitude of the effect was significantly reduced in the rabbits with PHT but not in sham-operated controls such that the greater effect in rabbits with PHT is lost (Figure 5b). L-NAME caused an increase in mean AOP and a reduction in cardiac index in both groups of rabbits which was not different between groups (-16 ± 5 ml.min^{-1} kg^{-1} in rabbits with PHT *c.f.* -24 ± 10 ml.min^{-1} kg^{-1} in controls). Following SB209670, heart rate was unaffected by L-NAME in either of the experimental groups.

Prior administration of the mixed ET receptor antagonist, SB209670 (10 mg.kg^{-1}) abolished the pulmonary pressor effect of ET-1 (in presence of L-NAME) in rabbits with PHT and sham-operated controls (Figure 6). ET-1 in the presence of SB209670 and L-NAME caused a systemic pressor effect in both groups with the magnitude of the peak response not being different between groups (an increase of 22.8 ± 4.0 mmHg in rabbits with PHT *c.f.* 23.3 ± 6.8 mmHg in controls).

Cardiac index was decreased in both groups with no difference in this effect between groups (-53 ± 8 ml.min^{-1} kg^{-1} in rabbits with PHT *c.f.* -70 ± 10 ml.min^{-1} kg^{-1} in controls). Heart rate was also decreased to a similar degree in both groups (-36 ± 10 b.p.m. in rabbits with PHT *c.f.* -33 ± 6 b.p.m. in controls).

Discussion

This is the first study to examine the *in vivo* pulmonary and systemic interactions between the ET-1 system and the NO system in the pulmonary circulation secondary to LVD. We provide evidence for modified regulation of pulmonary vascular tone relating to these endothelial factors in an *in vivo* model of PHT secondary to LVD. We have shown for the first time in this model, an increased responsiveness of the pulmonary hypertensive circulation to blockade of NOS and to both ET-1 and big ET-1. In addition, we have shown that the mixed ET_A/ET_B receptor antagonist SB209670 could

