Can intravenous endothelin-1 be used to enhance hypoxic pulmonary vasoconstriction in healthy humans?

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Background. Hypoxic pulmonary vasoconstriction (HPV) helps match pulmonary perfusion to ventilation. The peptide endothelin-1 (ET-1) may be involved in the cellular mechanisms of this response. We hypothesized that increasing plasma ET-1 concentration during hypoxia would enhance HPV in humans and might represent a strategy for improving gas exchange during single-lung anaesthesia or respiratory disease.

Methods. Nine healthy volunteers were each exposed twice to a 7-h protocol consisting of 1 h breathing air, 4 h of eucapnic hypoxia (end-tidal \( P_{O_2} \), 50 mm Hg), and 2 h of eucapnic euoxia (end-tidal \( P_{O_2} \), 100 mm Hg). Volunteers received a 7-h i.v. infusion of ET-1 during one protocol (1.0–2.5 ng kg\(^{-1}\) min\(^{-1}\)) and normal saline during the other. At intervals of 30–60 min, cardiac output and the maximum tricuspid pressure gradient during systole (\( \Delta P_{max,a,n} \), an index of HPV) were measured using Doppler echocardiography, systemic arterial pressure was measured using sphygmomanometry, and plasma samples were obtained to determine ET-1 concentration.

Results. During hypoxia, \( \Delta P_{max} \) increased for around 2 h before reaching a plateau. Compared with saline, ET-1 had no effect on \( \Delta P_{max} \), either at baseline or during hypoxia. ET-1 infusion slightly increased diastolic arterial pressure and reduced cardiac output, but had no specific effect on the change in these variables during hypoxia. During the final 1 h of hypoxia, plasma ET-1 concentration was 1.7 (0.4) pg ml\(^{-1}\) [mean (SD)] in the saline protocol and 21.9 (12.2) pg ml\(^{-1}\) in the ET-1 protocol.

Conclusions. ET-1 infusion seems unlikely to represent a therapeutic strategy for enhancing HPV during acute (<4 h) hypoxia.

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Hypoxic pulmonary vasoconstriction (HPV) was characterized in 1946 as a mechanism for optimizing gas exchange by matching pulmonary perfusion to ventilation.¹ Although now recognized as potentially pathophysiological in the context of global hypoxia,² ³ it is still regarded as a valuable response in the context of localized alveolar hypoxia. It has been estimated, for example, that local pulmonary vasoconstriction is capable of increasing arterial oxygen partial pressure (\( P_{A,O_2} \)) by up to 2.7 kPa in patients with ventilation–perfusion (V–Q) inequality due to chronic obstructive pulmonary disease (COPD) or acute respiratory distress syndrome (ARDS).⁴ In addition, during single-lung ventilation, when hypoxia due to shunting of blood through a collapsed lung represents the major challenge for the anaesthetist,⁵ HPV is regarded as a key mechanism for diverting blood flow towards the ventilated lung.⁶ ⁷

Given its importance during anaesthesia and in respiratory disease, there has understandably been interest in characterizing specific pharmacological enhancers of HPV. The vasoconstrictor almitrine has been shown to improve gas exchange in patients with COPD⁸ ⁹ or ARDS¹⁰ ¹¹ and to improve \( P_{A,O_2} \) during one-lung ventilation.¹² ¹³ However,
very few other compounds have so far been shown clearly to enhance HPV in man.

Endothelin-1 (ET-1) is a vasoactive peptide that has been widely implicated in the cellular mechanisms of HPV. The release of ET-1 from vascular endothelial cells throughout the body is enhanced by local hypoxia, and plasma ET-1 levels correlate with pulmonary artery pressure after rapid (<24 h) ascent to high altitude. Furthermore, a recent study has shown that despite having no effect on pulmonary artery pressure in healthy volunteers at sea level, the ET-1 receptor antagonist bosentan produced a significant reduction of established pulmonary hypertension when given shortly after arrival at altitude.

We hypothesized that increasing plasma ET-1 concentration during hypoxia might enhance HPV, potentially improving the efficiency of gas exchange in pulmonary disease or during single-lung anaesthesia. To test the assumption underlying this hypothesis, we studied the effects of i.v. ET-1 infusion on the pulmonary vascular response to isocapnic hypoxia in nine healthy humans.

