Altered Pulmonary Microvascular Reactivity to Norepinephrine in Canine Pacing-Induced Heart Failure

Mary I. Townsley, Vicki H. Pitts, Jeffrey L. Ardell, Zhuodao Zhao, Walter H. Johnson, Jr

Abstract Pulmonary hypertension in congestive heart failure causes medial hypertrophy in pulmonary vessels and thickening of the endothelial basement membrane. In this study, the functional consequences of such pulmonary vascular adaptations were evaluated. Heart failure was induced in dogs by rapid ventricular pacing (240 beats per minute) for 28 days, at which time left ventricular shortening fraction was decreased by 57% compared with that at baseline. Lung lobes from paced (n=56) and control dogs (n=68) were isolated and perfused with autologous blood. Total, arterial (Ra), and venous (Rv) vascular resistances were significantly increased and vascular capacitance decreased in lobes from paced animals compared with controls. Increment in Ra and Rv after intra-arterial boluses of norepinephrine were measured before and after sequential addition of the α1- and α2-receptor antagonists prazosin (16 μmol/L) and yohimbine (0.1 μmol/L) in the presence or absence of propranolol (5 μmol/L). Norepinephrine (1 to 40 μg) had little effect on Ra in the absence of propranolol, a pattern that persisted in control lobes after propranolol. However, when lobes from paced animals were pretreated with propranolol, norepinephrine increased Ra. Rv was increased after norepinephrine in control lobes, an effect that was enhanced in the paced group. In both groups, the increment in Rv was greater after propranolol. Irrespective of propranolol pretreatment, prazosin significantly attenuated, if not abolished, the response to norepinephrine. The enhancement in venous vascular reactivity in lobes from paced animals remained when venous pressure was elevated to 20 cm H₂O. In control lobes under conditions of elevated tone or when endothelium-dependent relaxing factor was blocked, responses to norepinephrine did not mimic those observed in the paced group. Microvascular permeability, as measured by the capillary filtration coefficient, was not altered in the paced group. We conclude that the pulmonary adaptations to 4 weeks of rapid ventricular pacing include functional changes in pulmonary hemodynamics and vascular reactivity but not in microvascular permeability. (Circ Res. 1994;75:47-356.)

Key Words • congestive heart failure • pulmonary venous hypertension • pulmonary microvascular permeability • pulmonary circulation • isolated lung • yohimbine • prazosin • propranolol

Congestive heart failure is associated with chronic exposure of the lung to high pulmonary vascular pressures. In part, this may be due to the increased circulating levels of humoral vasoconstrictors such as catecholamines and angiotensin II observed in animal models of heart failure and in humans with congestive heart failure.1-3 Although pulmonary capillary pressure (Pc), and therefore transcapillary fluid flux, would be predicted to be increased as a result, not all patients with congestive heart failure or high pulmonary vascular pressures secondary to mitral stenosis develop pulmonary edema.3 Several mechanisms could potentially contribute to protection of the lung against edema formation and alveolar flooding in congestive heart failure, including remodeling of the pulmonary microvascular exchange barrier to limit permeability to proteins and/or water. Thickening of the pulmonary endothelial basement membrane in heart failure4,5 supports this possibility. In addition, remodeling of pulmonary vascular smooth muscle mass and/or reactivity could be associated with an enhanced response of the pulmonary vasculature to circulating vasoconstrictor substances.6,7 Such an increase in pulmonary vascular reactivity could conceivably attenuate transmission of the left atrial pressure pulse wave to the pulmonary microcirculation. Although medial hypertrophy in both pulmonary arteries and veins has been reported in chronic pulmonary venous hypertension,8 the physiological consequences of such changes have not been evaluated.

We sought to quantify changes in pulmonary microvascular permeability and vascular adrenergic reactivity after development of congestive heart failure induced in dogs by rapid ventricular pacing.1 We hypothesized that heart failure and the resultant venous hypertension would cause a decrease in pulmonary microvascular permeability and an increase in vascular adrenergic reactivity. In lung lobes from control and paced animals, microvascular permeability was assessed with the capillary filtration coefficient (Kcc). Vascular reactivity was assessed by measuring changes in segmental pulmonary vascular resistance after intra-arterial bolus injections of norepinephrine. Further, the response to norepinephrine was evaluated in the presence and absence of pharmacological inhibitors of α1-, α2-, and β-adrenergic receptors. Portions of these studies have been briefly reported elsewhere.9,10

Materials and Methods

Pacing Model of Heart Failure

Conditioned, microfilaria-negative mongrel dogs (n=54, 22.3±0.5 kg, mean±SEM) were anesthetized with sodium
By guest on October 22, 2017 http://circres.ahajournals.org/ Downloaded from (30 mg/kg Tip, model 4011) and generator, was introduced into the right ventricle via the right jugular vein. Proper placement of the pacing lead was confirmed by the ability to capture ventricular rate, verified by surface ECG. A small subcutaneous pocket was created anterior to the first rib and a pacemaker generator (Medtronic, customSX-5985 or 8329) inserted. The pacing lead was tunneled subcutaneously to the pocket and attached to the generator, and the skin incisions were closed in layers. The dogs were given buprenorphine (0.2 mg IM) as needed during recovery for analgesia. Antibiotics (cefaclorin sodium, 500 mg PO twice daily) were administered for 5 days after surgery. Animals were allowed free access to food and water throughout the postoperative period and the subsequent pacing period.