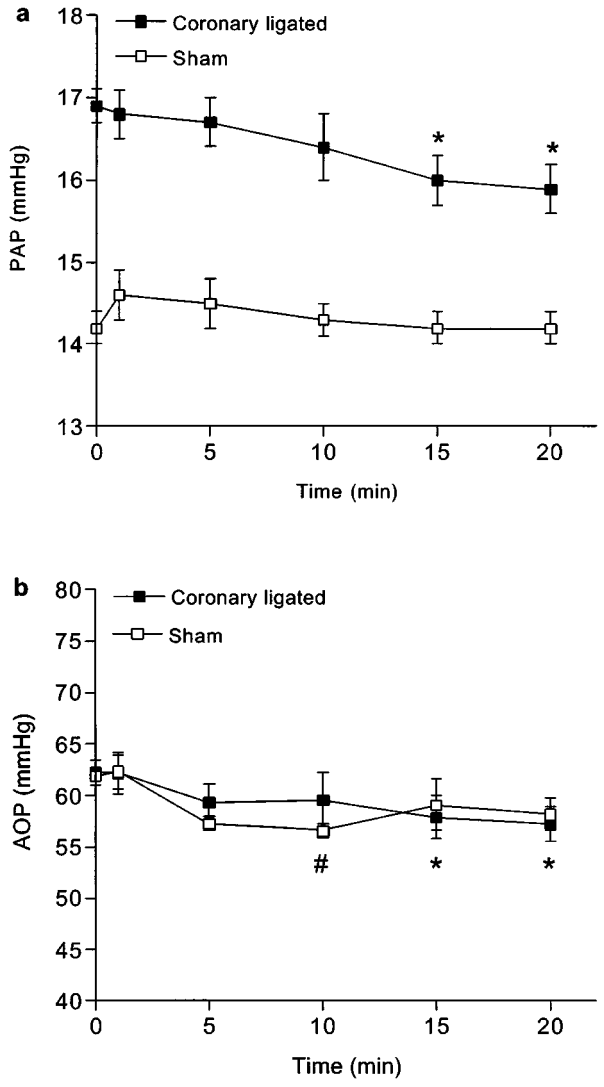

Figure 4 Effect of the mixed ET-1 receptor antagonist, SB209670 (10 mg kg^{-1}), on (a) mean PAP and (b) mean AOP in coronary ligated rabbits ($n=6$) and sham-operated controls ($n=7$). Data presented as mean ± s.e.mean. Statistical analysis was by repeated measures ANOVA followed by Dunnett's *post hoc* test. Changes in pressure compared to basal values, $*P<0.05$ for coronary ligated group and $\#P<0.05$ for controls.

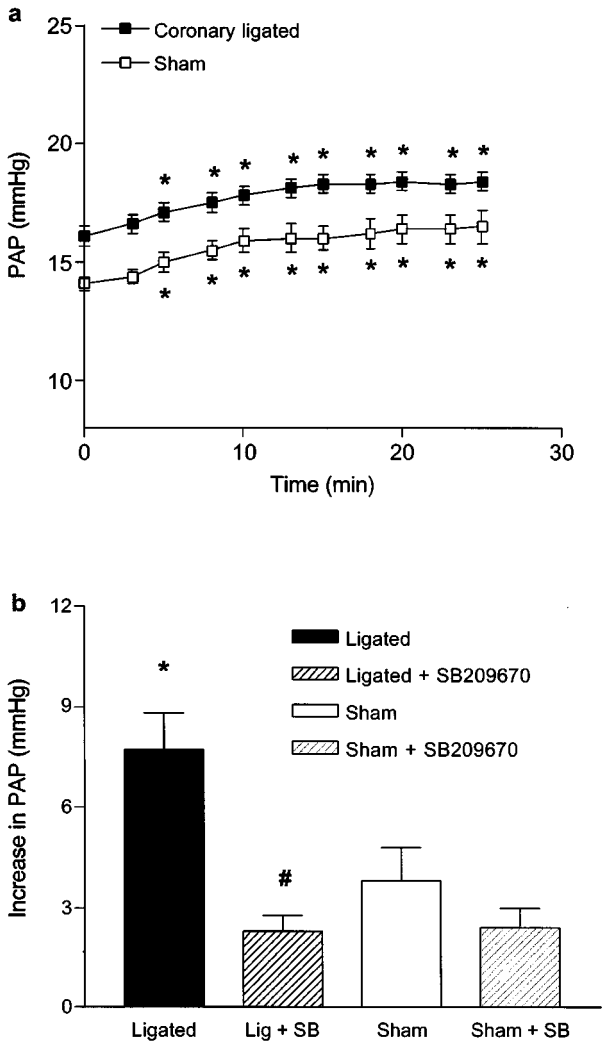

Figure 5 (a) Effect of L-NAME on mean PAP following SB209670 in coronary ligated rabbits ($n=6$) and sham-operated controls ($n=7$). Data shown as mean ± s.e.mean. Statistical analysis was by repeated measures ANOVA followed by Dunnett's *post hoc* test. Changes in pressure compared to basal values, $*P<0.05$. (b) Magnitude of response to L-NAME alone and following SB209670 (+SB). Data in absence of SB209670 is same as that presented in Figure 1c. For between group analysis one-way ANOVA followed by Tukey's *post hoc* test was applied. The response to L-NAME alone is greater in coronary ligated rabbits compared to controls ($n=13$ in each group $*P<0.05$) and the response to L-NAME following SB209670 is significantly reduced in the coronary ligated rabbits ($\#P<0.05$).

abolish the increased response to ET-1 following L-NAME in the pulmonary circulation of rabbits with PHT.

