Increased Sympathetic Nerve Activity in Pulmonary Artery Hypertension

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Background—This study tested the hypothesis that sympathetic nerve activity is increased in pulmonary artery hypertension (PAH), a rare disease of poor prognosis and incompletely understood pathophysiology. We subsequently explored whether chemoreflex activation contributes to sympathoexcitation in PAH.

Methods and Results—We measured muscle sympathetic nerve activity (MSNA) by microneurography, heart rate (HR), and arterial oxygen saturation (SaO₂) in 17 patients with PAH and 12 control subjects. The patients also underwent cardiac echography, right heart catheterization, and a 6-minute walk test with dyspnea scoring. Circulating catecholamines were determined in 8 of the patients. Chemoreflex deactivation by 100% O₂ was assessed in 14 patients with the use of a randomized, double-blind, placebo-controlled, crossover study design. Compared with the controls, the PAH patients had increased MSNA (67±4 versus 40±3 bursts per minute; P<0.0001) and HR (82±4 versus 68±3 bpm; P=0.02). MSNA in the PAH patients was correlated with HR (r=0.64, P=0.006), SaO₂ (r=−0.53, P=0.03), the presence of pericardial effusion (r=0.51, P=0.046), and NYHA class (r=0.52, P=0.033). The PAH patients treated with prostacyclin derivatives had higher MSNA (P=0.009), lower SaO₂ (P=0.01), faster HR (P=0.003), and worse NYHA class (P=0.04). Plasma catecholamines were normal. Peripheral chemoreflex deactivation with hyperoxia increased SaO₂ (91.7±1% to 98.4±0.2%; P<0.0001) and decreased MSNA (67±5 to 60±4 bursts per minute; P=0.0015), thereby correcting approximately one fourth of the difference between PAH patients and controls.

Conclusions—We report for the first time direct evidence of increased sympathetic nerve traffic in advanced PAH. Sympathetic hyperactivity in PAH is partially chemoreflex mediated and may be related to disease severity.

Key Words: hypertension, pulmonary □ nervous system, sympathetic □ catecholamines □ chemoreceptors

Pulmonary artery hypertension (PAH) is a rare, incurable disease of poor prognosis.¹ The pathophysiology of PAH remains incompletely understood. A series of biological abnormalities have been described that involve all the compartments of the pulmonary arterial wall,² but little is known about a possible participation of the sympathetic nervous system. Circulating catecholamines in PAH have been reported either to be increased³,⁴ or to remain within normal limits.⁵,⁶ These discrepancies may be related to the fact that circulating catecholamines are imperfect markers of sympathetic nervous system activity, being affected by a variety of processes, including changes in synthesis, metabolism, and facilitation of release by the peripheral nerve endings.⁷ Microneurography allows direct measurement of sympathetic nerve traffic directed to muscle circulation.⁸–¹¹ Muscle sympathetic nerve activity (MSNA) has been used previously to unravel the sympathetic component of abnormal neurohumoral activation in patients with left heart failure.¹¹ There has been no previous report of MSNA measurements in severe pulmonary hypertension without intrinsic alteration of left ventricular function.

We tested the hypothesis that sympathetic nerve activity is increased in PAH and determined resting MSNA in 17 patients with PAH and in 12 healthy controls matched for age, gender, and body mass index (BMI). Plasma catecholamine levels were also determined in 8 patients to assess whether these values correlated with direct sympathetic nerve traffic determinations in the patients with PAH.

Patients with PAH often present with mild to moderate decreases in arterial oxygenation, which are aggravated by exercise.¹² Chronic hypoxemia, as seen in patients with chronic obstructive pulmonary disease, is associated with increased MSNA and is improved by 100% oxygen administration.¹³ We tested the hypothesis that peripheral chemoreflex stimulation contributes to the activation of the sympathetic nervous system in 14 of the PAH patients using a...
randomized, double-blind, placebo-controlled, crossover study design. One hundred percent inspired oxygen was used to deactivate the peripheral chemoreceptors, and measures were compared with those recorded while patients breathed 21% oxygen.14,15 The results show a marked increase in sympathetic nerve activity in the patients with PAH that is partially chemoreflex mediated.

