Hypoxaemia and release of endothelin-1

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Abstract

Background - Secretion of the vasoconstrictor peptide endothelin-1 from vascular endothelium is increased by various stimuli. Whether hypoxaemia affects plasma levels of endothelin-1 in humans is unknown, but this may be important in the haemodynamic response to hypoxaemia. The plasma endothelin-1 concentrations in hypoxaemic humans has therefore been measured.

Methods - Plasma levels of endothelin-1 were measured by specific radioimmunoassay in 10 control subjects at rest and following 30 minutes of acute hypoxaemia (Sao2 75-80%) induced by breathing a nitrogen/oxygen mixture, and in 10 patients with hypoxaemic cor pulmonale.

Results - The plasma endothelin-1 concentration in control subjects was increased from a mean (SE) of 0·90 (0·11) pmol/l at baseline to 2·34 (0·34) pmol/l during hypoxaemia. In patients with cor pulmonale the plasma endothelin-1 concentration was 2·96 (0·34) pmol/l, raised in comparison with control subjects at rest but similar to levels in controls during hypoxaemia.

Conclusions - Plasma levels of endothelin-1 were increased by hypoxaemia in humans. The raised levels observed in patients with cor pulmonale may largely be attributable to the effects of hypoxaemia, although the pathophysiological significance of these observations remains to be established.

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Keywords: endothelin, cor pulmonale, hypoxaemia.

The endothelins are a group of structurally similar peptides synthesised by vascular endothelium. These peptides act via two specific receptors. Stimulation of the type A endothelin receptor (ETA), the main subtype found on vascular smooth muscle cells, results in profound and longlasting vasoconstriction, whilst stimulation of the type B receptor (ETB) causes release of vasodilator metabolites and transient vasodilation. Interestingly, however, in rat pulmonary resistance vessels in vitro ETB activation may be important in mediating endothelin-1 induced vasoconstriction. In humans, endothelin-1 is the most potent vasoconstrictor substance known through its relatively selective activation of the ETB receptor. A role in the maintenance of vascular tone is suspected but this remains speculative at present.

Synthesis of this peptide is increased by a number of humoral and physical stimuli, including hypoxia, which may be pathophysiologically relevant. In vitro studies have shown that acute hypoxia increases endothelin-1 production by human endothelial cells whilst, in experimental animals in vivo, hypoxia increases plasma concentrations of endothelin-1. The effect of hypoxaemia on endothelin-1 in humans is unknown but may be relevant in the cardiovascular adaptations to hypoxaemia and the circulatory abnormalities seen in conditions such as cor pulmonale.

We have therefore studied the effects of hypoxaemia on plasma endothelin-1 levels in healthy subjects and also in patients with hypoxaemic cor pulmonale.

Methods

Subjects

Normal controls

Ten young male volunteers of mean (SE) 28·1 (2·2) years were studied. None was taking prescribed medication and all had a normal clinical history and examination, 12-lead electrocardiogram, echocardiogram, and haematological and biochemical screen, and forced expiratory volume in one second (FEV1) of >90% predicted.

Cor pulmonale patients

Ten patients (six men) of mean (SE) age 73·4 (1·7) years with clinically stable cor pulmonale secondary to chronic obstructive pulmonary disease were studied. All had obstructive pattern spirometry (FEV1/forced vital capacity (FVC) <70%), arterial hypoxaemia while breathing air (Pao2 <8·0 kPa), and had or gave a history of having peripheral oedema despite normal left ventricular function (on echocardiogram or radionuclide ventriculography), normal renal function (serum creatinine <120 mmol/l), and normal serum albumin (>35 g/l). Patients with other significant cardiovascular disease were excluded.

In this group, FEV1 in litres was 0·75 (0·06) (range 0·36-1·11), FEV1 as % of predicted 33·8 (5·2) (range 19-61), Pao2 on air 6·30
Levels of endothelin-1 (0.34 kPa (range 4.35–7.80), and PaCO₂ on air 6.17 (0.50) kPa (range 3.84–8.80).

