Effects of Peripheral Chemoreceptors Deactivation on Sympathetic Activity in Heart Transplant Recipients

Agnieszka Ciarka, Boutaina Najem, Nicolas Cuylits, Marc Leeman, Olivier Xhaet, Krzysztof Narkiewicz, Martine Antoine, Jean-Paul Degaute, Philippe van de Borne

Abstract—Heart transplantation initially normalizes sympathetic hyperactivity directed at the muscle circulation. However, sympathetic activity increases with time after transplantation and the exact mechanisms responsible for sympathetic control in heart transplant recipients remain unclear. We examined the effects of peripheral chemoreflex deactivation caused by breathing 100% oxygen on muscle sympathetic nerve activity (expressed as number of burst per minute and mean burst amplitude), heart rate, and mean blood pressure in 13 heart transplant recipients, 13 patients with essential hypertension, and 10 controls. Heart transplant recipients disclosed the highest sympathetic activity, whereas it did not differ between controls and patients with essential hypertension (51±16 versus 37±14 versus 39±12 burst/min, respectively; P<0.05). Breathing 100% oxygen, in comparison with 21% oxygen, reduced sympathetic activity (−4±4 versus −1±2 burst/min, P<0.01; 85±9 versus101±8% of amplitude at baseline, P<0.001) and mean blood pressure (−4±5 versus +3±6 mm Hg; P<0.05) in heart transplant recipients, decreased sympathetic activity (−4±4 versus 0±3 burst/min, P<0.05; 90±16 versus101±9% of amplitude at baseline, P<0.05) in patients with essential hypertension, but did not reduce sympathetic activity (2±4 versus 3±3 burst/min, P=NS; 95±11 versus 95±13% of amplitude at baseline, P=NS) in control subjects. The sympathetic response to hyperoxia was more marked in heart transplant recipients than in controls (85±9 versus 95±11% of baseline amplitude; P<0.05). The decrease in sympathetic activity was most evident in patients with the longest time after heart transplantation (r=−0.75, P<0.01).


Key Words: chemoreceptors ■ sympathetic nervous system ■ transplantation

Congestive heart failure is associated with remarkably elevated muscle sympathetic nerve activity (MSNA).1 Heart transplantation restores a close to normal cardiac function but does not always normalize MSNA.2–5 Elevated MSNA after heart transplantation is associated with cyclosporine therapy6 and increases as a function of time after transplantation.5

Increased peripheral chemoreflex sensitivity has been demonstrated in humans and experimental animals with congestive heart failure.6–9 Whether this alteration in chemoreflex function is reversible when cardiac function is restored by heart transplantation is unknown. We hypothesized that increased peripheral chemoreceptor activation, possibly a lingering effect of heart failure, contributes to elevated MSNA in heart transplant recipients (HTRs). Accordingly, we studied the effects of hyperoxia, an intervention that acutely reducesafferent nerve traffic from the peripheral chemoreceptors, on MSNA in HTRs. Because the majority of HTRs are hypertensive10 and enhanced peripheral chemoreflex sensitivity has been observed in hypertensive humans and in animal models of hypertension,11–13 we also studied the effects of hyperoxia on MSNA in essential hypertension patients (EHPs). Last, we examined effects of hyperoxia on MSNA in 10 healthy subjects to determine whether tonic drive from peripheral chemoreceptors is increased in HTRs.

Subjects

Thirteen HTRs participated in the study (58±10 years, all men, body mass index [BMI] 26±4 kg/m²). Mean time after heart transplantation was 9±5 years (range, 2 to 15 years). All patients were on various combinations of immunosuppressive treatment including cyclosporine (n=12), methylprednisolone (n=10), tacrolimus (n=1), and mofetil mycophenolate (n=3), and had a normal left ventricular ejection fraction of 60±9% (range, 50% to 75%) determined by resting radionuclide angiography. Hypertension was treated with diuretics (n=8), β-blockers (n=5), calcium channel-blockers (n=8), angiotensin-converting enzyme inhibitors, and angiotensin II receptor antagonists (n=8). The origin of heart failure was ischemic heart disease (n=5), idiopathic dilated cardiomyopathy (n=5), valvular heart disease (n=2) and congenital heart defect (n=1). Patients had either no or modest left ventricular hypertrophy.

