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Evidence for a physiological role of pulmonary arterial baroreceptors in sympathetic neural activation in healthy humans

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Running title: Pulmonary arterial baroreceptors and sympathetic neural activity

Key points summary

- In an anaesthetized animal model, independent stimulation of baroreceptors in the pulmonary artery elicits reflex sympathoexcitation.
- In humans, pulmonary arterial pressure is positively related to basal MSNA under conditions where elevated pulmonary pressure is evident (e.g. high-altitude); however, a causal link is not established.
- Using a novel experimental approach, we demonstrate that reducing pulmonary arterial pressure lowers basal MSNA in healthy humans.
- This response is distinct from the negative feedback reflex mediated by aortic and carotid sinus baroreceptors when systemic arterial pressure is lowered.
- Afferent input from pulmonary arterial baroreceptors may contribute to sympathetic neural activation in healthy lowland natives exposed to high altitude.

Abstract

In animal models, distension of baroreceptors located in the pulmonary artery induces a reflex increase in sympathetic outflow; however, this has not been examined in humans. Therefore, we investigated whether reductions in pulmonary arterial pressure influenced sympathetic outflow and baroreflex control of muscle sympathetic nerve activity (MSNA). Healthy lowlanders (n=13; 5 females) were studied 4-8 days following arrival at high-altitude (4383m; Cerro de Pasco, Peru), a setting that increases both pulmonary arterial pressure and sympathetic outflow. MSNA (microneurography) and blood pressure (BP; photoplethysmography) were measured continuously during 1) ambient air breathing (Amb) and 2) a six-minute inhalation of the vasodilator nitric oxide (iNO; 40ppm in 21% O_2), to selectively lower pulmonary arterial pressure. A modified Oxford test was performed under both conditions. Pulmonary artery systolic pressure (PASP) was determined using Doppler echocardiography. iNO reduced PASP (24±3 vs 32±5 mmHg; P <0.001) compared to Amb, with a similar reduction in MSNA total activity (1369±576 to 994±474 a.u·min^{-1}; P = 0.01). iNO also reduced the MSNA operating point (burst incidence; 39±16 to 33±17 bursts·100 Hb^{-1}; P = 0.01) and diastolic operating pressure (82±8 to 80±8 mmHg; P < 0.001) compared to Amb, without changing heart rate (P= 0.6) or vascular-sympathetic baroreflex gain (P = 0.85). In conclusion, unloading of pulmonary arterial baroreceptors reduced basal sympathetic outflow to the skeletal muscle vasculature and reset vascular-sympathetic baroreflex control of MSNA downward and leftward in healthy humans at high-altitude. These data suggest the existence of a lesser-known reflex input involved in sympathetic activation in humans.

Lydia L Simpson completed her BSc in Sport and Exercise Sciences at the University of Birmingham (2014) and her MSc in Human and Applied Physiology at Kings College London (2016). Lydia is currently completing her PhD in integrative cardiovascular physiology at Bangor University examining neural control of blood pressure in chronic high-altitude hypoxia. Lydia has taken part in research expeditions to the Himalaya and the Peruvian Andes, where she has collected data in both lowland and high-altitude natives. In addition, Lydia has undertaken some of her doctoral training at the University of Alberta and Innsbruck University.

Jonathan Moore graduated from Edinburgh University (1990) with a BSc in Biological Sciences (Physiology). Then, he completed a PhD (1994) and postdoctoral training in cardiovascular studies at Leeds University School of Medicine, under the mentorship of Roger Hainsworth and Mark Drinkhill. Having investigated reflex responses from vagal afferent nerve endings located in the heart, coronary arteries, and pulmonary trunk of anaesthetized animals, now Jonathan's research focuses upon control and autonomic regulation of human sympathetic neural and blood pressure responses to stress, such as hypoxia and exercise. Jonathan lectures on human, environmental and exercise cardiovascular physiology at Bangor University School of Sport, Health and Exercise Sciences.

Introduction

In animal models, distension of pulmonary arterial baroreceptors, whilst carefully controlling the distending pressure to other major reflexogenic areas, elicits reflex sympathoexcitation and systemic vasoconstriction (Ledsome and Kan, 1977; Mcmahon *et al.*, 2000; Moore *et al.*, 2011). Strikingly, these baroreceptors, located at the pulmonary artery bifurcation and in the extrapulmonary artery branches, elicit sympathetic responses that contrast with reflex sympathoinhibition and the resulting systemic vasodilation elicited by isolated distension of carotid sinus baroreceptors in the same experimental preparation (Moore *et al.*, 2011). Moreover, an interaction exists between pulmonary arterial and carotid sinus baroreceptors, whereby altering pressure within the pulmonary arteries acutely resets the vascular limb of the carotid sinus baroreflex with unaltered reflex gain (Moore *et al.*, 2011). In contrast to these studies, a role for pulmonary arterial baroreceptors in sympathetic activation in humans has been largely unexplored, likely due to the difficulty in isolating a physiological stimulus to the pulmonary circulation.