Methods

Patients
Seventeen patients with PAH (10 women and 7 men; mean±SEM age, 53±4 years) gave informed consent to the study, which was approved by the Ethics Committee of Erasme University Hospital. Three of the patients were in New York Heart Association (NYHA) functional class II, 9 in NYHA class III, and 5 in NYHA class IV. Their BMI was 25±1 kg/m². PAH was idiopathic in 14 patients and associated with fenfluramine intake in 3 patients. The patients were treated with prostacyclin analogues (intravenous epoprostenol, n=6; subcutaneous treprostinil, n=1; oral beraprost, n=2), calcium channel blockers (n=2), an endothelin-1 antagonist (bosentan; n=1), furosemide (n=10), bumetamide (n=2), spironolactone (n=2), acenocumarol (n=12), and dobutamine (n=2). Two patients had undergone an atrial septostomy 7 and 24 months before inclusion in the study. The mean delay between onset of PAH symptoms and inclusion in the study was 48 months (range, 2 to 108 months).

Subjects
Twelve healthy subjects matched for age (50±4 years), gender (7 were female), and BMI (22±2 kg/m²) served as controls. All of them had a normal physical examination. None was taking any medication.

Measurements
All measurements were performed under quiet resting supine conditions. MSNA was recorded continuously in the patients and the controls by obtaining multunit recordings of postganglionic sympathetic activity, measured from a nerve fascicle in the peroneal nerve posterior to the fibular head.10 Electric activity in the nerve fascicle was measured with the use of tungsten microelectrodes (shaft diameter 200 μm, tapering to an uninsulated tip of 1 to 5 μm). A subcutaneous reference electrode was inserted 2 to 3 cm away from the recording electrode, which was inserted into the nerve fascicle. The neural signals were amplified, filtered, rectified, and integrated to obtain a mean voltage display of sympathetic nerve activity.15

Blood pressure (BP) was measured every 3 minutes with a Physiocontrol Colin BP-8800 sphygmomanometer (Colin Press Mate, Colin Corporation), and arterial oxygen saturation (SaO2) (Nellcor, N100C pulse oximeter, Medical Resource) and heart rate (HR) (ECG Monitoring, Siemens Medical) were monitored continuously during the microneurographic recordings. HR and MSNA were recorded online on a Power Macintosh with a MacLab 8/8 data acquisition system (AD Instruments) for subsequent analysis.

Cardiac echocardiographic studies were performed in the patients with the use of an Agilent Technology Sonos 5500 ultrasound system with standard S3 imaging transducer (Hewlett-Packard). All measurements were performed following the recommendations of the American Society of Echocardiography.16,17 Left ventricular outflow tract diameter was measured by 2-dimensional echocardiography. Pulsed-wave Doppler allowed measurements of stroke volume and cardiac output at the level of the left ventricular outflow tract. Peak tricuspid regurgitant jet velocity was determined by continuous-wave Doppler to calculate systolic tricuspid pressure. A 7.5F flow-directed thermocatheter Swan-Ganz catheter (Baxter) was inserted percutaneously in the patients under local anesthesia into an internal jugular or subclavian vein for measurements of resting right atrial pressure, pulmonary artery pressure, pulmonary artery occluded pressure, and cardiac output. Pulmonary vascular resistance was calculated as follows: (mean pulmonary artery pressure – pulmonary artery occluded pressure) / cardiac output.

Exercise capacity was determined by a 6-minute walk test,18 followed by an estimation of dyspnea with the use of the Borg scale.19 Echocardiography, right heart catheterization, and exercise testing data were obtained within 48 hours of microneurographic examination, with the patients being clinically stable and with unchanged treatment.