Pacing at 240 beats per minute was initiated 1 to 2 days after recovery from surgery; rate was controlled via an external programmer (Medtronic, model 9710). Maintenance of pacing was monitored daily by palpation and verified by a biweekly echocardiogram. The echocardiograms were recorded on videotape at baseline before the initiation of pacing and biweekly thereafter for subsequent analysis of left ventricular shortening fraction (LVSF). All such recordings were made while the dogs were awake, lying quietly in a right lateral decubitus position. Pacing was maintained until LVSF in sinus rhythm fell to approximately 50% of the baseline value. LVSF was calculated as the difference in left ventricular end-diastolic and end-systolic diameters divided by the end-diastolic diameter.

**Controls**

Controls consisted of microfilaria-negative mongrel dogs (n = 68, 19.9 ± 0.5 kg) studied acutely. In one subset of control animals, echocardiograms were recorded just before the experiment while the animals were awake and lying in a right lateral decubitus position.

**Terminal Experiment**

After an overnight fast, analgesia was induced in all animals by ketamine hydrochloride (200 mg IM). Heparin (10 000 units IV) was then administered, followed by sodium pentobarbital (<15 mg/kg IV in the paced animals, up to 30 mg/kg IV in controls) until a surgical plane of anesthesia was induced. Once the initial anesthetic regimen was completed, anesthesia was maintained by intravenous administration of a-chloralose. We have found that this protocol adequately maintains a surgical plane of anesthesia without marked cardiovascular depression, even in most of the paced group. An oral airway was introduced as soon as relaxation was apparent, and ventilation was initiated (15 mL/kg tidal volume, 15 breaths per minute). A carotid artery was cannulated for measurement of systemic arterial blood pressure (SAP) and arterial blood gases and for blood withdrawal. Central venous pressure (CVP) was measured via a catheter placed in the superior vena cava via the jugular vein. A catheter was advanced into the left ventricle via a femoral artery for measurement of left ventricular end-diastolic pressure (LVEDP). In vivo measures of blood gases, SAP, CVP, and LVEDP could not be made in some paced animals because of anesthetic-induced cardiovascular collapse. Vascular pressures and blood gases were measured only in one subset of control animals. After the in vivo measurements, the lung lobes were isolated.

**Isolated Lung Preparation**

Surgical details of the lower left lung lobe isolation and cannulation have been described previously. Briefly, the left chest was opened via an incision at the fifth intercostal space. The left upper and middle lobes were excised for measurement of blood-free extravascular lung water (see below). The lower left lung lobe was then isolated. In two animals, the upper right lobe was also isolated for ex vivo perfusion, although these lobes were used for different protocols within this experiment. We routinely isolate and perfuse up to three separate lobes from each animal, always for separate protocols. There are no differences in either vascular hemodynamics or permeability between lobes in the canine lung. For each lobe, a mixture of 200 mL blood, removed via the carotid cannula, and 100 mL sterile Earle’s buffer solution was used to fill the perfusion system. Use of diluted blood for lobar perfusion has no effect on microvascular permeability, as evidenced by a lack of change in the capillary filtration coefficient over a wide range of hematocrit and plasma protein concentrations. Similarly, the magnitude and distribution of vascular resistance measured in these lobes were not different from those observed in lobes perfused with whole blood.

Lobes were rapidly excised and wide-bore plastic cannulas tied into the lobar artery, vein, and bronchus. Lobes were suspended from a counterbalanced force transducer (Grass model FT-10). The weight recording system was set to provide 1.0 cm of deflection per gram weight gain. Lobes were ventilated throughout the study with 30% O2/5% CO2 at a rate of 6 breaths per minute, unless otherwise noted (see protocols outlined below). End-expiratory pressure was set to 2 cm H2O, while tidal volume was set to produce peak inspiratory pressures ranging from 8 to 10 cm H2O. The recirculating blood in the perfusion system was warmed to 37°C. Perfusion pH was measured and corrected to 7.35 to 7.40 with the addition of sodium bicarbonate, unless otherwise noted.

**Lobar Hemodynamics**

Arterial (Pa) and venous (Pv) pressures (cm H2O) were measured via thin catheters placed at the orifices of the inflow and outflow catheters, respectively, and zero pressure was referenced to the level of the lung hilum. Pv was set to 4 to 5 cm H2O, and blood flow was increased to the maximal level that could be attained while the lobe was kept in an isogravimetric state. Thereafter, blood flow was held constant. Pcv was measured by the double vascular occlusion technique. Blood flow (Q) was measured by timed collection of venous effluent in a graduated cylinder and referenced either to 100 g initial lobe wet weight or to 1 g blood-free dry weight, the latter obtained from the lung water analysis detailed below. All pressures and lung weights were continuously recorded on either a Grass or Beckman polygraph. Total pulmonary vascular resistance (Rt) was calculated as Rt = (Pa – Pv)/Q. Pcv was used to partition Rt into precapillary (Ra) and postcapillary (Rv) resistances: Rv = (Pa – Pcv)/Q and R = (Pcv – Pv)/Q, respectively. A rapid venous occlusion was used to evaluate total vascular compliance (Ct) as described by Linehan et al. On venous occlusion, venous pressure rises abruptly to equal that upstream of the largest pulmonary veins and thereafter at a slower but linear rate dependent on the compliance of the vasculature and the rate of blood flow into the vascular compartment. Ct is then calculated as Ct = Q/[the linear rate of ΔPv/Δt after rapid venous occlusion].

**Evaluation of Permeability and Transvascular Fluid Exchange**

The capillary filtration coefficient (Kf,c), used as a measure of microvascular permeability, was evaluated after an 8 to 10 cm H2O step increase in Pv. The rate of weight gain became constant after approximately 7 to 10 minutes. The (AW/Δt) between minutes 10 and 15 was used to calculate Kf,c by the equation Kf,c = (AW/Δt)/ΔPc. ΔPc was calculated as the difference in Pcv measured before and at the end of the 15-minute elevated pressure period. If the lobe was not isogravimetric before the Kf,c maneuver, the pre-Kf,c rate of weight gain was subtracted from that measured at elevated pressure, and the difference was used to calculate Kf,c. Kf,c was expressed in mL min−1 cm H2O−1 per 100 g initial lobe wet weight.