Some indirect support for a sympathoexcitatory reflex originating from baroreceptors in the pulmonary circulation of humans is evident in the literature. First, although a causal relationship has not been established, pulmonary arterial pressure is positively related to basal MSNA under conditions of elevated pulmonary arterial pressure, including exposure to high-altitude hypoxia (Duplain *et al.*, 1999), in heart failure (Ferguson *et al*., 1990) and in pulmonary arterial hypertension (Velez-Roa *et al.*, 2004). Second, approximately a quarter of single-unit muscle sympathetic efferent fibres display an increase in neural activity during non-hypertensive lower body positive pressure (LBPP), a manoeuvre that increases central venous pressure and right-heart filling, and a decrease in neural activity

during non-hypotensive lower body negative pressure (LBNP), which reduces right-sided filling pressure (Millar *et al.*, 2013; Millar *et al* 2015; Incognito *et al.*, 2018). Together, given the strong evidence from animal studies and supporting evidence in humans, we believe that a potential role for pulmonary arterial baroreceptors in sympathetic neural activation in humans requires investigation.

Therefore, the aim of the present study was to investigate the mechanistic role of pressure sensitive receptors in the pulmonary circulation in control and regulation of sympathetic outflow in humans. To investigate this, we employed a novel experimental paradigm to isolate the pulmonary baroreceptors. Healthy lowlanders were studied at high-altitude, a setting known to increase pulmonary arterial pressure (Naeije*.,* 1992). Basal MSNA and baroreflex control of MSNA were assessed whilst breathing ambient air and during inhalation of the selective pulmonary vasodilator nitric oxide (NO) (Frostell *et al*., 1991; Pepke-Zaba *et al.*, 1991), to reduce the pressure stimulus to baroreceptors in the pulmonary circulation, without altering the stimulus to systemic arterial baroreceptors. Based on available evidence in animals, we hypothesised that reducing pulmonary artery pressure would reduce MSNA in healthy humans at high-altitude and reset baroreflex control of MSNA.

Methods

Ethical approval

All experimental procedures had Institutional Review Board approval from the University of British Columbia (HS17-02687) and conformed to the latest revision of the *Declaration of Helsinki,* except for registration in a database. Prior to participation, all subjects provided written informed consent. The present study

formed part of the Global REACH expedition to the Universidad Peruana Cayetano Heredia's Instituto de Investigacions de Altura (4383m; Cerro de Pasco, Peru) in July 2018. Participants took part in a number of other studies; however, care was taken to ensure no overlap existed between studies and the present study addressed a distinct *a priori* research question.

Participants

Thirteen lowlanders (5 females) mean age (28 ± 7 yrs), height (1.7 ± 0.1 m) and weight (71 ± 7 kg) free from cardiovascular, respiratory, metabolic and neurological disease were recruited. Participants rapidly ascended from sea-level (SL) to 4383m over the course of 9–10 h by motor vehicle and were studied 4-8 days (5 ± 2 days) following arrival at 4383m (High altitude, HA; Cerro de Pasco, Peru; barometric pressure, 455 ± 0.7 mmHg). All participants were asked to abstain from caffeine, alcohol and vigorous exercise for at least 12 hours prior to the experimental session and arrived at the laboratory a minimum of 4 hours after a light meal. On the day of testing, participants completed the Lake Louise Acute Mountain Sickness (AMS) questionnaire (Roach *et al.*, 2018) to evaluate symptoms of AMS. Twelve participants reported a Lake Louise Score (LLS) ≤3 and did not have clinically defined AMS. One subject was administered medication for treatment of AMS 4 days following arrival at high-altitude [two doses of 125mg acetazolamide over 24 hours], but was tested following a 72 hour washout period. The washout allowed sufficient clearance time (i.e. >48 h) before experimental data were collected, since the reported half-life for acetazolamide is ~10 h (Ritschel *et al.*, 1998) and this low-dose quantity is reported to be 90–100% excreted within 24 h of administration (Richalet *et al.*, 2005). This subject reported a LLS score of 5 on the day of testing and was,

therefore, experiencing mild AMS at the time of participation. None of the participants were taking any prescription or over-the-counter medication at the time of participation. Based upon self-reporting, three females were tested in the early-follicular phase, one in the late-follicular phase of their menstrual cycle and one in the low-hormone phase of oral contraceptive use.

Experimental protocol

Following arrival at the laboratory, subjects rested in the supine position and an antecubital venous cannula was inserted for subsequent drug administration. Following instrumentation, acquisition of an acceptable MSNA signal, and a period of stabilisation, a single modified Oxford test was performed to determine vascular-sympathetic baroreflex function during ambient air breathing (Amb). Briefly, the modified Oxford test involved bolus injection of sodium nitroprusside followed 90 seconds later by phenylephrine, as described previously in detail (Rudas *et al*., 1999; Simpson *et al*., 2019). Participants were then transferred to breathing room air via a mouthpiece and noseclip. Following a period of stabilisation, subjects rested for 5 minutes to determine baseline cardiovascular and pulmonary hemodynamics and sympathetic neural activity. Subjects were then switched to breathing a gas mixture containing 21% oxygen and 40ppm Nitric Oxide (iNO) from a Douglas bag. Following six minutes of iNO, a second modified Oxford test was performed. During the modified Oxford test, subjects continued to breathe iNO. Modified Oxford tests were separated by a minimum of 20 minutes.