Plasma Catecholamines (n=8)
Blood samples for plasma epinephrine and norepinephrine were obtained in 8 patients within 24 hours after the MSNA recordings in carefully standardized supine resting conditions and while patients were taking their usual medications. Samples were collected in prechilled heparinized tubes (10 mL) and immediately placed on ice, then centrifuged at 4°C. Epinephrine and norepinephrine levels were assayed by high-performance liquid chromatography.20

Chemoreflex Deactivation (n=14)
We compared the effects of 21% and 100% oxygen administration on MSNA, HR, BP, and SaO2. The gases were administered for 15 minutes via a nonrebreathing mask at identical flow rates with the use of a double-blind, randomized, placebo-controlled, crossover study design. A 30-minute recovery period was observed between interventions. Measurements taken during the last 5 minutes of each intervention were averaged for the comparison.

Data Analysis
Sympathetic bursts were identified by careful inspection of the voltage neurogram by a trained observer (A.C.), blinded to subject and intervention. Sympathetic activity was expressed as burst frequency per minute and as integrated sympathetic activity, which corresponds to burst frequency multiplied by mean burst amplitude and is expressed in arbitrary units (AU). Integrated sympathetic activity depends on neural signal amplification, which varies from one recording site to another but remains constant throughout each experiment. Therefore, burst frequency permits comparison of sympathetic nerve activity between different subjects (patients versus controls), whereas both burst frequency and integrated sympathetic activity are used to assess the effects of hyperoxia on sympathetic activity in patients with PAH.

Statistical Analysis
Results are expressed as mean±SEM. Effects of 100% oxygen were determined by Student paired t test (2 tailed) in patients. Comparisons between patients and controls and between patients with different therapy were performed by unpaired t tests (2 tailed). Relationships between parameters were estimated by linear regression analysis.

Results
Echocardiography of the patients showed enlarged right ventricle telediastolic diameter (40±3 mm), with variable degrees of septal shift. Cardiac output and stroke volume calculated from echocardiography were low (3.3±0.2 L/min and 43±4 mL, respectively), and tricuspid gradient was increased to 83±3 mm Hg. A pericardial effusion was present in 10 of the patients. All the patients had normal left ventricular ejection fraction (70±3%). As shown in the Table, the hemodynamic profile of the patients was typical of advanced PAH,1 with a mean pulmonary artery pressure ~3 times normal, decreased cardiac output, increased right atrial pressure, and normal pulmonary artery occluded pressure. Exercise capacity was limited, with a 6-minute walk distance of ~300 m and increased Borg dyspnea.
score, consistent with advanced NYHA functional class. SaO₂ was decreased, around >90%.

Plasma catecholamines were within the normal range, with epinephrine at 0.038 ± 0.005 ng/mL (normal: 0 to 0.16 ng/mL) and norepinephrine at 0.56 ± 0.008 ng/mL (normal: 0 to 0.8 ng/mL). However, as shown in Figures 1 and 2, MSNA was markedly higher in PAH patients than in the controls (67 ± 4 versus 40 ± 3 bursts per minute; \( P < 0.0001 \)), even after correction for HR (83 ± 4 versus 60 ± 5 bursts per 100 heartbeats; \( P = 0.002 \)). HR was also higher in the PAH patients (82 ± 4 versus 68 ± 3 bpm; \( P = 0.02 \)), whereas SaO₂ (91.6 ± 0.8% versus 97.0 ± 0.3%; \( P = 0.0003 \)) and mean BP (81 ± 3 versus 89 ± 2 mm Hg; \( P = 0.046 \)) were lower.

MSNA was positively correlated with HR, the presence of pericardial effusion (\( r = 0.51 \), \( P = 0.046 \)), and NYHA class and inversely correlated with SaO₂ (Figure 3). There was no correlation between MSNA and the echocardiographic estimation of cardiac output and pulmonary artery pressures, invasive hemodynamic variables, or dyspnea score.