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weight or per 1 g blood-free dry weight; the density of the filtered fluid was assumed to be 1 g/mL.

Blood-free extravascular lung water (EVLW) was measured by a modification of the method devised by Pearce et al. Briefly, lobes were homogenized with 100 mL distilled water. Then aliquots of blood, lung homogenate, and homogenate supernatant (obtained after centrifugation of homogenate at 15 000 rpm for 1 hour) were weighed and then dried to constant weight (65°C). Total hemoglobin was measured in both blood and supernatant, allowing correction of lung water for blood water. EVLW is reported as milliliters per gram blood-free dry weight. Total lobar blood-free dry weights for the perfused lobes were also obtained in this analysis.

Isolated Lung Protocols

In all lobes from control and paced animals, irrespective of the protocol, the distribution of vascular resistance, Ct, and Kc were measured after the maximal isovameric blood flow had been established. After the Kc maneuver and when a stable hemodynamic state had been established, the distribution of vascular resistance was measured before and at the peak response after intra-arterial bolus injections of norepinephrine (bolus volume was <40 μL). Vascular pressures were allowed to return spontaneously to the prenorepinephrine level before the next dose was administered. The vascular responses to norepinephrine were evaluated by one of the following protocols:

1. The baseline dose response to norepinephrine (1 to 40 μg) was evaluated in lobes from both control (n=12) and paced (n=13) animals, in which lobar Pv was maintained at 4 to 5 cm H2O throughout the study. Some of the lobes in each group were not ventilated but rather statically inflated with room air to a lobar airway pressure of 3 cm H2O. However, since there were no differences in the response to norepinephrine with respect to ventilation regimen, the results were combined.

2. A similar dose response to norepinephrine was evaluated in control (n=8) and paced (n=6) groups with acute venous hypertension. In these lobes, Pv was increased to 20 cm H2O after the baseline measurements but before any administration of norepinephrine and was kept at this level during the entire dose response curve.

3. The response to norepinephrine (10 and 40 μg) was evaluated in lobes from control (n=17) and paced (n=19) animals after selective adrenergic blockade. Responses obtained in protocol 1 are included in this overall summary. In other control (n=19) and paced (n=20) groups, lobar responses were measured 30 minutes after the addition of propranolol (5 μmol/L) to the perfusate to block β-adrenergic responses. In subsets of these lobes, after measurement of baseline responses, norepinephrine challenge was repeated after the sequential addition of the α1-adrenergic antagonist prazosin (16 μmol/L) and yohimbine (0.1 μmol/L); an α2-antagonist, as described below. In each case, the antagonist was allowed to circulate for 30 minutes before any challenge with norepinephrine. The specific order of α-antagonist was as follows: (a) In control (n=5) and paced (n=6) groups in which lobes were not treated with propranolol, prazosin was added first, followed by yohimbine. (b) In other control (n=7) and paced (n=10) groups, lobes were pretreated with propranolol, then prazosin was added first, followed by yohimbine. (c) In final control (n=5) and paced (n=7) groups, lobes were also pretreated with propranolol, but the order in which the α-antagonists were introduced was reversed.

4. β-Adrenergic receptor-mediated responses were further evaluated in separate control (n=8) and paced (n=6) groups. In these lobes, prazosin (16 μmol/L) and yohimbine (0.1 μmol/L) were added to the perfusate to block α1- and α2-adrenergic receptors. Responses to norepinephrine were then evaluated in these lobes before and after total vascular resistance was increased approximately twofold with the addition of 10% KCl.

5. Interactions of α1-adrenergic receptors and endothelium-derived relaxing factor, or nitric oxide, were investigated in control (n=9) and paced (n=5) groups. Propranolol (5 μmol/L) and prazosin (16 μmol/L) were added to restrict responses to those involving only α1-adrenergic receptors. After 30 minutes, the response to intra-arterial boluses of 10, 40, and 100 μg norepinephrine was evaluated. After recovery, N^-nitro-L-arginine methyl ester (L-NAME, 0.3 μmol/L) was added to the perfusate to inhibit nitric oxide synthesis, and norepinephrine challenge was repeated 30 minutes later.

6. In separate groups of control lobes, the response to norepinephrine was evaluated when tone was elevated. After baseline measurements and after responses to norepinephrine were evaluated at a Pv of 20 cm H2O in one group of control lobes (n=6), Ppv was returned to 5 cm H2O and the lobe allowed to reequilibrate. Vascular tone was then increased by addition of KCl to the perfusate in sufficient quantity to increase total vascular resistance approximately threefold. When lobar hemodynamics had stabilized, challenge with norepinephrine (10 and 40 μg) was repeated. Results in these lobes were compared with those in the control group under protocol 1 (basal tone). A separate group of control lobes (n=7) was pretreated with prazosin (5 μmol/L) and yohimbine (0.1 μmol/L) 30 minutes before the challenge with norepinephrine to isolate responses to α1-adrenergic receptors. Responses to norepinephrine (10 and 40 μg) were evaluated before and after tone was increased with KCl as outlined above.