Methods

Subjects and protocols

Nine healthy volunteers [two females, seven males, age 23.9 (20–29) yr, mean (range)] participated in the study, which was approved by the Central Oxford Research Ethics Committee (COREC). All gave written, informed consent. Each volunteer undertook two 7-h protocols, separated by at least 1 week. The two protocols were identical in terms of end-tidal gas control, consisting of 1 h breathing normal room air, followed by 4 h of alveolar hypoxia [end-tidal partial pressure of oxygen (P\textsubscript{\text{ET}}\textsubscript{o})=50 mm Hg] and then 2 h of controlled euoxia (P\textsubscript{\text{ET}}\textsubscript{o}=100 mm Hg). With the exception of the first 1 h of each protocol, the end-tidal partial pressure of carbon dioxide (P\textsubscript{\text{ET}}\textsubscript{c}O\textsubscript{2}) was held constant throughout, at each volunteer’s normal value breathing air.

During the ET-1 protocol, volunteers received a continuous 7-h i.v. infusion of ET-1. During the control protocol, volunteers received a continuous 7-h infusion of normal saline. The two protocols were otherwise identical. Five volunteers underwent the ET-1 protocol first, and four underwent the control protocol first. Females were asked to participate only during the first 14 days of their menstrual cycle.

During both protocols, transthoracic Doppler echocardiography was used to determine cardiac output and the maximum pressure gradient across the tricuspid valve during systole. Changes in this gradient are widely used as an index of HPV in human studies. Systemic arterial pressure was also measured regularly using automated oscillometric sphygmomanometry, arterial oxygen saturation was measured continuously using a pulse oximeter, and venous blood samples were obtained regularly for determination of plasma ET-1 levels.

Gas control

Experiments took place in a purpose-built normobaric chamber, in which respired gas was sampled continuously using a nasal catheter. Inspired and end-tidal partial pressures of oxygen and carbon dioxide were recorded breath-by-breath using a gas analyser (Datex Engstrom, Finland), and accurate maintenance of predetermined end-tidal gas composition was achieved by computer-automated adjustment of the chamber gas composition approximately every 5 min, as described in detail elsewhere.

Doppler echocardiography

Echocardiographic measurements were made every 30–60 min using a Hewlett-Packard (Palo Alto, CA, USA) Sonos 5500 ultrasound machine with an S4 two-dimensional transducer (2–4 MHz), and recorded continuously to s-VHS video cassettes for subsequent off-line analysis. During and for at least 5 min before each measurement, volunteers were lying on a couch in the left lateral position. Heart rate and respiratory waveform were recorded, and all measurements were made as close as possible to end-expiration.

Tricuspid valve maximal pressure gradient

Pulmonary vascular tone was assessed using a standard technique that has been extensively validated. Briefly, using an apical four-chamber view, the maximum velocity of a regurgitant jet of blood through the tricuspid valve was measured during systole and the maximum systolic pressure gradient across the valve (ΔP\textsubscript{max}) was calculated using Bernoulli’s equation. Right atrial pressure is unaffected by sustained hypoxia, so changes in ΔP\textsubscript{max} reflect changes in systolic pulmonary artery pressure and have been used in numerous studies to assess HPV non-invasively in humans. At each time point, ΔP\textsubscript{max} represents the average of 5–10 velocity measurements, recorded within a 5-min period.

Cardiac output

Using an apical five-chamber view, the velocity of blood flow through the aortic valve was measured during systole. Cardiac output was calculated using the aortic valve diameter, measured using a parasternal long axis view. At each time point, cardiac output represents the average of 3–5 measurements, recorded within a 5-min period.

Endothelin-1 administration

During the ET-1 protocol, pharmaceutical grade human ET-1 (Clinalfa AG, Switzerland) was infused continuously for 7 h through an indwelling venous cannula in the forearm. For the first 30 min, the infusion rate was 1.0 ng kg\textsuperscript{-1} min\textsuperscript{-1} and arterial pressure was measured every 5 min. The rate was then increased gradually over the next 30 min to a maximum of 2.5 ng kg\textsuperscript{-1} min\textsuperscript{-1}. In five volunteers, ET-1 produced a mild local inflammatory response, and in seven
volunteers, the infusion produced some local muscular discomfort. These symptoms resolved within a few hours of the end of the infusion, but were controlled when necessary during the experiment by varying the rate of ET-1 infusion. The total volume of infusion was 59 (15) ml [mean (SD)]. During the control protocol, a similar volume of normal saline was infused at a constant rate.