Methods

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Comparison of effects of 100% oxygen breathing between HTR and control group was performed by ANOVA for repeated measurements with group and time (baseline before 100% oxygen and 100% oxygen) as factors. Relationships between variables were estimated by linear regression analysis. A χ² test was used to compare the proportion of patients on different classes of hypertensive treatment in the HTRs and EHPs.

**Results**

HTRs, EHPs, and control subjects were matched for gender, age, and BMI (P=0.07). There was no difference in the antihypertensive treatment regimen between HTRs and EHPs (P>0.24).

HTRs and EHPs presented the same MBP, which was increased in both groups in comparison with the control subjects (Figure 1). HTRs disclosed a higher MSNA than the controls, and also had faster heart rates (HRs) and slightly larger arterial blood oxygen saturations in comparison with the EHPs (Figure 1).

Breathing 100% oxygen decreased MSNA and MBP in HTRs (Table 1, Figure 2). Peripheral chemoreceptor inhibition with 100% oxygen decreased MSNA, but did not change MBP and HR in EHPs (Table 2, Figure 3). In control subjects, 100% oxygen in comparison with 21% oxygen provoked a decrease in HR and did not change MSNA or blood pressure (Table 3, Figure 4). Acute hyperoxia did not change respiratory rate and respiratory movement amplitude in HTRs, EHPs, and controls (Table 1, Table 2, and Table 3).

The reduction in MSNA during 100% oxygen breathing was not different between HTRs and EHPs (ANOVA P=0.29 for percent of baseline burst amplitude), whereas the MSNA response to hyperoxia was more marked in the HTRs than in the control subjects (85±9 versus 95±11% of baseline burst amplitude, ANOVA P<0.05).

The reduction in MSNA during 100% oxygen breathing was most evident in patients with the longest time after transplantation (Figure 5). Oxygen saturation was not related to transplantation time (r=-0.33). The decrease in MBP was not related to the decrease in MSNA (r=0.16).

In HTRs, plasma creatinine was 1.9±0.6 mg/dL (range, 1.3 to 3.4 mg/dL), and there was no relation between plasma creatinine and MSNA at baseline (r=0.05).

**Discussion**

This is the first study to our knowledge to demonstrate that peripheral chemoreflex deactivation by hyperoxia decreases MSNA and MBP after cardiac transplantation. Heart transplantation initially decreases the MSNA related to the heart failure state, because it returns to normal values within 1 year after the surgical procedure, although this normalization does not seem to be permanent. Only a few studies have used direct recording of nerve traffic to unravel sympathetic activation after heart transplantation. These studies revealed that cyclosporine and time from heart transplantation contribute to MSNA increase in HTRs. Our study confirms previous observations of increased MSNA in long-term HTRs. It provides a new insight into sympathetic regulation after heart transplantation. The demonstration that peripheral chemoreceptor deactivation decreases resting MSNA and blood pressure in normoxemic HTRs is the first main finding.
of our study. The demonstration of a higher peripheral chemoreceptor contribution to MSNA in HTRs than in healthy controls is the second important finding of our study. The reduction in MSNA in response to hyperoxia was nearly 3-times larger in the HTRs than in the controls, despite the fact that both groups disclosed strictly identical baseline arterial blood oxygen saturations. This suggests that the tonic peripheral chemoreceptor drive to MSNA is increased in HTR. Moreover, the concomitant decrease in MSNA and MBP in response to hyperoxia in HTR suggests a causal interaction, because an isolated reduction in MBP rather would be expected to elicit a baroreflex-related increase in MSNA.

Animal studies also reveal that acute hyperoxia decreases HR and blood pressure probably through changes in sympathetic activity. Human studies report a decrease in MSNA during short breathing periods of 3 to 4 minutes of hyperoxia. However, longer exposure to oxygen was reported to decrease heart rate but not MSNA in healthy subjects.