Inhaled Nitric Oxide

Inhaled NO rapidly diffuses across the alveolar-capillary membrane into the pulmonary vascular smooth muscle where it activates soluble guanylate cyclase and

induces vascular smooth muscle relaxation (Steudel *et al.,* 1999). NO that diffuses into the intravascular space rapidly binds to haemoglobin, which serves to inactivate NO (Rimar & Gillis, 1993) and prevent systemic vasodilation (Frostell *et al*., 1991; Pepke-Zaba *et al.*, 1991). Therefore, inhaled NO acts as a selective pulmonary vasodilator. NO was diluted in 100% nitrogen (N_2) in a Douglas bag, to prevent the production of nitrogen dioxide. Immediately prior to inhalation, the NO/N_2 gas mixture was titrated with 100% Oxygen (O_2) to obtain a gas mixture containing 21% O_2, 79% N_2 and 40ppm NO (ML206; ADInstruments, Colorado Springs, CO, USA). O_2 and NO gas concentrations were verified and nitrogen dioxide concentration was measured to ensure levels remained below 5ppm.

Experimental measurements

Cardiovascular and pulmonary haemodynamics

Heart rate (HR) and blood pressure (BP) were continuously recorded using electrocardiogram (ECG; Lead II) and finger photoplethysmography, respectively (Finometer MIDI, Finapres Medical Systems BV, Amsterdam, Netherlands). Systolic (SBP), diastolic (DBP) and mean (MAP) pressures were calculated on a beat-by-beat basis from the finger arterial pressure waveform. Finometer values were calibrated against the average of three brachial artery blood pressure measurements taken during the baseline period. Peripheral oxygen saturation (SpO_2) was determined using finger pulse oximetry (Nellcor, Medtronics, USA). Echocardiograhy was used to assess left ventricular stroke volume (SV) and pulmonary artery systolic pressure (PASP). Images were obtained using a commercially available system (Vivid Q, GE, Fairfield, CT, USA) and stored for subsequent off-line analysis. Images were acquired from parasternal long and short- axis and apical four- and five-

chamber views in line with the American Society of Echocardiography guidelines for the assessment of the right and left heart (Lang *et al.*, 2005, 2015; Rudski *et al.*, 2010). SV was calculated from the Doppler signal using the velocity-time integral and the aortic cross section area (π*aortic diameter2$\cdot 4^{-1}$). Cardiac output (Qc) was calculated as the product of HR and SV. PASP was quantified as the maximum systolic pressure gradient across the tricuspid valve added to right atrial pressure estimated from the collapsibility of the inferior vena cava, in line with the guidelines of the American Society of Echocardiography (Rudski *et al.*, 2010). To derive pressure, the modified Bernoulli equation ($4\cdot V^2$) was applied to the peak systolic regurgitation jet velocity measured via continuous wave Doppler (Rudski *et al.*, 2010).

Muscle sympathetic nerve activity

Multi-unit MSNA was recorded from the peroneal nerve via microneurography as previously described (Hagbarth & Vallbo, 1968; Sundlof & Wallin, 1978). The MSNA signal was confirmed by pulse-synchronous activity that responded to end-expiratory apnea but not to startle stimuli or skin stroking (Delius *et al.*, 1972). Nerve signals were acquired (Neuroamp EX headstage, ADInstruments, Sydney, Australia), amplified (100,000x), filtered (band pass 700-2,000Hz), rectified and integrated (decay constant 0.1s) (LabChart Pro v8.3.1, ADInstruments, Sydney, Australia). No adverse events or complications occurred during or following the microneurography procedure in any subject.

Data analyses

All haemodynamic data were sampled at 1 KHz using a commercial data acquisition software (LabChart Pro v8.3.1, ADInstruments, Sydney, Australia) and stored for

offline analysis. The raw MSNA signal was sampled at 10 KHz. Multi-unit bursts of MSNA were identified using an automated detection algorithm (Chart Pro 8.3.1) and confirmed using established criteria (White *et al.*, 2015) by a trained observer (LLS), who performed data analysis whilst blinded to the condition. To account for differences in microelectrode position, burst amplitude data were normalised by assigning a value of 100 to the largest burst observed during baseline. All other bursts were calibrated against this value. Resting MSNA was quantified as burst frequency (burst·min^{-1}), burst incidence (burst·100HB^{-1}), mean burst amplitude (a.u) and total activity (mean burst amplitude x burst frequency [au·min^{-1}]) and total MSNA (mean burst amplitude x burst incidence [burst·100HB^{-1}]). A single sonographer (TGD), who was blinded to the condition, analyzed echocardiography images.