Drugs

Norepinephrine (MW 205.6), propranolol (MW 295.8), prazosin (MW 419.9), yohimbine (MW 390.9), phenylephrine (MW 203.7) hydrochloride, and L-NAME (MW 269.7) were obtained from Sigma Chemical Co. Drugs were prepared daily as stocks in sterile 0.9% saline. Aliquots of the propranolol, prazosin, yohimbine, and L-NAME stocks were added to the perfusate (300 mL) to achieve the circulating concentrations cited above. Doses of norepinephrine and phenylephrine are quoted as micrograms injected. The bolus doses of norepinephrine (1 to 40 μg) injected into the 300-mL perfuse volume are equivalent to circulating concentrations ranging from 1.6x10^-5 to 6.5x10^-3 mol/L.

After the final responses to norepinephrine were measured, 2 to 4 mg papaverine hydrochloride were added to the perfusate to facilitate vasodilation; this dose of papaverine is insufficient to alter pulmonary microvascular permeability. When a new hemodynamic steady state was reached, the final measures of the vascular resistance distribution, Ct, and Kc were obtained.

Data are presented as mean±SEM. Statistical differences were evaluated by ANOVA, with a Student-Newman-Keuls post hoc test to identify specific differences.

Results

In the heart failure group, animals were paced for an average of 28±1 days. All paced animals appeared to tolerate this intervention relatively well for several weeks. Overt signs of distress such as dyspnea and apathy did not appear until the last few days of pacing and did not appear in all animals. At the time of the terminal experiment, ascites and airway fluid were evident in 24 (44%) and 22 (40%) of the 54 paced animals, respectively. Pleural effusions were observed less frequently (9%), although pericardial effusion was seen in 33 (61%) of the paced animals. Body weight at the terminal study (22.8±0.5 kg) was not significantly different from the initial weight.
As a result of rapid ventricular pacing, significant changes were observed in both left ventricular function and left ventricular hemodynamics, as shown in Table 1. Pacing resulted in a decrease in sinus rhythm LVSF from 33.6±1.1% to 13.9±0.4% (P<.05). In control animals, sinus LVSF was no different from the prepacing value in the paced animals. The pacing-induced reduction in LVSF was accompanied by a 10-fold increase in LVEDP and a 5-fold increase in CVP compared with controls (P<.05). Despite these changes, SAP in sinus rhythm was not different in the two groups. Maintenance of normal SAP in the paced group despite substantial pacing-induced depression of cardiac performance is probably a result of our use of ketamine as a preanesthetic. Ketamine is an excitatory anesthetic, resulting in sympathetic activation in dogs.22 As a consequence of this pacing protocol, the pulmonary circulation was exposed to higher than normal pulmonary Pv. Fig 1 shows the time course of changes in left atrial pressure in four additional awake, paced dogs in whom left atrial catheters were implanted during the initial sterile surgery. Although mean left atrial pressures rose only modestly over the pacing period, it is clear that the peak pressures were increased substantially within the first week in these animals.

As a consequence of the hypertension accompanying pacing, EVLW in the paced group was 37% higher on average than in controls (P<.05, Table 1). In addition, systemic oxygenation was modestly impaired in the paced group, in that arterial PO2 was 31% less than in controls (P<.05). When lung lobes from these dogs were isolated, the initial lobar wet weight in the paced group was found to be nearly double that in controls (P<.05), as shown in Table 2. Since there were no differences in Ked, our measure of microvascular permeability, as a function of ex vivo perfusion time in any group, the baseline and final values were averaged for intergroup comparisons. The average Ked in lobes from paced animals was not different from that in controls when calculated on the basis of lobar wet weight (0.073±0.007 versus 0.074±0.004 mL·min⁻¹·cm H₂O⁻¹·100 g⁻¹, respectively). This similarity between groups remained when Ked was referenced to the lobar blood-free dry weight (0.0049±0.0004 versus 0.0042±0.0002 mL·min⁻¹·cm H₂O⁻¹·g dry weight⁻¹, respectively).

The summary of baseline lobar hemodynamics in the control and paced groups is shown in Table 2. These data represent hemodynamic measurements made before any antagonist administration and challenge with norepinephrine. Lobar Pa and Pc were statistically higher and the measured blood flow rate lower in lobes from paced animals compared with controls (P<.05). Similarly, Q/100 g wet weight was significantly lower in the paced group. Since pulmonary blood flow in isolated lung lobes is commonly normalized to 100 g wet weight of lung11,14 and since EVLW and initial lung weight were increased in the paced group, the normalized blood flows could potentially have been biased by the difference in tissue water. However, the substantial difference in normalized blood flow remained even

### Table 1. In Vivo Measurements of Cardiovascular Function and Gas Exchange

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Paced</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals in group</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>19.9±0.5</td>
<td>22.8±0.5*</td>
</tr>
<tr>
<td>SAP, mm Hg</td>
<td>115±6 (5)</td>
<td>122±3 (45)</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>2.0±0.5 (5)</td>
<td>10.2±1.0* (43)</td>
</tr>
<tr>
<td>LVSF, %</td>
<td>26.5±2.7 (6)</td>
<td>13.9±0.4* (53)</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>3.9±0.9 (6)</td>
<td>39.9±1.8* (50)</td>
</tr>
<tr>
<td>EVLW, mL/g</td>
<td>3.93±0.06 (56)</td>
<td>5.39±0.12* (50)</td>
</tr>
<tr>
<td>Po2, mm Hg</td>
<td>103±5 (5)</td>
<td>71±2* (47)</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>29±1 (5)</td>
<td>28±1 (50)</td>
</tr>
<tr>
<td>pH</td>
<td>7.44±0.02 (5)</td>
<td>7.40±0.01 (50)</td>
</tr>
</tbody>
</table>

SAP indicates systemic arterial pressure; CVP, central venous pressure; LVSF, left ventricular shortening fraction; LVEDP, left ventricular end diastolic pressure; and EVLW, extravascular lung water expressed per gram blood-free dry lung weight. Data are mean±SEM for control and paced animals during the terminal experiment. Pressure measurements were obtained in sinus rhythm. Numbers in parentheses represent the numbers of animals included in this mean. *P<.05 vs control group.