Previous observations of MSNA reduction during hyperoxia in humans could be explained by acclimatization to the laboratory environment. Our control subjects decreased their MSNA during 100% oxygen breathing; however, their MSNA decreased also during 21% oxygen, similar to what was observed in the study by Narkiewicz et al. HTRs are at risk for systemic hypertension. The cumulative probability of hypertension reaches up to 77% at the fourth year after transplantation. Hypertension appears within weeks or months after the surgical procedure and is of multifactorial origin. Moreover, it does not respond to single antihypertensive agents and requires combined antihypertensive therapy. Cyclosporine is implicated in the pathogenesis of hypertension, because it has been demonstrated to cause sympathetic nerve activation and is also well-documented as a cause of chronic nephropathy. However, patients who underwent transplantation who do not receive cyclosporine can also have hypertension develop. Alternative mechanisms such as abnormal renin-angiotensin-aldosterone-system (RAAS) activity could contribute to the development of hypertension in HTRs.

### Figure 1
Comparison of mean blood pressure (MBP), heart rate (HR), sympathetic nerve activity (MSNA), and arterial blood oxygen saturation (Sat) in control subjects (white bars), heart transplant recipients (HTRs, black bars), and treated essential hypertensive patients (EHPs, hatched bars) during room air breathing. Comparison by ANOVA, with Bonferroni contrasts a posteriori.

### Table 1
Effects of 100% Oxygen Breathing on MSNA, Mean Burst Amplitude (% Changes From Before Intervention), Heart Rate, MBP, Arterial Saturation, Respiratory Rate, and Respiratory Movement Amplitude in Heart Transplant Recipients (n=13)

<table>
<thead>
<tr>
<th>Variables</th>
<th>21% Oxygen</th>
<th>100% Oxygen</th>
<th>P Interaction</th>
<th>21% vs 100% Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNA, burst per min</td>
<td>Before</td>
<td>During</td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td></td>
<td>51 ± 17</td>
<td>50 ± 16</td>
<td>53 ± 16</td>
<td>48 ± 17</td>
</tr>
<tr>
<td>Mean burst amplitude, au</td>
<td>100 ± 0</td>
<td>101 ± 8</td>
<td>100 ± 0</td>
<td>85 ± 9</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>74 ± 11</td>
<td>76 ± 13</td>
<td>74 ± 12</td>
<td>73 ± 12</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>106 ± 11</td>
<td>109 ± 13</td>
<td>109 ± 14</td>
<td>105 ± 12</td>
</tr>
<tr>
<td>Arterial saturation, %</td>
<td>96 ± 1</td>
<td>96 ± 2</td>
<td>96 ± 2</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>Respiratory rate, breath per min</td>
<td>16 ± 2</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>Respiratory movement amplitude, au</td>
<td>0.52 ± 0.05</td>
<td>0.55 ± 0.05</td>
<td>0.51 ± 0.04</td>
<td>0.50 ± 0.05</td>
</tr>
</tbody>
</table>

au indicates arbitrary units; bpm, beats per minute; MBP, mean blood pressure; MSNA, muscle sympathetic nerve activity.
rone system responsiveness to fluid retention are therefore also postulated to contribute to the pathogenesis of blood pressure elevation. Our study further improves the understanding of the physiopathology of arterial hypertension after heart transplantation by demonstrating that tonic peripheral chemoreceptor activation contributes to sympathetic activity and blood pressure in these patients.

The third important finding of our study is that there is no difference in the MSNA response to peripheral chemoreceptor deactivation between HTRs and treated elderly patients with essential arterial hypertension. We also report for the first time to our knowledge that acute hyperoxia decreases MSNA in patients with essential hypertension but fails to decrease arterial blood pressure in this clinical setting.

Several studies have investigated chemoreceptor function in animal models of hypertension and in hypertensive patients. Spontaneously hypertensive rats exposed to hypoxia present increased carotid chemoreceptor discharge in comparison with normotensive Wistar rats and Wistar–Kyoto rats. Studies in young, untreated, borderline hypertensive humans demonstrated exaggerated ventilatory and MSNA response to hypoxia and also a decrease in arterial blood pressure and total peripheral resistance during peripheral chemoreceptor deactivation by acute hyperoxia. None of these studies determined, however, the effects of hyperoxia on MSNA in EHPs.