Vascular-sympathetic baroreflex function

Vascular-sympathetic baroreflex gain was estimated from the relationship between 1) DBP and MSNA burst probability and 2) DBP and total MSNA during a “gold standard" modified Oxford test. All DBP values were assigned to a 3 mmHg bin to reduce the statistical impact of non-baroreflex influences. The percentage of cardiac cycles associated with a burst of MSNA (ranging from 0-100%) was calculated for each DBP bin to give values for MSNA burst probability. To determine the relationship between total MSNA and DBP the sum of normalized burst amplitudes for each DBP bin was determined. This value was then divided by the number of bursts within that DBP bin, to calculate mean burst amplitude (Usselman *et al.*, 2015). Mean burst amplitude was then multiplied by the burst probability to calculate total MSNA. The slope of the linear relationship was determined by weighted linear regression analysis, and this value provided an index of vascular-sympathetic baroreflex gain. Only slopes with (i) at least five data points and (ii) R≥

0.5 were included in the group mean data (Simpson *et al*., 2019). Vascular-sympathetic baroreflex gains for rising and falling pressures were not determined independently. The vascular-sympathetic baroreflex operating point was taken as the average values for DBP and MSNA burst incidence or Total MSNA during 5 minutes of the Amb condition immediately prior to the start of iNO,and during the last 5 minutes of iNO. Participants breathed with a mouthpiece during both periods. Furthermore, baroreflex gain was also assessed from the slope of linear regression analyses relating burst probability and total MSNA to corresponding spontaneous fluctuations in DBP during each of these 5-minute periods. Only values that met the previously described criteria were included in subsequent statistical analyses.

Statistical analyses

Significant vascular-sympathetic baroreflex slopes were not obtained in 2 out of 13 participants (R≥ 0.5) and a modified Oxford test was not performed in one participant during iNO; therefore, repeated measure comparisons for vascular-sympathetic baroreflex gain are limited to 10 participants. Spontaneous baroreflex slopes were not obtained in 5 out of 13 participants (R≥ 0.5); therefore, repeated measure comparisons for spontaneous baroreflex gain are limited to 8 participants. Values for cardiovascular haemodynamics and sympathetic neural activity during iNO were calculated by averaging over the last 5 minutes of NO inhalation and were compared to the 5 minutes ambient air breathing immediately preceding the start of inhalation. Pulmonary haemodynamics were determined between minutes one and two, and between minutes five and six of NO inhalation. The duration of inhalation had no effect on pulmonary haemodynamics (i.e. PASP values determined after one minute were not different from those measured after five minutes). Therefore, values for PASP presented in results, figures and tables are determined from those

measurements taken at each time-point. The effects of iNO on cardiovascular and pulmonary hemodynamics, MSNA, and sympathetic baroreflex gain were assessed using paired t-tests. Normality was assessed using Shapiro-Wilk test, and data that was not normally distributed underwent $\log_{10}$ transformation prior to analysis. All statistical analyses were performed using Prism 7.03 (GraphPad software, USA) and statistical significance was set at $P<0.05$ *a priori*. Group data are reported as means (± SD).

Results

Effect of iNO on pulmonary, cardiovascular haemodynamics and basal sympathetic neural activity

By design, inhalation of NO reduced PASP compared to Amb (Table 1). MSNA total activity was also significantly reduced during iNO. An example of MSNA and haemodynamic data recorded in one subject during Amb and iNO is presented in Figure 1. The relationship between MSNA total activity and PASP during Amb and iNO is shown in Figure 2. There was, however, no significant relationship between the ΔPASP and ΔMSNA total activity (r = 0.32, P = 0.29), or ΔPASP and ΔMSNA burst frequency (r = 0.27, P = 0.38). The reduction in total activity was mediated by a reduction in MSNA burst frequency with no change in mean burst amplitude compared to Amb (Table 1). There was a small, but significant, reduction in systemic SBP, DBP and MAP during iNO compared to Amb, with no change in HR, SV, Qc or SpO_2 (Table 1).

Effect of iNO on vascular-sympathetic baroreflex function

Inhalation of NO significantly reduced diastolic operating pressure (82 ± 8 to 80 ± 8 mmHg; P < 0.001) and MSNA operating point (burst incidence, 39 ± 15 to 33 ± 17

bursts·100HB^{-1}; *P* = 0.01: total MSNA, 1882 ± 862 to 1479 ± 891 a.u·100HB^{-1}; *P* = 0.02), indicating a leftward and downward resetting of the vascular-sympathetic baroreflex during iNO (Figure 3). The mean slope of the linear portion of the baroreflex curve was similar during iNO and Amb, regardless of whether MSNA was quantified as burst probability (-3.4 ± 1.7 to -3.5 ± 1.8 %·mmHg^{-1}; *P* = 0.85) or total MSNA (-204 ± 108 to -216 ± 83 au·mmHg^{-1}; *P* = 0.7), indicating no differences in vascular-sympathetic baroreflex gain (Figure 3). Similarly, the mean slope of the linear portion of the relationship between MSNA and spontaneous changes in arterial pressure, was comparable during the iNO and Amb conditions, regardless of whether MSNA was quantified as burst probability (-3.0 ± 1.5 %·mmHg^{-1} to -3.2 ± 0.9 %·mmHg^{-1}; P = 0.84) or total MSNA (-186 ± 135 to -197 ± 144 au·mmHg^{-1}; *P* = 0.88)

Discussion

The key novel findings from this study are twofold: 1) lowering arterial pressure in the pulmonary circulation alters the prevailing level of MSNA, providing first-in-human evidence of afferent input from pulmonary arterial baroreceptors regulating sympathetic neural outflow; and 2) pulmonary arterial pressure influences the set-point of the vascular-sympathetic baroreflex, providing a mechanism contributing to vascular-sympathetic baroreflex resetting during high-altitude exposure.