The alterations in PAP observed, most likely reflect changes in pulmonary vascular resistance (PVR) for a number of reasons. Firstly, in experiments where there was a difference in pulmonary response the increases in AOP were not different and could not have passively affected the pulmonary pressure. Secondly, cardiac index decreased and again could not explain the differences in pulmonary responses. Finally, preliminary experiments showed that interventions which resulted in PAP changes failed to alter left ventricular end diastolic pressure, which can be used as an index of left atrial pressure. Therefore assuming that left atrial pressure does not change and changes in cardiac index were not different between the groups, we can assume that changes in PAP will be proportional to changes in PVR.

The pulmonary vascular endothelium is thought to play a central role in the control of local vessel tone through the release of vasoactive factors including NO and ET-1. It is not surprising therefore that endothelial dysfunction has been proposed as a potential mechanism in disease states involving a dysfunctional vasculature such as is found in PHT (Loscalzo, 1992). Indeed acetylcholine mediated NO production has been used *in vivo*, to identify endothelial dysfunction as a possible mediating factor in the development of pulmonary vascular disease in man (Porter *et al*., 1993).

There is in fact, conflicting reports in the literature in relation to NO levels in PHT. In rats with PHT secondary to either chronic hypoxia or monocrotaline injection there is

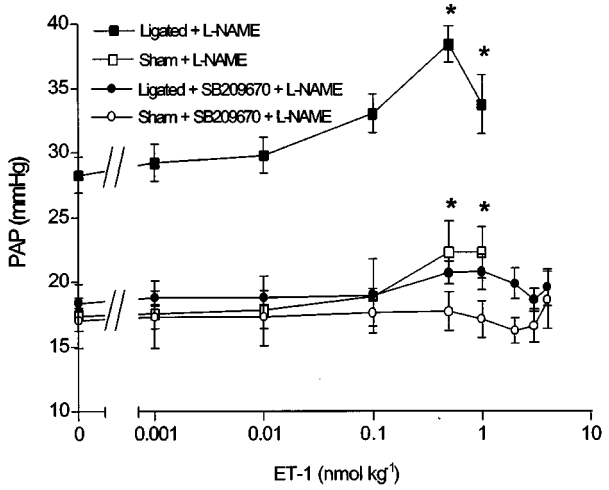

Figure 6 Effect of ET-1 following L-NAME in the presence and absence of the mixed ET-1 receptor antagonist, SB209670 (10 mg kg^{-1}), in coronary ligated rabbits ($n=6$) and sham-operated controls ($n=7$). Data in absence of SB209670 is same as that presented in Figure 2a. Data shown as mean ± s.e.mean. Statistical analysis was by repeated measures ANOVA followed by Dunnett's *post hoc* test. Changes in pressure compared to basal values, $^*P<0.05$.

evidence suggesting an increased (Xue *et al*., 1994; Madden *et al*., 1995) or a decreased NO production (Adnot *et al*., 1991; Mathew *et al*., 1995). Likewise, in patients with PHT there are reports of both decreased and increased expression of eNOS in the pulmonary circulation (Giaid & Saleh, 1995; Xue & Johns, 1995). One possible explanation for the greater pulmonary pressor effect of NOS inhibition in the rabbits with PHT compared to the controls, may be that basal NO production is elevated in PHT. In support of this possibility, it has been shown that the expression of endothelial NOS is enhanced in proliferating endothelial cells, a feature which would be evident in the vascular remodelling associated with PHT (Arnal *et al*., 1994). Indeed, we have evidence of pulmonary vascular remodelling in this rabbit model of PHT (Deuchar *et al*., 1998).