In both protocols, blood samples were withdrawn every 60 min from a second indwelling venous cannula, positioned in the forearm not being used for infusion of ET-1 or saline. Samples were anticoagulated with EDTA and spun for 15 min at 3000 rpm to obtain plasma, which was stored immediately at −20°C. The ET-1 concentration in each sample was subsequently determined in duplicate by enzyme-linked immunosorbent assay (QuantiGlo human ET-1 immunoassay, R&D Systems, UK).

Statistics
Repeated measured analysis of variance was used to determine whether changes in measured variables occurred during hypoxia, and whether any such changes differed in the ET-1 protocol, compared with the control protocol. Paired Student’s t-tests were used to compare individual time points both within and between protocols. Statistical significance was accepted at the 95% confidence level (P<0.05). Values are reported as mean (sd).

Results

Gas control
Steps between air breathing and hypoxia, and between hypoxia and euoxia, were completed within <5 min, and both $P_{\text{IO}2}$ and $P_{\text{E}02}$ were maintained at desired levels throughout all periods of gas control (Fig. 1). During hypoxia, arterial oxygen saturation was 83.2 (0.7)% in the ET-1 protocol and 82.9 (0.7)% in the control protocol [mean (SD); P>0.4].

Plasma ET-1 concentrations
At baseline (0 h), there was no difference between plasma ET-1 concentration in the control and ET-1 protocols [1.0 (0.4) and 1.3 (0.7) pg ml$^{-1}$, respectively; P>0.07]. These values are within the normal range provided by the assay manufacturers (0.5–2.3, mean 1.1 pg ml$^{-1}$).

The changes in plasma ET-1 levels during each protocol are summarized in Table 1, and have been reported in more detail elsewhere.$^{33}$ Briefly, plasma ET-1 concentration did not change during the first 1 h of the control protocol, but rose significantly after the onset of hypoxia (P<0.01). On return to euoxia, plasma ET-1 levels returned to baseline within 1 h.

In the ET-1 protocol, plasma ET-1 concentration increased throughout the infusion. However, this increase was most marked during the first 4 h of infusion, after which a steady state appeared to be reached.$^{33}$ At every time point except baseline (0 h), ET-1 levels were significantly higher in the ET-1 protocol than in the control protocol (P<0.02).

Systemic cardiovascular responses
Cardiac output decreased modestly during the first 30 min of both protocols, but the decrease was statistically significant only in the ET-1 protocol (P<0.03). This trend appeared to continue during the 30 min preceding hypoxia...
in the ET-1 protocol, but not in the control protocol. There was, however, no significant difference between cardiac output at 30 and 60 min in either protocol (P = 0.08 and P = 0.2 for ET-1 and control protocols, respectively).

Hypoxia produced a significant increase in both cardiac output and heart rate (P < 0.01; Fig. 2), but not stroke volume (P > 0.1). After the onset of hypoxia, the initial increase in cardiac output was followed in both protocols by a short plateau and then by a further gradual increase that continued throughout hypoxia, but reversed rapidly upon returning to euoxia. Cardiac output was lower during the ET-1 protocol than during the control protocol (P < 0.02 and P < 0.05, respectively), but ET-1 had no detectable effect on systolic arterial pressure (P > 0.3).

Pulmonary vascular responses

During the 1 h preceding hypoxia, there was no change in ΔP_{max} in either protocol (P > 0.2; Fig. 4A) and no difference between the ET-1 and the control protocols (P > 0.7), indicating that neither ET-1 nor saline infusion per se had a significant effect on pulmonary vascular tone.

In both protocols, hypoxia increased ΔP_{max} (P < 0.01). The increase began within 30 min and continued for at least 2 h, before reaching a plateau. There was no significant difference between the changes in ΔP_{max} in the ET-1 and control protocols (P > 0.8), and ET-1 did not significantly alter the effect of hypoxia on ΔP_{max}, compared with control (P > 0.05; Fig. 4A).