Sympathetic neural activation by pulmonary arterial baroreceptors

The existence of vagal afferent fibres whose firing is related to pulmonary arterial pressure changes, has been known for a long time (Bianconi & Green, 1959; Coleridge & Kidd, 1961). In anesthetized non-hypoxic animals, isolated distension of these baroreceptors, located at the pulmonary artery bifurcation and in the extrapulmonary artery branches, elicit increases in sympathetic neural activity

(Ledsome and Kan, 1977; Moore *et al.*, 2011). Furthermore, pulmonary arterial baroreceptor distension resets carotid baroreflex control of the peripheral vasculature (Moore *et al.*, 2011) to operate at higher levels of sympathetic nerve activity and systemic pressure. Until now, however, the physiological role of these receptors in humans has not been investigated, presumably due to the technical difficulty in isolating a physiological stimulus to the pulmonary vasculature in a closed-loop system. In an attempt to overcome this challenge, we studied healthy lowlanders at high-altitude, before and during inhalation of nitric oxide (iNO), an intervention shown to reduce pulmonary arterial pressure without altering systemic arterial pressure (Frostell et al., 1991; Pepke-Zaba *et al.*, 1991). With this approach, pulmonary arterial systolic pressure was lowered by 20%, which was accompanied by a 25% reduction in MSNA total activity. However, the magnitude of the response (i.e. ∆MSNA) was not related to the magnitude of stimulus (i.e. ∆PASP), which is likely due to individual differences in both the location of the operating point on the stimulus-response curve and the responsiveness of the reflex (i.e. gain). Whilst the reduction in MSNA during iNO also occurred in parallel to a small reduction in systemic arterial pressure, this would have been expected to evoke an increase in MSNA via arterial baroreflex buffering. Strikingly, MSNA total activity was remarkably stable in one subject who did not display a reduction in PASP during iNO. Therefore, we interpret that the reduction in MSNA observed during iNO was mediated by unloading of the pulmonary arterial baroreceptors. Notably, this response is directionally opposite to the sympathetic response elicited during unloading of the systemic arterial baroreceptors.

As the human circulation is an integrated closed-loop system, changes to reflexogenic areas other than the pulmonary artery, may have influenced the MSNA

response. Thus, several alternative interpretations of our data require discussion. First, an increase in atrial pressure is proposed to activate single-unit MSNA in healthy humans (Millar *et al.*, 2013; Incognito *et al.*, 2018). However, in contrast, when a stimulus is localized precisely to the left atria-pulmonary vein junction of anaesthetized dogs, atrial receptor activation has no effect on sympathetic efferent activity in lumbar nerves, which is an analogue of human MSNA (Karim *et al*., 1972). Notably, neither atrial receptor stimulation (Carswell et al., 1970) nor changes in ventricular filling (Drinkhill et al., 2001) affect systemic vascular resistance in anaesthetized dogs. Thus, whilst we acknowledge differences may exist between anaesthetized dogs and conscious humans, in our view, the intrathoracic receptors most likely to elicit the observed MSNA response are those located in the pulmonary artery and its bifurcation (Moore *et al.*, 2004*a*, 2004*b*). Furthermore, studies that have employed invasive haemodynamic measurements report no change in right atrial pressure and pulmonary capillary wedge pressure from sea level to high-altitude (6100m), despite an increase in pulmonary artery mean pressure from 15 to 24 mmHg (Reeves *et al*., 1987; Groves *et al*., 1987). Therefore, right and left atrial pressure would not necessarily be expected to change during the reduction in PASP observed during iNO in the present study. Second, we cannot reject some contribution by the arterial baroreflex. Notably, arterial baroreceptors respond to deformation of the arterial wall and not arterial pressure *per se* (Angell-James, 1971). Therefore, a small change in stroke volume, independent of arterial pressure, could alter baroreceptor afferent activity and influence the observed MSNA response (Lacolley *et al.,* 1992; Taylor *et al*., 1995; Fu *et al*., 2008). Closer inspection of our data, however, reveals a variable change in SV during iNO; that is, SV increased in eight participants, and decreased in five. Despite this, MSNA was reduced in 11 out

of 13 participants. Along with the previously discussed small decrease in arterial blood pressure, this variability in the SV response suggests that the arterial baroreflex did not mediate the observed decrease in MSNA.