Patients treated by prostacyclin analogues (n = 9) had faster HR (93 ± 5 versus 69 ± 4 bpm; \( P = 0.003 \)), higher MSNA (76 ± 4 versus 57 ± 6 bursts per minute; \( P = 0.009 \)), lower SaO₂ (90 ± 1% versus 94 ± 1%; \( P = 0.01 \)), and worse NYHA functional class (3.4 ± 0.2 versus 2.8 ± 0.2; \( P = 0.04 \)) than patients without prostacyclin therapy (n = 8).

Patients who were either on no therapy (n = 4) or on only diuretic therapy (n = 3) also exhibited higher MSNA than the control subjects (56 ± 7 bursts per minute for patients versus 40 ± 3 bursts per minute for controls; \( P = 0.02 \)).

Administration of hyperoxia to the patients increased SaO₂ from 91.7 ± 1% to 98.4 ± 0.2% (\( P < 0.0001 \)). This increase was accompanied by a reduction in HR from 81 ± 5 to 75 ± 5 bpm (\( P < 0.0015 \)) and a decrease in MSNA burst frequency from 67 ± 5 to 60 ± 4 bursts per minute (\( P = 0.0015 \)) and burst amplitude from 616 ± 72 to 530 ± 66 AU (\( P = 0.0007 \); Figure 4). During hyperoxic breathing, the PAH patients exhibited mean BP (both 81 ± 3 mm Hg; \( P = 0.76 \)) and HR (75 ± 5 versus 68 ± 3 bpm; \( P = 0.24 \)) similar to those of the controls.
but their MSNA remained elevated (60±4 versus 40±3 bursts per minute; \(P=0.0009\)) despite a higher \(\text{SaO}_2\) (98.4±0.2% versus 97.0±0.3%; \(P=0.0008\)). Changes in MSNA during 100% oxygen administration did not correlate with baseline oxygen saturation levels.

**Discussion**

We report the first direct evidence of increased sympathetic nerve traffic in patients with PAH.

Early reports of PAH included testing for reversibility with tolazoline, an \(\alpha\)-receptor blocker, on the assumption that the disease would be initiated and perpetuated by increased pulmonary vasoactivity and that increased sympathetic nervous system tone could be involved.\(^{21}\) Although tolazoline indeed partially decreased pulmonary vascular resistance in a proportion of patients, it has since been realized that PAH is rather a disease of pulmonary arteriolar remodeling, related to a series of abnormal signaling mechanisms, among which the angiopoietin-1-BMPR2 system has been recently suggested to play a central role.\(^{22,24}\) However, sympathetic activation may participate in pulmonary arteriolar remodeling. The pulmonary circulation is richly endowed with sympathetic nerve endings,\(^{25,26}\) which seem to play an important role in vascular growth and differentiation,\(^{27}\) albeit by yet undefined mechanisms. Sympathetic activation related to exercise, cold exposure, or emotions may transiently increase pulmonary vascular resistance in PAH patients, possibly aggravating vessel wall tension–induced remodeling.\(^{25}\) Indirect supporting evidence is provided by epidemics of PAH reported after the release on the market of amphetamine derivative anorexigen, such as aminorex\(^{28}\) and fenfluramines.\(^{29}\) High-altitude pulmonary edema is a particular condition that differs from PAH by acute vasoconstriction and minimal and rapidly reversible pulmonary vascular remodeling. However, interestingly, there is evidence that subjects susceptible to high-altitude pulmonary edema have exaggerated peripheral sympathetic response to hypoxia as well as increased pulmonary vasoconstriction compared with controls. The sympathetic activation of these subjects is directly correlated with systolic pulmonary artery pressure.\(^{30}\)