A small proportion of the increase in ΔP_{max} during hypoxia in this study can be attributed to the measured increase in cardiac output, rather than to active pulmonary vasoconstriction. Using published data describing the effect of changes in cardiac output on ΔP_{max},^{34} we subtracted from the measured ΔP_{max} the contribution attributable to changes in cardiac output (for details see Discussion). Figure 4A shows changes in this calculated index (ΔP'_{max}). Despite the lower cardiac output in the ET-1 protocol, the magnitude and time course of changes in ΔP'_{max} and ΔP_{max}
are very similar, and the principal findings of the study are independent of which index of pulmonary vascular tone is used.

Discussion

Since its first description over 60 yr ago, HPV has been the subject of considerable debate, with some authors regarding the phenomenon as largely vestigial in the adult human, deriving from life in utero, and others believing it to be an important means of optimizing gas exchange in the normal human lung.

Whatever its teleological or functional significance in the healthy lung, there is increasing evidence of a role for HPV in lung injury and anaesthesia. It has been reported to contribute to the maintenance of $P_{aO_2}$ in a range of clinical settings, including COPD and ARDS, chronic bronchial obstruction, and particularly during one-lung ventilation. In anaesthetized patients exposed to unilateral hypoxia before elective surgery, HPV has been reported to reduce blood flow to the hypoxic lung by around 40% within 15–60 min. In fact, there is reason to believe that these studies may substantially underestimate the potential contribution of HPV. First, the intensity of HPV may increase over time. In isolated lungs of the rabbit exposed to sudden but sustained unilateral hypoxia, HPV reduced shunt by around 50% at 30 min, but by almost 100% after 6 h. This dramatic reduction was associated with a gradual increase in $P_{aO_2}$, and represents a second, slow phase of HPV in vivo. A similar slow phase of the pulmonary vascular response to hypoxia has now been described in man, with an initial rapid phase of HPV being followed by a more gradual vasoconstriction that is not maximal until at least 2–4 h after the onset of hypoxia. Secondly, it may be possible to enhance the magnitude of HPV pharmacologically. As discussed above, the respiratory stimulant and vasoconstrictor almitrine, which reportedly mimics hypoxia by acting on cellular pathways involved in pulmonary oxygen-sensing, has been shown to improve $P_{aO_2}$ in patients with respiratory disease, and is effective in both preventing and treating hypoxaemia during single-lung ventilation.

On the basis of these beneficial effects of almitrine during hypoxia, we hypothesized that the vasoactive peptide ET-1 might also enhance the pulmonary vascular response to hypoxia. ET-1 has been widely implicated in the cellular mechanisms of HPV, but its role remains controversial, with no clear consensus emerging from numerous studies in a range of animal preparations. In healthy volunteers, ET-1 antagonist bosentan substantially reduced established HPV when taken by healthy volunteers after a long-term of 15 min of hypoxia. In contrast, a more recent study showed that the dual ET A/ET B antagonist BQ123 decreased pulmonary vascular resistance during euoxia, but had no additional effect on pulmonary vasoconstriction during 15 min of hypoxia.

The major finding of the current study is that ET-1 infusion did not significantly alter the pulmonary vascular tone during hypoxia, although previous studies have examined its effect during euoxia. In healthy volunteers, ET-1 has been shown to induce pulmonary vasoconstriction at infusion rates $\geq 3.7$ ng kg$^{-1}$ min$^{-1}$ but not at lower rates of 1.0 or 1.9 ng kg$^{-1}$ min$^{-1}$. These findings concur with our observation that infusion at a rate of $\leq 2.5$ ng kg$^{-1}$ min$^{-1}$ did not change $\Delta P_{max}$ before the onset of hypoxia in the ET-1 protocol, a feature of our study that allowed assessment of the effect of elevated ET-1 per se during hypoxia, rather than elevated baseline pulmonary vascular tone.