From our present data, it is impossible to state whether the observed increased peripheral chemoreceptor drive in HTRs is caused by heart transplantation per se or by post-transplant hypertension. Although increased peripheral chemoreceptor drive could be a lingering effect of heart failure, it could also be a result of hypertension, which commonly develops in HTRs. The observation that hyperoxia decreases MSNA in both HTRs and EHPs supports the latter hypothesis. However, the finding that hyperoxia decreased MBP only in HTR suggests an impact of other factors specific to patients after heart transplantation.

Studies in young patients with borderline hypertension showed increased MSNA and elevated plasma norepinephrine levels, whereas MSNA was not increased in elderly hypertensive patients. In our study, we did not find a difference in baseline MSNA between EHPs and matched control subjects. This finding, however, is not unexpected,

<table>
<thead>
<tr>
<th>Variables</th>
<th>21% Oxygen</th>
<th>100% Oxygen</th>
<th>P Interaction 21% vs 100% Oxygen</th>
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</thead>
<tbody>
<tr>
<td>MSNA, burst per min</td>
<td>41 ± 14</td>
<td>40 ± 14</td>
<td></td>
</tr>
<tr>
<td>Mean burst amplitude, au</td>
<td>100 ± 0</td>
<td>101 ± 9</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>61 ± 9</td>
<td>61 ± 9</td>
<td></td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>109 ± 13</td>
<td>109 ± 14</td>
<td></td>
</tr>
<tr>
<td>Arterial saturation, %</td>
<td>95 ± 1</td>
<td>95 ± 2</td>
<td></td>
</tr>
<tr>
<td>Respiratory rate, breath per min</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
<td></td>
</tr>
<tr>
<td>Respiratory movement amplitude, au</td>
<td>0.5 ± 0.03</td>
<td>0.5 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Recordings show ECG, HR, MBP, saturation, MSNA (neurogram), and respiratory activity (respiration) in a heart transplant recipient during 21% oxygen (left panel) and 100% oxygen (right panel). Hyperoxia increases arterial blood saturation and decreases MSNA and MBP.

Figure 3. Recordings show ECG, HR, MBP, saturation, MSNA (neurogram), and respiratory activity (respiration) in essential hypertensive patients during 21% oxygen (left panel) and 100% oxygen (right panel). Arterial blood saturation increases whereas MSNA decreases and MBP remains unchanged during 100% oxygen breathing.

Table 2. Effects of 100% Oxygen Breathing on MSNA, Mean Burst Amplitude (% Changes From Before Intervention), Heart Rate, MBP, Arterial Saturation, Respiratory Rate, and Respiratory Movement Amplitude in Treated Patients With Essential Arterial Hypertension (n=13)
because our EHPs were of an advanced age, with established arterial hypertension, and were using antihypertensive treatment. Although diuretics do not seem to affect sympathetic activity and calcium channel-blockers tend to increase MSNA, some studies demonstrated beneficial effects of beta-blockers, angiotensin-converting enzyme II inhibitors, and selective angiotensin II receptor blockade on sympathetic activity in hypertensive subjects. Therefore, we cannot exclude that normalization of MSNA in our EHPs was caused by pharmacological antihypertensive treatment.

The decrease in MSNA in response to chemoreflex inhibition was positively related to the duration after heart transplantation. We speculate that cyclosporine-related arterial baroreceptor attenuation and possibly the duration of systemic hypertension after the surgical procedure, may play a role.

End-stage renal failure is accompanied by increased MSNA, which remains elevated in patients after renal transplantation with diseased native kidneys, but decreases during peripheral chemoreceptor deactivation by acute hyperoxia. In our study, HTRs presented moderately elevated plasma creatinine levels. However, renal failure does not seem to be a key component of sympathetic activation in HTR, because creatinine levels did not correlate with sympathetic overactivity.