### Table 2. Baseline Hemodynamics in Isolated Lung Lobes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Paced</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of lobes</td>
<td>68</td>
<td>56</td>
</tr>
<tr>
<td>Pa, cm H₂O</td>
<td>14.8±0.3</td>
<td>17.7±0.5*</td>
</tr>
<tr>
<td>Pc, cm H₂O</td>
<td>10.1±0.2</td>
<td>11.6±0.3*</td>
</tr>
<tr>
<td>Pv, cm H₂O</td>
<td>4.4±0.1</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>Q, L/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual</td>
<td>0.50±0.01</td>
<td>0.44±0.01*</td>
</tr>
<tr>
<td>Per 100 g wet wt</td>
<td>1.27±0.04</td>
<td>0.63±0.03*</td>
</tr>
<tr>
<td>Per g dry wt</td>
<td>0.075±0.002</td>
<td>0.042±0.002*</td>
</tr>
<tr>
<td>Ct, mL/cm H₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per 100 g wet wt</td>
<td>2.14±0.08</td>
<td>1.03±0.06*</td>
</tr>
<tr>
<td>Per g dry wt</td>
<td>0.12±0.01</td>
<td>0.07±0.01*</td>
</tr>
<tr>
<td>Lobar wet wt, g</td>
<td>40.2±1.3</td>
<td>78.2±4.2*</td>
</tr>
<tr>
<td>Lobar blood-free dry wt, g</td>
<td>6.9±0.2</td>
<td>11.3±0.5*</td>
</tr>
</tbody>
</table>

Pa indicates arterial pressure; Pc, capillary pressure; Pv, venous pressure; Q, blood flow; and Ct, total pulmonary vascular compliance. Data are mean±SEM in lobes from control and paced animals. *P<.05 vs controls.

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**Fig 1.** Graph showing left atrial pressures measured in four awake, paced animals plotted as a function of pacing time. Both mean and peak pressures measured during the cardiac cycle are shown. *P<.05 compared with the prepacing value (at 0 days).
when the blood-free dry lung weight was used as the basis for that normalization. The baseline distribution of total vascular resistance, normalized to both wet and dry weight, is shown in Fig 2. Overall, both Ra and Rv were increased in the paced group compared with controls. All resistances decreased (P<.05) when venous pressures were increased by 10 cm H2O during the measurement of Kt, (data not shown), although Rv remained significantly elevated in lobes from paced animals compared with controls. In concert with the alterations in vascular resistance, Ctk was significantly decreased in the paced group (Table 2).

Protocols 1 and 2

The most striking finding of this study was the alteration in vascular reactivity to norepinephrine in the paced group, detailed in Table 3. At a Pv of 5 cm H2O, norepinephrine had little effect on Ra in control lobes and induced only a small, though significant, dose-dependent increase in Ra in lobes from paced animals. Although the ΔRa after 1, 4, and 40 μg norepinephrine was greater in lobes from paced animals studied at a Pv of 20 cm H2O compared with that at the lower Pv, there was no dose-dependent response in this group. In contrast to the arterial response, in control lobes at a Pv of 5 cm H2O, norepinephrine induced moderate but dose-dependent vasoconstriction and an enhanced, dose-dependent increase in Rv (P<.05) in lobes from paced animals. When Pv was elevated to 20 cm H2O in control lobes, the pulmonary venous response to norepinephrine was significantly attenuated compared with that in the low-Pv group (P<.05 for 1 and 40 μg; P=.06 for 4 and 10 μg). In contrast, in the paced group, the increase in Rv induced by norepinephrine was clearly sustained in lobes studied at 20 cm H2O and even enhanced at the highest dose.

Protocols 3 and 4

Fig 3 shows the overall response to norepinephrine with and without β-adrenergic receptor blockade. The ΔRa in response to norepinephrine clearly was enhanced in the paced group but not in controls when lobes were pretreated with the β-antagonist (top panel, right). In contrast, propranolol significantly enhanced the vasoconstrictor response in both groups (bottom panel, right). Note that the resistance scale for ΔRv is different from that used for ΔRa in the top panel of Fig 3. Fig 4 illustrates the effects of selective α-adrenergic antagonists on ΔRv in subsets of these lobes. The pattern of changes in ΔRa after α-blockade was essentially the same (data not shown). The norepinephrine-induced vasoconstriction was totally abolished after the addition of prazosin, irrespective of whether the lobes

Table 3. Changes in Ra and Rv After Norepinephrine Challenge in Control and Paced Groups