Alternatively, the greater pressor response to L-NAME could also reflect an increased vasoconstrictor sensitivity to endogenous ET-1 in the rabbits with PHT. It is not possible to exclude either of these potential explanations from the data obtained in these experiments. Indeed, similar observations have been made in *in vitro* studies using pulmonary resistance arteries from this rabbit model of PHT. These demonstrated that L-NAME potentiates the contractile response to ET-1 in vessels from coronary artery ligated rabbits, whilst having no effect on ET-1-induced vasoconstriction in vessels from sham-operated controls. However, as in this study, it is not possible to rule out either a greater ability to produce NO or an altered sensitivity of the vasoconstrictor ET receptors for the observed difference in the response (Docherty & MacLean, 1998).

In the human pulmonary circulation, production and clearance of ET-1 is balanced such that there is no net arterio-venous gradient (Dupuis *et al*., 1996). Evidence does suggest that increased ET-1 production and activity may play a role in the pathophysiology of PHT (Stewart *et al*., 1991; MacLean, 1998; 1999). For instance an increased ET-1 production has been shown in the lungs of patients with heart failure and this ET-1 spillover was found to correlate with pulmonary vascular resistance in these patients (Tsutamoto *et al*., 1994).

Surprisingly, we have previously shown exogenously administered ET-1 to have no effect on mean PAP in either rabbits with PHT or the sham-operated controls despite a potent systemic vasoconstrictor effect in both groups (Deuchar *et al*., 1998). Similar effects to exogenous ET-1 have been found in a group of patients with LVD (Cowburn *et al*., 1998). The difference in response to exogenous ET-1 between the systemic and pulmonary circulations may reflect a difference between these vascular beds relating to the regulation of vascular tone by the ET_A and ET_B receptor populations present. Thus the contribution of ET receptor subtypes mediating vasoconstriction may vary between the systemic and the pulmonary circulation. Indeed, various *in vitro* studies have shown evidence of a heterogeneous population of ET_A and ET_B receptors present in the pulmonary vasculature of various species leading to atypical responses to ET-1 in these vessels (Docherty & MacLean, 1998; McCulloch *et al*., 1998). Also, the pulmonary circulation is the main site for removal of ET-1 from the circulation, a function attributed to the ET_B receptors located on the endothelium (Fukuroda *et al*., 1994). The possibility that this may account for the differing effects between the pulmonary and systemic circulations can not be ruled out.

Another inconsistency which required investigation was that studies on isolated human, rabbit and rat pulmonary arteries have demonstrated that ET-1 is a potent vasoconstrictor in these vessels (Docherty & MacLean, 1998; McCulloch *et al*., 1998). This is inconsistent with the absence of a pulmonary pressor effect to i.v. ET-1 in humans and rabbit (Cowburn *et al*., 1998; Deuchar *et al*., 1998). We hypothesized that this lack of effect *in vivo* in comparison to the *in vitro* findings, may be explained by considering the location of the ET receptors. *In vitro*, ET-1 is able to act at the vascular smooth muscle and endothelial ET receptors simultaneously resulting in vasoconstriction. However, exogenous administration of ET-1 *in vivo* would initially activate the vasodilator endothelial ET_B receptors thereby masking the effect of vasoconstrictor ET-1 receptors on the underlying vascular smooth muscle cells. We have shown that by blocking NO production and thereby the predominant role of the endothelial ET_B receptors *in vivo* to exogenous ET-1, a pulmonary vasoconstrictor response was uncovered in both groups of rabbits. The magnitude of this response was greater in the rabbits with PHT, suggesting an increased responsiveness of the pulmonary vasculature to exogenous ET-1 at the vasoconstrictor receptors.