Peripheral chemoreceptors are primarily influenced by a decrease in arterial blood oxygen saturation, but they respond also to an increase in arterial carbon dioxide content. Acute hyperoxia selectively suppresses the activity of peripheral chemoreceptors. This allows the contribution of resting peripheral chemoreflex drive on MSNA and blood pressure to be determined. However, breathing 100% oxygen can also increase ventilation in normal subjects. Central chemoreceptor activation during hyperoxia, known as the Haldane effect, may play a role because oxygenated hemoglobin has a lower transport capacity for tissue CO2 than does nonoxygenated hemoglobin. Subsequently, an increase of CO2 in brain tissue may result in stimulation of central chemoreceptors. In mitigation, however, first, it is very unlikely that central chemoreflex activation played an important role in our study because this reflex increases not only ventilation but also MSNA and MBP, in contrast to what we observed in our HTRs and EHPs. We cannot exclude, however, the possibility that the Haldane effect may have limited the size of the decrease in MSNA and MBP we observed.

Sympathetic nerve traffic to the periphery is modulated by respiration. In normal individuals and in patients with heart failure, sympathetic activity is reduced during hyperventilation. However, this reflex is impaired in patients with heart failure, presumably due to a blunted baroreflex sensitivity.

Table 3. Effects of 100% Oxygen Breathing on MSNA, Mean Burst Amplitude (% Changes From Before Intervention, Heart Rate, MBP, Arterial Saturation, Respiratory Rate, and Respiratory Movement Amplitude in Control Subjects (n=10)

<table>
<thead>
<tr>
<th>Intervention Variables</th>
<th>21% Oxygen</th>
<th>100% Oxygen</th>
<th>P Interaction 21% vs 100% Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNA, burst per min</td>
<td>37±16</td>
<td>36±16</td>
<td>35±15</td>
</tr>
<tr>
<td>Mean burst amplitude, au</td>
<td>100±0</td>
<td>95±13</td>
<td>100±0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>64±10</td>
<td>65±10</td>
<td>65±9</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>98±10</td>
<td>98±11</td>
<td>99±9</td>
</tr>
<tr>
<td>Arterial saturation, %</td>
<td>96±2</td>
<td>96±2</td>
<td>96±2</td>
</tr>
<tr>
<td>Respiratory rate, breath per min</td>
<td>13±3</td>
<td>15±2</td>
<td>15±2</td>
</tr>
<tr>
<td>Respiratory movement amplitude, au</td>
<td>3.21±1.23</td>
<td>3.45±1.29</td>
<td>3.51±1.32</td>
</tr>
</tbody>
</table>

Figure 4. Recordings show ECG, HR, MBP, saturation, MSNA (neurogram), and respiratory activity (respiration) in a control subject during 21% oxygen (left panel) and 100% oxygen (right panel). The increase in arterial blood saturation is accompanied by the reduction in HR. However, MSNA and MBP remain unchanged.

Figure 5. Linear regression analysis between time after heart transplantation (years) and the reduction in MSNA (expressed as a percentage of baseline values) during administration of 100% oxygen.
failure, MSNA is affected by the breathing pattern and is inversely related to tidal volume and is directly related to breathing frequency. Thus, changes in pulmonary stretch receptor activation affects MSNA. However, the reduction in MSNA we observed during peripheral chemoreflex suppression cannot be explained by an augmented stimulation of pulmonary stretch receptors, because hyperoxia did not affect respiratory rate or the amplitude of respiratory movements in our study.

Perspectives
Heart transplantation decreases but does not normalize the ventilatory response to exercise, which remains excessive in comparison with healthy subjects. Peripheral chemoreceptors are known to intervene in exercise hyperpnea. Whether peripheral chemoreceptor sensitivity is increased in HTRs is unknown, and this will need further studies on the ventilatory and MSNA response to hypoxia. We speculate that increased peripheral chemoreceptor sensitivity could correlate with the excessive ventilatory response to exercise in HTRs. In conclusion, our study demonstrates that peripheral chemoreceptors contribute to MSNA and blood pressure in HTRs, as well as to MSNA in elderly patients with essential arterial hypertension. Effects of hyperoxia on MSNA are more marked in HTRs than in control subjects. The contribution of peripheral chemoreceptors to MSNA is directly related to the time from heart transplantation.

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