Inhalation of NO resulted in a small, non-significant increase in SpO_2; therefore, a reduced peripheral chemoreceptor drive may have mediated the reduction in MSNA. We cannot ignore this possibility, although we and others have demonstrated no change in MSNA with administration of 100% oxygen at high-altitude (Hansen & Sander, 2003; Simpson *et al.*, 2019). Furthermore, we did not observe a significant relationship between the change in SpO_2 and change in MSNA ($r = -0.16$, $P = 0.61$) in the present study. Finally, whilst any NO that enters the circulation is rapidly inactivated (Rimar & Gillis, 1993), an increased central NO bioavailability, via the nitrate-nitrite-NO pathway may have influenced the MSNA response (Owlya *et al.,* 1997; Young *et al.*, 2009; Notay *et al.*, 2017). However, no change in plasma nitrite levels were previously reported in healthy subjects during a 60 minute inhalation of 25ppm iNO (Westfelt *et al.*, 1995).

Interaction between pulmonary arterial baroreceptors and arterial baroreflex

Interestingly, the reduction in MSNA total activity was mediated by a reduction in burst occurrence, with no change in burst amplitude. Kienbaum et al. (2001) proposed two central sites for modulation of sympathetic outflow, where the arterial baroreflex determines the occurrence of sympathetic bursts, however, other peripheral inputs largely determine the size of those bursts. As such, it appears that afferent input from pulmonary arterial baroreceptors alter sympathetic outflow by modulating baroreflex control of MSNA. Indeed, we demonstrated an interaction

between the two groups of baroreceptors, whereby unloading of the pulmonary arterial baroreceptors (i.e. lowering pulmonary arterial systolic pressure) did not change the MSNA responsiveness to acute increases and decreases in arterial pressure (i.e. gain), but reset sympathetic neural activity and diastolic pressure to lower levels. Thus, there was a downward and leftward resetting of the arterial baroreflex control of MSNA. This is consistent with work in experimental animals that report an upward and rightward resetting of the carotid baroreflex control of vascular resistance, with no change in gain, during increases in pulmonary arterial pressure (Moore et al., 2011).

In the present study, we observed a small, but significant, reduction in diastolic blood pressure (~2mmHg) during reductions in pulmonary arterial systolic pressure. Using individual stimulus-response relationships, we estimate that such a reduction in diastolic pressure should increase the probability of a burst by around 5 to 6% (i.e. 5 - 6 bursts·100Hb^{-1}), via engagement of the arterial baroreflex. In fact, we observed the opposite, a reduction of 6 bursts·100Hb^{-1}. We interpret a lack of reflex sympathoexcitation as further confirmation that the set-point of the vascular-sympathetic baroreflex is reset when the prevailing arterial pressure in the pulmonary circulation changes. Recently, we observed an upward resetting of the vascular-sympathetic limb of the arterial baroreflex during chronic exposure (10-21 days) of healthy lowlanders to high-altitude (Simpson *et al.*, 2019). Furthermore, reducing peripheral chemoreceptor drive, via administration of 100% oxygen, did not reverse this resetting, leaving the potential mechanism(s) unclear. The mechanism presented and discussed here, therefore, may play a role; that is, afferent feedback from pulmonary arterial baroreceptors contributes to resetting of arterial baroreflex regulation of MSNA and blood pressure control at high-altitude.

Experimental limitations

First, the present study characterizes the neural response to a reduction in pulmonary arterial pressure only. Primarily, this was due to the difficultly in elevating pulmonary arterial pressure independently in conscious humans. All the same, a study performed under non-hypoxic conditions observed marked suppression of MSNA during rapid volume infusion, which acutely raised pulmonary pressure (Pawelczyk *et al*, 2001); however, sympathoinhibition in that setting is the net effect of the integration of multiple reflex inputs. In contrast, experiments in non-hypoxic animals demonstrated sympathoexcitation in response to incremental increases in pulmonary arterial pressure over a physiological range. Notably, careful control of pressure to the aortic and carotid baroreceptors eliminated baroreflex buffering of measured response (Moore et al., 2011), something which very difficult to accomplish in humans. Second, we investigated sympathetic vasomotor outflow to the skeletal muscle vasculature only and acknowledge that outflow to other vascular beds may exhibit differential reflex responses (Morrison 2001). Third, under ambient conditions, a modified Oxford test was performed prior to a participant being placed on the mouthpiece, whereas during the iNO intervention the test was performed whilst the subject breathed with a mouthpiece. Breathing through a mouthpiece alone, therefore, may have influenced the vascular-sympathetic baroreflex. However, MSNA 'set-point' was determined from a period when the participants were spontaneously breathing through a mouthpiece for both conditions; furthermore, an index of spontaneous vascular-sympathetic baroreflex gain was determined during these same periods. Notably, inhalation of NO did not alter baroreflex gain, regardless of the method of determination. In our view, therefore, it is unlikely that breathing via a mouthpiece has confounded our interpretation. Fourth, we did not

measure ventilation, which has known influences on MSNA (Hagbarth & Vallbo, 1968; Somers et al., 1989) and vascular-sympathetic baroreflex gain (Van De Borne *et al.*, 2000). However, Scherrer and colleagues (Scherrer *et al.*, 1996) demonstrated that a 12 minute inhalation of NO (40ppm), in healthy lowlanders at a similar altitude (4559m), had no effect on ventilation or end tidal CO_2. Fifth, due to the unknown time course of recovery, the order of conditions was not counterbalanced in the present study; therefore, we cannot rule out a potential order effect on our results.