<table>
<thead>
<tr>
<th>Norepinephrine Dose, μg</th>
<th>1</th>
<th>4</th>
<th>10</th>
<th>40</th>
</tr>
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<tbody>
<tr>
<td>ΔRa, cm H2O · L⁻¹ · min⁻¹ · 100 g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pv=5 cm H2O</td>
<td>0±0.2</td>
<td>0.1±0.4</td>
<td>0±0.3</td>
<td>0.3±0.4</td>
</tr>
<tr>
<td>Pv=20 cm H2O</td>
<td>0.5±0.2</td>
<td>0.2±0.1</td>
<td>0.7±0.4</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>Paced</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pv=5 cm H2O</td>
<td>−0.2±0.5</td>
<td>−0.7±0.4</td>
<td>0.2±0.8</td>
<td>1.4±0.9*</td>
</tr>
<tr>
<td>Pv=20 cm H2O</td>
<td>2.9±1.4†</td>
<td>5.0±2.5††</td>
<td>4.1±2.7</td>
<td>5.9±1.8††</td>
</tr>
<tr>
<td>ΔRv, cm H2O · L⁻¹ · min⁻¹ · 100 g⁻¹</td>
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</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pv=5 cm H2O</td>
<td>0.6±0.2</td>
<td>1.0±0.4</td>
<td>1.6±0.3</td>
<td>3.7±0.6*</td>
</tr>
<tr>
<td>Pv=20 cm H2O</td>
<td>−0.9±0.2†</td>
<td>0.1±0.2</td>
<td>0.6±0.4</td>
<td>1.4±0.6†</td>
</tr>
<tr>
<td>Paced</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pv=5 cm H2O</td>
<td>3.3±1.0†</td>
<td>8.3±1.8†</td>
<td>13.7±2.8‡</td>
<td>12.9±2.1*‡</td>
</tr>
<tr>
<td>Pv=20 cm H2O</td>
<td>7.9±2.4†</td>
<td>13.5±2.9‡</td>
<td>24.5±7.9‡</td>
<td>34.4±10.8*‡</td>
</tr>
</tbody>
</table>

Responses shown are absolute changes in precapillary (Ra) or postcapillary (Rv) vascular resistances (ΔRa or ΔRv), taken as the difference between the resistance at the peak of the norepinephrine response and the prechallenge baseline resistance. Lobar Pv was set to either 5 or 20 cm H2O during the entire dose response curve.

*P<.05 denotes a significant dose-dependent effect of norepinephrine; †P<.05 vs Pv=5 cm H2O within the same group; ‡P<.05 vs the paired response in controls.
Fig 3. Bar graphs showing lobar responses to intra-arterial injection of norepinephrine studied in two groups: in the absence (left) or presence (right) of propranolol (5 μmol/L). Increments in pulmonary arterial resistance ($\Delta R_A$, top) and venous resistance ($\Delta R_V$, bottom) are shown for each group. *P<.05 compared with controls within the same treatment group; **P<.01 compared with the corresponding value in the absence of propranolol.

were pretreated with propranolol. In contrast, when the order of the $\alpha$-antagonists was reversed, the $\Delta R_V$ response on the whole was not significantly altered by the addition of yohimbine (middle panel, right) but was abolished after the addition of prazosin (bottom panel, right). Together, these data from Figs 3 and 4 suggest some enhancement of $\beta$-adrenergic receptor-mediated responses in the paced group in addition to the clear enhancement in $\alpha_1$-adrenergic responsiveness. However, there was no significant difference in the response to norepinephrine between the two groups when both $\alpha_1$- and $\alpha_2$-adrenergic receptors were blocked (Fig 4, bottom panel, left). One could envision that this could be due to the relatively low tone of the lobar vasculature. However, in separate control and paced groups in which lobes were pretreated with prazosin and yohimbine, the responses to norepinephrine were not different at basal and increased tone, as shown in Table 4.

Protocol 5

Blockade of nitric oxide synthase with L-NAME did not alter baseline total pulmonary vascular resistance in lobes from either control (9.0±0.9 after L-NAME versus 8.5±0.9 cm H$_2$O·L$^{-1}$·min$^{-1}$·100 g$^{-1}$) or paced animals (24.6±2.9 after L-NAME versus 26.2±3.5 cm H$_2$O·L$^{-1}$·min$^{-1}$·100 g$^{-1}$), nor was the distribution of resistance altered by L-NAME in either group. When both $\alpha_1$- and $\beta$-adrenergic receptors were blocked in these lobes, norepinephrine had little if any effect on $R_A$. Further, in neither group did L-NAME substantially modify the arterial response to norepinephrine (data not shown). A small but statistically significant enhancement of the venoconstrictor response was observed but only in the control group and then only at the highest norepinephrine dose studied, as shown in Fig 5.

Protocol 6

A final concern was whether increases in active tone per se could contribute to the enhanced vasoreactivity in the paced group. This is a rational concern, given the documented increases in circulating humoral vasoconstrictor in this model$^{1-3}$ and the high baseline resistance in lobes isolated from paced animals (Fig 2). The paired responses to norepinephrine in control lobes at basal and increased tone are shown in Table 5. KCl increased lobar arterial and venous resistance by threefold to fivefold, irrespective of any pretreatment with $\beta$- or $\alpha_2$-agonists. It is clear that when tone in control lobes was increased to levels commonly observed in the paced group, the response to norepinephrine was not enhanced.

Discussion

Congestive heart failure is characterized by chronic exposure to high pulmonary $P_v$ and increased circulating levels of vasoactive mediators,$^{1-3}$ leading to increases in pulmonary $P_c$, the primary force promoting transcapillary fluid filtration. When $P_c$ is acutely elevated in the normal lung, pulmonary edema is prevented by interstitial and lymphatic safety factors as long as $P_c$ remains less than approximately 24 cm H$_2$O (18 mm Hg), but it accumulates in a pressure-dependent fashion above that threshold.$^{25}$ However, not all congestive heart failure patients or those with pulmonary hypertension secondary to mitral stenosis develop pulmonary edema.$^{2}$ In our paced group, EVLW was variable, ranging from 3.76 to a high of 7.62 mL/g blood-free dry weight, and airway fluid was noted in only 40% of this group. EVLW was inversely related to systemic arterial $P_O_2$ in a rough
Table 4. Effects of Norepinephrine After α-Adrenergic Blockade at Low and High Tone