PROTOCOL
Informed consent was obtained to the protocol previously approved by the Tayside committee for medical research ethics. Baseline venous blood samples were taken into chilled EDTA tubes after 30 minutes supine rest while breathing air. Control subjects only were then rendered hypoxaemic (steady state SaO₂ 75–80% measured by pulse oximetry) for 30 minutes by breathing a variable nitrogen/oxygen mixture before taking a further blood sample.

ENDOTHELIN-1 ASSAY
Samples were centrifuged at 2000 rpm at 4°C for 15 minutes and stored at –70°C. Assay was carried out using a commercially available radioimmunoassay kit (Nicholls Institute Diagnostics, San Juan Capistrano, CA, USA) after solid phase extraction on C18 silica columns. The lower limit of detection was 0.4 pmol/l and the intra-assay coefficient of variation was 4.5%. The anti-endothelin antibody used was 100% specific for endothelin-1 and had cross-reactivity of 96% with endothelin-3 and 7% with proendothelin.

DATA ANALYSIS
After testing for normality of distribution, between group comparisons were made by analysis of variance followed by Duncan’s multiple range testing with p<0.05 considered significant. Results are expressed as mean (SE) with 95% confidence intervals (CI) for significant differences.

Results
The plasma endothelin-1 concentration in normoxaemic controls was 0.90 (0.11) pmol/l. After 30 minutes hypoxaemia it increased significantly to 2.34 (0.34) pmol/l (95% CI for mean difference 0.41 to 2.48). In patients with cor pulmonale, the plasma endothelin-1 concentration was 2.96 (0.34) pmol/l, significantly greater than control subjects when normoxaemic (95% CI for mean difference 1.02 to 3.09) but not when hypoxaemic.

The plasma endothelin-1 concentration therefore increased 2.6-fold in response to hypoxaemia in controls and was raised 3.3-fold in patients with cor pulmonale compared with normoxaemic controls. Results from individual subjects with sample means are depicted in the figure.

Discussion
These findings indicate that hypoxaemia increases plasma levels of endothelin-1 in humans. In patients with cor pulmonale, endothelin-1 was increased to levels comparable to those in normal subjects rendered acutely hypoxaemic. It is interesting to note that baseline levels in controls were similar to those observed in other series, and that the increase following hypoxaemia in patients with cor pulmonale is comparable to the rise seen in normal subjects at high altitude.

These observations in humans are largely confirmatory of in vitro and animal studies which have shown hypoxia to be a potent stimulus for endothelin-1 synthesis and gene expression. In normal humans Therkelson et al found that 15 minutes of hypoxaemia caused only a small increase in plasma endothelin-1 which was not statistically significant, perhaps due to the shorter duration of the stimulus. Abnormally high levels of endothelin-1 have been described in a series of patients with pulmonary hypertension of varying aetiology, not all of whom were hypoxaemic, and thus other stimuli may also be implicated. In the present series, by excluding subjects with other significant cardiovascular diseases such as hypertension and congestive heart failure, we feel hypoxaemia is the most significant stimulus responsible for increased endothelin-1 levels in cor pulmonale. The in vitro evidence would suggest that the increased endothelin-1 levels observed were due to increased synthesis although, as endothelin-1 clearance mechanisms in humans have not been fully characterised, the possibility of decreased removal cannot be discounted.

The potent vasoactive properties of endothelin-1 may also be responsible for some of the circulatory abnormalities seen during hypoxaemia, although plasma levels may not accurately reflect local concentrations and hence vasoconstrictor activity. Whether endothelin-1 acts as a mediator of acute hypoxic pulmonary vasoconstriction is unknown. Support for this hypothesis might be drawn from studies using endothelin receptor blockers which can attenuate acute hypoxic pulmonary vasoconstriction in rats and prevent development.
of pulmonary hypertension following chronic hypoxia. These drugs now need to be tested in humans where it is likely that endothelin-1 plays a significant part in the cardiovascular response to hypoxaemia.

Manipulation of the endothelin system may therefore be a useful measure in patients with hypoxaemic lung disease, either to prevent or treat the cardiopulmonary consequences of chronic hypoxaemia.

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