Fig 4 (A) Mean $\Delta P_{max}$ during the control and ET-1 protocols. (B) Calculated $\Delta P_{max}$ (termed $\Delta P_{max}$; see Results) after a subtraction from the measured change in $\Delta P_{max}$ of a direct contribution from the change in cardiac output during each protocol. Volunteers breathed air for the first 1 h of each protocol; black bar indicates eucapnic hypoxia ($P_{aO_2}=50$ mm Hg); grey bar indicates eucapnic euoxia ($P_{aO_2}=100$ mm Hg); open bar indicates 7-h infusion of ET-1 or saline. Symbols represent mean (SD), n=9.
response to sustained hypoxia. There are several possible interpretations of such a finding. First, ET-1 may be assumed to play no major aetiological role in human HPV over this time course. Secondly, however, it is possible that ET-1 receptors in the pulmonary circulation are normally saturated during hypoxia, so no further enhancement by exogenous ET-1 is possible. Thirdly, it is possible that exogenous peptide is simply unable to access its site of action, particularly given the known tendency for ET-1 to be secreted from the abluminal face of endothelial cells, directly on to the smooth muscle cells below.50

In opposition to this latter possibility that ET-1 was unable to access its site of action on vascular smooth muscle cells, we observed the characteristic cardiovascular effects of ET-1 infusion during our current study. During the first 30 min of both protocols, cardiac output decreased modestly. It is possible that a longer control period would have resulted in a more stable cardiac output at baseline, that is, before the onset of ET-1 infusion. However, in the control protocol, cardiac output was stable for at least 30 min before hypoxia. In the ET-1 protocol, cardiac output did appear to decrease during the 30 min preceding hypoxia (although this decrease did not reach statistical significance), but this seems likely to represent the anticipated systemic effect of ET-1 infusion. Cardiac output was significantly reduced by ET-1 infusion, compared with saline, and this finding is in keeping with several previous reports. Sorensen and colleagues,51 for example, infused ET-1 into healthy volunteers for 1 h at a rate of 2.5 ng kg\(^{-1}\) min\(^{-1}\), and described no change in systolic arterial pressure, but an increase in diastolic pressure (\(\sim 8\%\)) and a decrease in heart rate (\(\sim 14\%\)). In a similar study, Kiely and colleagues47 infused ET-1 at 1.9, 3.7, and 7.5 ng kg\(^{-1}\) min\(^{-1}\), and also described a dose-dependent increase in diastolic arterial pressure and a decrease in both heart rate and cardiac output. These effects, which are very similar to those reported in the current study, seem likely to be mediated mainly via the ET\(_A\) receptor, since BQ123 was unable to access its site of action on vascular smooth muscle cells, we observed the characteristic cardiovascular effects of ET-1 mediated mainly via the ET\(_A\) receptor, since BQ123 was unable to access its site of action on vascular smooth muscle cells below.47

Given the substantial difference between cardiac output in the ET-1 and control protocols, an important question is whether this difference could represent an important cofounder in the assessment of changes in \(\Delta P_{\text{max}}\) during hypoxia. To address this question, we used data from a study in which diurnal variations in cardiac output and \(\Delta P_{\text{max}}\) were measured in 33 resting healthy humans.34 Cardiac output was seen to vary by up to \(\sim 2.5\) litre min\(^{-1}\), but the associated change in \(\Delta P_{\text{max}}\) was only 0.61 (0.74) [mean (sd)] mm Hg min litre\(^{-1}\). These data were used in the current study to subtract from the measured change in \(\Delta P_{\text{max}}\) a contribution attributable to the change in cardiac output (from time zero, \(\Delta Q\)), according to the equation

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\Delta P_{\text{max}}^* = \Delta P_{\text{max}} - (\Delta Q \times 0.61).
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Calculated changes in \(\Delta P_{\text{max}}^*\) are shown in Figure 4b. Importantly, the magnitude and time course of changes in \(\Delta P_{\text{max}}\) and \(\Delta P_{\text{max}}\) are very similar, and the principal findings and conclusions of this study are independent of whether \(\Delta P_{\text{max}}\) or \(\Delta P_{\text{max}}^*\) is used as an index of pulmonary vascular tone.

In summary, we have demonstrated that, compared with saline infusion, i.v. ET-1 infusion increases diastolic arterial pressure and decreases cardiac output, but does not alter the pulmonary vascular response, during acute (<4 h) generalized, eucapnic alveolar hypoxia in healthy volunteers. If it is assumed that the pulmonary vascular response to regional hypoxia would be similarly unaltered by exogenous ET-1, these findings suggest that ET-1 infusion is unlikely to represent a therapeutic strategy for enhancing HPV during acute (<4 h) hypoxia.

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