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th></th>
<th>Paced Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔRa</td>
<td></td>
<td>ΔRa</td>
<td></td>
</tr>
<tr>
<td>Baseline Ra</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal tone</td>
<td>5.7 ± 1.0</td>
<td>-0.2 ± 0.2</td>
<td>11.7 ± 1.7</td>
<td>-0.4 ± 0.4</td>
</tr>
<tr>
<td>Increased tone</td>
<td>12.5 ± 2.1</td>
<td>0.2 ± 0.4</td>
<td>22.0 ± 5.1</td>
<td>0.9 ± 1.0</td>
</tr>
</tbody>
</table>

|                  | ΔRv           |                  |             |                  |
| Baseline Rv      |               |                  |             |                  |
| Basal tone       | 3.5 ± 0.5     | 0.2 ± 0.2        | 13.0 ± 2.3  | 0.0 ± 0.5        |
| Increased tone   | 8.2 ± 1.6     | -0.7 ± 0.4       | 32.1 ± 5.1  | 0.2 ± 1.0        |

Ra and Rv indicate precapillary and postcapillary vascular resistances, respectively. Responses to norepinephrine are compared in lobes from control and paced animals pretreated with prazosin (16 μmol/L) and yohimbine (0.1 μmol/L) to isolate the β-adrenergic receptor response to norepinephrine. Lobes were studied at basal tone and when tone had been increased by addition of 10% KCl to the perfusate. Baseline resistances (cm H2O·L⁻¹·min⁻¹·100 g⁻¹) are those absolute resistances measured after α-antagonist pretreatment but before norepinephrine challenge. ΔRa and ΔRv are the absolute changes in vascular resistance (cm H2O·L⁻¹·min⁻¹·100 g⁻¹) induced by norepinephrine.

**Bar graphs showing increments in venous resistance (ΔRv) after norepinephrine evaluated in the presence of propranolol (5 μmol/L) and prazosin (16 μmol/L) to isolate responses to those involving α2-adrenergic receptors. ΔRv was measured both before (Baseline) and after treatment of the lobes with N⁵-nitro-L-arginine methyl ester (L-NAME) (0.3 mmol/L) to block nitric oxide synthesis. *P < .05 compared with baseline responses; #P < .05 compared with controls.**

Arterial hypertension are thought to be associated with arteriolar necrosis and obliteration of capillary exchange area. In this study, however, we found no change in Kf in the paced group, which implies that pulmonary microvascular permeability and exchange area remain unaffected. We have previously reported up to 10-fold higher Kf measured after chemical lung injury or after brief periods of acute pulmonary venous hypertension (Pv > 50 cm H2O). Further, with the same methodology in isolated canine skeletal muscle, Kf values 10-fold lower than that reported here are typically observed. Thus, we believe that if remodeling secondary to this pacing protocol were sufficient to markedly alter the pulmonary exchange barrier, we would be able to measure such changes. In fact, we can predict from our earlier work that 80% to 90% of the lung mass (predominantly exchange area) would need to be obliterated to match the increment in total lobar vascular resistance observed in the paced group and that Kf would have been reduced nearly 10-fold as a result. Our results suggest that the hemodynamic changes observed in the paced group can best be explained by either structural changes in extra-alveolar vascular walls or active vasoconstriction rather than a decrease in pulmonary capillary density. We cannot exclude the possibility, however, that barrier remodeling sufficient to alter pulmonary microvascular permeability and/or exchange area may require ventricular pacing for a longer time than that tested in our model.

Medial thickening has been observed in both pulmonary arteries and veins in chronic pulmonary venous hypertension. Circulating levels of vasoactive humoral agents are increased in congestive heart failure, as noted above, which may also play a role in the hypertension. However, whether vascular reactivity per se remains normal is unclear. In some models of pulmonary arterial hypertension, pulmonary vasoreactivity is significantly altered. We hypothesized that pulmonary vascular adrenergic reactivity would be altered in the paced group. The normal canine pulmonary vasculature possesses both α- and β-adrenergic receptors but few or no α2-adrenergic receptors. In the present study, in the absence of any adrenergic antagonist, norepi-
norepinephrine evoked a predominantly venoconstrictor response, which was enhanced in the paced group. This norepinephrine-induced vasoconstriction appeared to be primarily an α₁-adrenergic response in both groups, since prazosin essentially abolished the increase in Rv observed after norepinephrine. The contribution of α₂-adrenergic receptors to the norepinephrine-induced vascular response was minimal, as evidenced by the insignificant vasoconstrictor response remaining after α₁- and β-adrenergic blockade (Fig 4). Further, there was minimal enhancement of vasoconstriction in the presence of L-NAME, and then only at the highest dose in control lobes (Fig 5), suggesting that activation of endothelial α₁-adrenergic receptors and subsequent nitric oxide release do not attenuate norepinephrine-induced constriction in either group.