The potential contribution of sympathetic nervous system activity to PAH pathophysiology to our knowledge has been previously explored in only 4 studies, all of which relied on measurements of circulating catecholamines. One study reported a nonsignificant increase in circulating catecholamines that correlated positively with pulmonary vascular resistance and circulating endothelin and negatively with survival.\(^{8}\) The other studies reported circulating norepinephrine to be either increased\(^{3,4}\) or normal.\(^{5}\) These discrepancies might be explained by the fact that plasma catecholamines are affected by neuronal release, reuptake, spillover, and degradation and are therefore insensitive markers of sympathetic nerve outflow in pathological conditions.\(^{7}\) In the present study, normal circulating catecholamines were found in the presence of markedly increased MSNA, further underscoring their limited usefulness as indicators of sympathetic nervous system activity.

Our study population included patients with idiopathic PAH and patients with PAH associated with fenfluramine intake. PAH associated with fenfluramine intake is very similar to idiopathic PAH in clinical course, histopathology, and response to therapy. It is also, with idiopathic PAH, the only PAH category in which BMP2R mutations have been described.\(^{3,24}\) We therefore believe that our study group is reasonably homogeneous from a diagnostic point of view. The majority of our patients were treated with prostacyclin analogues, calcium channel blockers, an endothelin-1 antagonist, and diuretics. Higher sympathetic nervous activity was observed in patients treated with prostacyclins. Prostacyclins could have affected the sympathetic nervous system by an associated decrease in \(BP\).\(^{31,32}\) In our study, patients treated by prostacyclin analogues were also in a worse clinical state, with more advanced NYHA functional class and lower \(\text{SaO}_2\). PAH patients who were untreated (\(n=4\)) or treated only with diuretics (\(n=3\)) also had higher than normal MSNA. Taken together, these observations suggest that disease state rather than prostacyclin therapy was the main cause of sympathoexcitation. It may be added that diuretic therapy also may have caused an increase in MSNA due to hypovolemia and associated baroreflex activation. Increased right atrial pressure in our patients may argue against this mechanism.

Sympathetic nerve activity has been shown repeatedly to be increased in patients with left ventricular failure.\(^{11,15,33}\) The causes of sympathetic nervous system activation in left heart failure remain incompletely understood, but increased filling pressures, decreased cardiac output, and impaired baroreflex control appear to play a role.\(^{11,34}\) Severe PAH leads to right ventricular failure and consequently to low systemic cardiac output, as occurs in left ventricular failure. This in turn could activate compensatory sympathetic response. This may mean that PAH represents a pathophysiological model of sympathetic response similar to that described in left ventricular failure. Moreover, increased sympathetic drive could induce potential adverse effects on myocytes of both failing ventricles, as shown by chronic troponin leakage in patients with left ventricular failure and primary pulmonary hypertension, a finding invariably related to poor prognosis.\(^{3,5,36}\)
The decrease of MSNA and HR after administration of 100% oxygen suggests that peripheral chemoreflex activation contributes in part to increased MSNA in PAH patients. To our knowledge, there are no previous studies providing direct evidence of decrease in sympathetic activation by hyperoxic breathing in PAH. A study on pulmonary vascular impedance in patients with pulmonary hypertension of various etiologies reported a decrease in circulating norepinephrine after hyperoxic breathing. In our patients hyperoxia on average corrected one fourth of abnormally increased MSNA, indicating that most of the increased sympathetic activity in PAH patients is not the consequence of mild hypoxemia-related chemoreflex activation. Interestingly, sympathetic and HR responses to hyperoxic breathing in our patients with PAH contrast with those of patients with congestive left ventricular failure, in whom we observed that hyperoxia did not improve sympathetic hyperactivity. This may be explained by the fact that patients with PAH are often more hypoxemic than patients with left ventricular failure.

In conclusion, we report direct evidence of increased sympathetic nerve traffic in advanced PAH. Sympathetic hyperactivity is partially chemoreflex mediated and may be correlated with disease severity.

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