The suggestion that endothelial release of NO masks the effects of exogenous ET-1 was investigated further by examining the effects of exogenous big ET-1. ET-1 is converted from big ET-1 *via* the activity of ECE located within or on the surface of endothelial cells (Schweizer *et al*., 1997). ET-1 produced by the endothelium from big ET-1 is paracrine in nature with as much as 80% being released abluminally towards the underlying vascular smooth muscle cells (Wagner *et al*., 1992) explaining the lack of vasodilator response due to little or no activation of the endothelial ET_B receptors (Haleen *et al*., 1993). Our results are consistent with these observations, as big ET-1 increased PAP even in the

absence of L-NAME, while ET-1 itself does not. In the presence of L-NAME big ET-1 induced a more potent pulmonary pressor response in the rabbits with PHT. In addition to the increased responsiveness of the vasoconstrictor ET-1 receptors uncovered following L-NAME administration being an explanation, it is also possible that there could be an increased ECE activity in the rabbits with PHT leading to higher relative ET-1 levels irrespective of whether ET-1 clearance is reduced or unaffected. Indeed in a separate study carried out on patients with heart failure with elevated PAP it was shown that there was an increase in the ratio of ET-1 to big ET-1 in the plasma as compared to patients with normal PAP (Cowburn *et al*., 1998). It is not known whether this is due to differences in clearance of ET or an increased ECE activity in these patients. Also in experiments carried out on isolated rat lungs it was shown that hypoxia increased the conversion of big ET-1 to ET-1 suggesting an increase in ECE activity (Lal *et al*., 2000).

The increasing availability of ET receptor antagonists which are both receptor specific and non specific has provided a useful tool for investigating a potential role for ET-1 in the pathophysiology of many diseases (Benigni & Remuzzi, 1999). There have been some encouraging results from studies using ET antagonists in animal models of PHT with reports of both reversal of PHT and prevention of its development (Willette *et al*., 1997; Chen *et al*., 1995).

Having uncovered a response to ET-1 with L-NAME we aimed to look at the effect of a mixed ET_A/ET_B receptor antagonist on both the raised basal PAP and the increased response to ET-1 seen in rabbits with PHT. The rationale for using SB209670 is that we have previously shown, in isolated rabbit, human and rat small pulmonary arteries, that selective ET_A antagonists or selective ET_B antagonists fail to inhibit contractile responses to ET-1, whilst SB209670 is a potent and competitive inhibitor of such responses (McCulloch *et al*., 1996; 1998; Docherty & MacLean, 1998; MacLean *et al*., 1998).

SB209670 induced a fall in the PAP in the rabbits with PHT, while having no effect on PAP in sham-operated rabbits. Although this effect was small it represents a 37% reduction in the difference in mean PAP between rabbits with PHT and sham operated controls when compared to basal values. This finding together with the increased response to ET-1 following NOS inhibition provides support for a pathophysiological role for ET-1 in this model. In the rabbits with PHT, i.v. administration of SB209670 significantly reduced the subsequent pulmonary pressor response to L-NAME. Firstly, this may imply that under basal conditions the pulmonary endothelial ET_B receptors are responsible for an increase in NO production in this model of PHT. However, it is possible that by blocking NOS we are unmasking a greater underlying vasoconstriction mediated *via* endogenous ET-1 as opposed to the removal of an increased vasodilator function through NO production. Thus by blocking NOS the greater increase in PAP in PHT rabbits may reflect either an increase in basal NO production and/or a greater underlying response to endogenous ET-1.

The pulmonary response to ET-1, uncovered by L-NAME, was abolished in both groups following the administration of SB209670. Therefore, SB209670 given i.v. is not only potentially antihypertensive in the diseased pulmonary vasculature but is also effectively blocking the response to further increases in plasma ET-1 levels as may be evident in cases of PHT, providing further support for the potential use of ET antagonists in the management of PHT associated with LVD.

In conclusion, we have shown evidence to implicate a modified contribution of endothelial derived factors in the control of pulmonary vascular tone in rabbits with PHT secondary to LVD. We have demonstrated that blockade of NOS unmasks a greater pulmonary pressor response to exogenously administered ET-1 and big-ET. These findings together with the promising effects of the mixed ET_A/ET_B receptor antagonist suggest a possible role for ET-1 in the aetiology of PHT in this model and supports further work for developing ET-1 receptor antagonists as a potential therapy in the treatment of PHT associated with heart disease.

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