The enhancement of the pulmonary vasoconstrictor response in paced animals does not appear to be due to attenuation of β-adrenergic responsiveness, since norepinephrine administration after pretreatment with propranolol in control lobes did not evoke the magnitude of response observed in the paced group. Conclusions regarding the possibility of pacing-induced enhancement of pulmonary vascular β-adrenergic responsiveness are more problematic. The enhancement in constrictor responses to norepinephrine after propranolol (Fig 3) does suggest that β-adrenergic receptors mediate a portion of the overall vascular response to this agonist in the canine lung and further, that this contribution may be proportionately larger in the paced group. However, the responses to norepinephrine after α-adrenergic blockade were no different in lobes from control and paced animals (Fig 4, bottom panel, left), even at high tone (Table 4). Several possible explanations may pertain. First, differences in the degree of heart failure and the baseline lobar vascular resistances could underlie the enhancement observed in Fig 3. Although there were no differences in LVSVF or LVEDP between the two paced groups, baseline Ra and Rv in the paced group that ultimately received propranolol were 40% to 60% higher than those that would not receive the β-antagonist. However, since there were no such differences in resistances between the comparable control groups, this explanation seems unlikely. A second and more likely possibility is that β-receptors are involved in the overall response to norepinephrine but that a substantial vasodilator contribution could not be documented when tone was increased (Table 4) because of the experimental protocol, i.e., either the method or extent of increasing tone. Given the amount of KCl used to increase tone in our experiments, we estimate that circulating [K⁺] would have risen to a maximum of 25 mmol/L. One could argue that the resultant depolarization might limit ligand-mediated vascular relaxation. However, even in the face of maximum K⁺-induced smooth muscle depolarization, force generation can be inhibited by vasodilators such as isoproterenol. In separate control lobes, we could detect no difference in the response to isoproterenol when tone was increased to a similar level with either KCl (n=2) or phentolamine (n=2, data not shown). However, the extent of tone elevation could be a limitation. Hyman and Kadowitz showed that the vasodilator response to isoproterenol and nitroglycerin is dependent on the level of pulmonary vascular tone and that norepinephrine induces significant vasodilation after β-adrenergic blockade at high tone (lobar arterial pressure of 50 cm H₂O on average). In our experiments, when tone was increased, lobar arterial pressures averaged only 32 to 36 cm H₂O. Thus, it is possible that substantially higher tone would be required to demonstrate significant β-adrenergic vasodilation in our model. A final possibility is that pulmonary vascular β-adrenergic responsiveness is truly not enhanced in pacing-induced heart failure. Mathew et al recently reported that relaxation of canine pulmonary artery rings with isoproterenol was impaired after the development of pacing-induced heart failure. Since responses to forskolin were preserved, these authors suggested that coupling of the β-adrenergic receptor to cAMP-dependent relaxation might be altered after pacing. Given the responses to norepinephrine shown in Fig 4, this scenario may not pertain in the pulmonary circulation as a whole. Since we cannot specifically exclude this possibility given our data, this issue will be the focus of further investigation.

One could envision that, in vivo, a heightened venous response to norepinephrine could play a protective role...
in that any increment in venous resistance would attenuate retrograde transmission of the left atrial pressure pulse wave to the pulmonary capillaries. However, this hypothesis would pertain only if the exacerbated adrenergic response persisted when pulmonary venous pressures were elevated. This seemed particularly important, since both the pressor response to sympathetic nerve stimulation in the feline lung and histamine-induced venoconstriction in the canine lung were significantly attenuated when pulmonary PV was acutely elevated. In our study, the effect of high PV (PV set to 20 cm H2O) in lobes from control animals was similar, in that the venoconstrictor response was attenuated or ablated. In contrast, the enhanced venoconstrictor effect of norepinephrine was maintained at high PV in the paced group. This observation suggests that pacing-induced pulmonary vascular remodeling leads to a “pressure reserve” or the ability to effectively contract at higher distending pressures such as that observed in peripheral arterial hypertension. This hypothesis remains an attractive explanation for our results, since pulmonary vascular medial thickening has been documented in chronic pulmonary venous hypertension.

The mechanisms underlying the altered pulmonary vascular adrenergic reactivity in pacing-induced pulmonary hypertension are unclear at this time. It is possible that the elevated plasma norepinephrine concentrations seen in heart failure may contribute, in part, because lung adrenergic receptor density appears to be regulated by circulating catecholamines. While we cannot address this latter point from our data, we can discuss several other potential mechanisms for the enhancement, including elevation of active pulmonary vascular tone and depression of endothelium-dependent relaxation. In the feline pulmonary circulation, adrenergic responses are enhanced when vascular tone is elevated. In contrast, our data would suggest that increases in tone secondary to elevations in humoral vasoconstrictor concentrations cannot explain our results. When vascular tone was increased threefold to fivefold in control lobes, no modulation of the norepinephrine response was evident, even in the presence of α2- and β-adrenergic blockade. Similarly, depression of endothelium-dependent nitric oxide synthesis does not appear to be a viable explanation, since the norepinephrine response was not substantially modified by treatment with L-NAME. As additional evidence, pulmonary arteries isolated from dogs subjected to a pacing regimen similar to that used in our experiments show normal responses to acetylcholine and bradykinin, two endothelium-dependent vasodilators that stimulate nitric oxide synthesis.

We conclude that as heart failure develops and the pulmonary circulation is exposed to chronically high PV, significant changes in pulmonary vascular hemodynamics and adrenergic reactivity result, while the microvascular exchange barrier remains unaffected. The increased sensitivity of the pulmonary vasculature to norepinephrine, characterized primarily by an increase in α1-adrenergic reactivity (References 9 and 10 and the present study), implies that significant remodeling of the pulmonary vascular α1-adrenergic receptor system occurs after the development of pacing-induced heart failure. Although the persistence of the heightened reactivity to norepinephrine at elevated PV is suggestive, whether these early functional changes are accompanied by frank structural remodeling per se cannot specifically be addressed from the present studies. Further, we cannot conclude whether this enhanced responsiveness in the paced group is specific for adrenergic receptor pathways or part of a more general nonspecific response to pulmonary venous hypertension. Regardless, we can predict from our data that the pulmonary vascular response to elevated norepinephrine levels in vivo would result in some elevation of pulmonary Pc and thus contribute to edema formation. However, the potential for other vasoconstrictor inputs directed at the arterial vascular compartment, for example, circulating angiotensin II and alveolar hypoxia, to modulate such an edemogenic effect should not be ruled out.

Acknowledgments

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References


Altered pulmonary microvascular reactivity to norepinephrine in canine pacing-induced heart failure.

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