Implications and Significance

The present study represents an important first step to bridge a gap in evidence between human and animal studies. Consistent with data in non-hypoxic anaesthetized dogs, MSNA was greater when pulmonary arterial pressure was higher in conscious humans, albeit under HA hypoxia. We propose that a sustained increase in pulmonary arterial pressure evokes a reflex input to central nervous system areas controlling sympathetic vasoconstrictor outflow. This input, therefore, acts as signal for sympathetic restraint of hypoxic vasodilatation, thus preserving arterial pressure homeostasis at high-altitude. The same pathway could link increased right ventricular outflow and elevated pulmonary arterial pressure during exercise to sympathetic restraint of muscle blood flow, so that blood pressure is maintained. Still, this speculation requires investigation. Furthermore, it is uncertain how this input pathway contributes to beat-by-beat control of the vasoconstrictor outflow when pulmonary arterial pressure is normal. More study, therefore, is required to distinguish low-pressure pulmonary baroreceptor control of sympathetic outflow from classical negative feedback reflex control originating from arterial baroreceptors located in the systemic circulation.

Conclusion

This study provides evidence that supports a physiological role for pulmonary arterial baroreceptors in sympathetic activation in humans, at least when there is a sustained elevation in arterial pressure in the pulmonary circulation. Taking advantage of a novel experimental approach, we observed a reduction in basal MSNA during acute lowering of pulmonary arterial pressure; this is opposite to the sympathoexcitatory effect when there is a reduction of systemic arterial pressure. Furthermore, lowering arterial pulmonary pressure influences the set-point of the vascular sympathetic baroreflex. Finally, this study illustrates some of the technical challenges encountered when studying sympathetic neural responses to stimulation of different reflex inputs in conscious humans, and highlights the importance of developing experimental approaches to overcome such challenges.

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ADDITIONAL INFORMATION

Competing Interests

None

Author Contributions

LLS, JPM, MS contributed to conception of the work. LLS, JPM, MS, SJO, CDS, MMT, JA, and PNA contributed to the design of the work. LLS, JPM, MS, CDS, JSL, VLM, AS, CG, ST, SAB, TGD and ALD contributed to acquisition and analysis of the data. LLS, JPM, MS contributed to the interpretation of the data and LLS, JPM and MS wrote and critically revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons included as an author qualify for authorship, and all those who qualify for authorship are listed.

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Table 1. Haematological, cardiovascular and pulmonary haemodynamics and muscle sympathetic nerve activity (MSNA) during ambient air breathing (Amb) and during inhalation of nitric oxide (iNO). Group (n=13) data are presented as mean (± SD) (♦ SpO_2 for n=11). Statistical comparisons performed using paired t-tests.

Table 1.

	Amb (n=13)	iNO (n=13)	*P* Value
Pulmonary haemodynamics			
Pulmonary systolic artery pressure (mmHg)	32 ± 5	26 ± 4	**<0.001**
Cardiovascular haemodynamics			
SpO_2 (%) ♦	82 ± 3	85 ± 5	0.07
Heart rate (bpm)	75 ± 18	73 ± 17	0.6
Systolic BP (mmHg)	130 ± 17	128 ± 17	**0.002**
Diastolic BP (mmHg)	82 ± 8	80 ± 8	**<0.001**
Mean arterial pressure (mmHg)	101 ± 11	100 ± 11	**0.0034**
Stroke volume (mL)	71.4 ± 16.3	73.9 ± 18.3	0.13
Cardiac output ($L \cdot min^{-1}$)	5.3 ± 1.3	5.3 ± 1.2	0.81
Total peripheral resistance ($mmHg \cdot L \cdot min^{-1}$)	20.9 ± 5.6	19.9 ± 4.8	0.76
Muscle sympathetic nerve activity			
Burst frequency ($bursts \cdot min^{-1}$)	29 ± 13	23 ± 13	**0.008**
Burst incidence ($bursts \cdot 100HB^{-1}$)	39 ± 15	33 ± 17	**0.01**
Mean burst amplitude (au)	48 ± 11	46 ± 16	0.17
Total activity ($au \cdot min^{-1}$)	1369 ± 576	994 ± 474	**0.01**
Total MSNA ($au \cdot 100HB^{-1}$)	1882 ± 862	1479 ± 891	**0.02**

Figure 1.

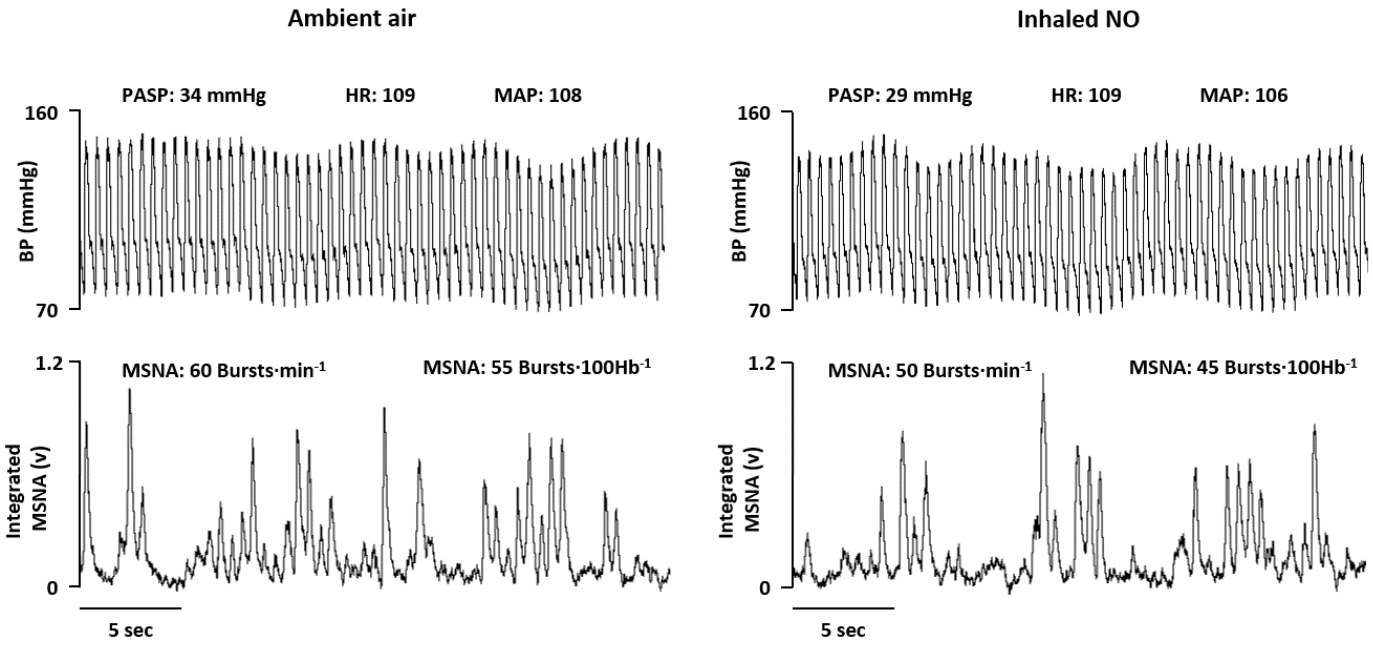

Figure 1. Example recording of MSNA and blood pressure. One representative subject during ambient air breathing and during inhalation of nitric oxide (NO).

Figure 2.

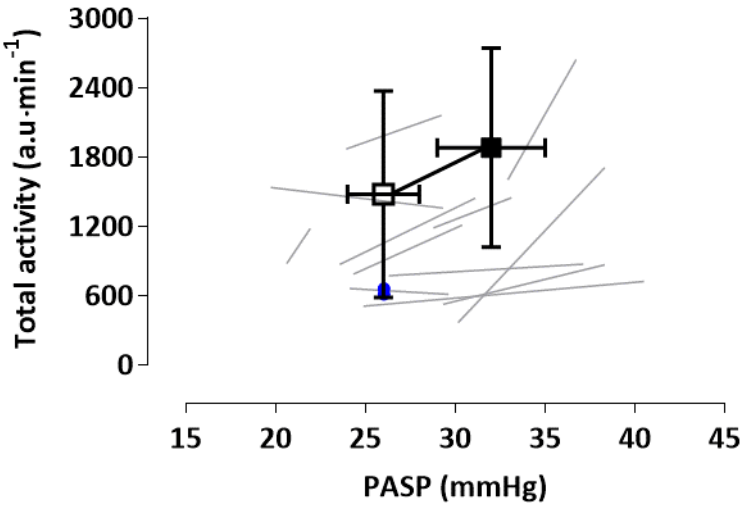

Figure 2. Group average pulmonary arterial systolic pressure (PASP) and corresponding MSNA total activity during ambient air breathing (Amb) and inhalation of nitric oxide (iNO). Grey lines represent each individual's MSNA response to changes in PASP and black line represents average MSNA response to changes in PASP. Data from one participant who did not display a change in PASP during iNO is highlighted in blue Statistical comparisons performed using paired t-tests.

Figure 3. Vascular-sympathetic baroreflex function: A) Average regression line for relationships (n=10) between DBP and MSNA burst probability and DBP and total MSNA from a modified Oxford test during ambient air (Amb) and inhaled nitric oxide (iNO). The operating points are indicated by symbols and error bars (mean ± SD) * Vascular-sympathetic baroreflex set-point $P<0.05$ versus Amb. The vascular-sympathetic baroreflex was reset downward during iNO. The slope of the relationship between DBP and MSNA was similar during Amb and iNO, indicating no difference in vascular-sympathetic baroreflex gain (paired t-test). B) Individual and mean (n=10) slopes for the relationship between DBP and MSNA burst probability and DBP and Total MSNA.