9
Mutation of the von Hippel-Lindau Gene Alters Human Cardiopulmonary Physiology

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Abstract Intracellular responses to hypoxia are coordinated by the von Hippel-Lindau – hypoxia-inducible factor (VHL-HIF) transcriptional system. This study investigated the potential role of the VHL-HIF pathway in human systems-level physiology. Patients diagnosed with Chuvash polycythaemia, a rare disorder in which VHL signalling is specifically impaired, were studied during acute hypoxia and hypercapnia. Subjects breathed through a mouthpiece and ventilation was measured while pulmonary vascular tone was assessed echocardiographically. The patients were found to have elevated basal ventilation and pulmonary vascular tone, and ventilatory, pulmonary vasoconstrictive and heart rate responses to acute hypoxia were greatly increased, as were heart rate responses to hypercapnia. The patients also had abnormal pulmonary function on spirometry. This study's findings demonstrate that the VHL-HIF signalling pathway, which is so central to intracellular oxygen sensing, also regulates the organ systems upon which cellular oxygen delivery ultimately depends.

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1 Introduction

Intracellular responses to hypoxia are coordinated by the hypoxia-inducible factor (HIF) family of transcription factors (Semenza 2004). HIF is primarily regulated via its interaction with the von Hippel-Lindau tumour suppressor protein (VHL) (Maxwell *et al*. 1999). Oxygen-dependent hydroxylation of HIF increases its affinity for VHL (Jaakkola *et al*. 2001) which then binds to HIF, so targeting it for proteasomal degradation. Under hypoxic conditions this hydroxylation and degradation are slowed, resulting in accumulation of HIF and up-regulation of HIF-dependent genes.

Chuvash polycythaemia (CP) is a rare disorder caused by a point mutation in VHL that diminishes its binding affinity for hydroxylated HIF (Ang *et al*. 2002). HIF target genes including erythropoietin are pathologically up-regulated, resulting in congenital erythrocytosis. CP provides a unique opportunity to explore the molecular mechanisms underlying cardiopulmonary physiology. A recent publication reported this study's major findings and included data not shown here (Smith *et al*. 2006). The spirometry and hypercapnia protocol data have not previously been published.

2 Methods

These methods have been described in detail elsewhere (Smith *et al*. 2006). Three patients with CP were compared with a normal control group (six age- and sex-matched healthy volunteers) and a polycythaemia control group (three sex-matched patients with polycythaemia unrelated to VHL). The CP patients and polycythaemia control subjects had been chronically venesected to a normal haematocrit and were iron-deficient, although none had recently been venesected. The CP patients (age 22.3 ± 5 yr) did not differ significantly in age, height, weight or BMI from the normal control group (24.2 ± 5 yr). The study was approved by the Oxfordshire Clinical Research Ethics Committee, and each participant provided written informed consent.

Pulmonary function tests were performed using a handheld spirometer (Micro Medical Ltd, Kent, UK) and peak flow meter (Vitalograph Ltd, Buckingham, UK). Each subject then undertook six protocols. The first two protocols determined responses to very mild hypoxia. An initial five min of euoxia (end-tidal partial pressure of oxygen, $P_{ET_{O_2}}$, of 100 mmHg) preceded 10 min hypoxia at $P_{ET_{O_2}}$ 70 mmHg, followed by a final 5 min of euoxia. The second two protocols determined responses to moderate hypoxia using a stimulus $P_{ET_{O_2}}$ of 50 mmHg. The end-tidal partial pressure of carbon dioxide, $P_{ET_{CO_2}}$, was maintained close to each subject's baseline air-breathing value throughout the hypoxia protocols. The final two protocols determined responses to euoxic hypercapnia ($P_{ET_{CO_2}}$ 5 mmHg above each subject's baseline value).

Gas control was achieved using dynamic end-tidal forcing (Robbins *et al*. 1982). Subjects breathed through a mouthpiece and ventilation was measured using a

turbine volume-measuring device. During the first of each pair of protocols, pulmonary vascular tone was assessed continuously by echocardiography (Smith *et al*. 2006). In this technique, Doppler measurements are used to determine the maximum systolic pressure gradient across the tricuspid valve (ΔP_{max}), a standard index of pulmonary vascular tone. Cardiac output was determined echocardiographically during the second protocol in each pair.

Differences between groups were assessed using Student's unpaired t-test (Microsoft Excel) and univariate repeated measures ANOVA (SPSS statistical package), and were considered significant at the $p < 0.05$ level.

3 Results

3.1 Pulmonary Function Tests

Mean arterial P_{CO_2} was 6 mmHg lower in the CP patient group than in the normal control group (data not shown; $p < 0.05$). In the CP patients, forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and peak expiratory flow rate (PEFR) were all approximately 80% of predicted values (adjusted for age, sex, height and ethnicity), and were significantly lower than for the normal controls (Table 1; $p < 0.05$). However, the FEV_1/FVC ratio was preserved in the CP patients.

3.2 Hypoxia Protocols

These results have been reported previously (Smith *et al*. 2006). Both mild and moderate hypoxia provoked a greater increase in ventilation in CP patients than in normal control subjects ($p < 0.05$). Baseline pulmonary arterial pressure in the CP patients was almost double that of the control group ($p < 0.01$). The ΔP_{max} response was much more vigorous in the CP patient group with both mild and moderate hypoxia ($p < 0.01$). Upon transition to mild hypoxia, heart rate increased 3.2-fold more in the CP patient group ($p < 0.01$); the corresponding difference was not statistically significant with moderate hypoxia. Changes in cardiac output did not differ significantly between the two groups.

Table 1 Pulmonary function tests. Values are mean ± SD. Asterisks indicate significant differences between each control group and the CP patient group (*, $p < 0.05$; **, $p < 0.01$)

	CP Patients (n = 3)	Normal Controls (n = 6)	Polycythaemia Controls (n = 3)
FEV_1 (% predicted)	80.4 ± 6.5	98.4 ± 6.5 **	99.4 ± 6.3 *
FVC (% predicted	80.8 ± 4.8	99.3 ± 9.3 *	102.8 ± 3.4 **
FEV_1/FVC ratio	0.85 ± 0.01	0.85 ± 0.05	0.81 ± 0.04
PEFR (% predicted)	83.6 ± 9.5	102.6 ± 7.0 *	99.3 ± 11.6

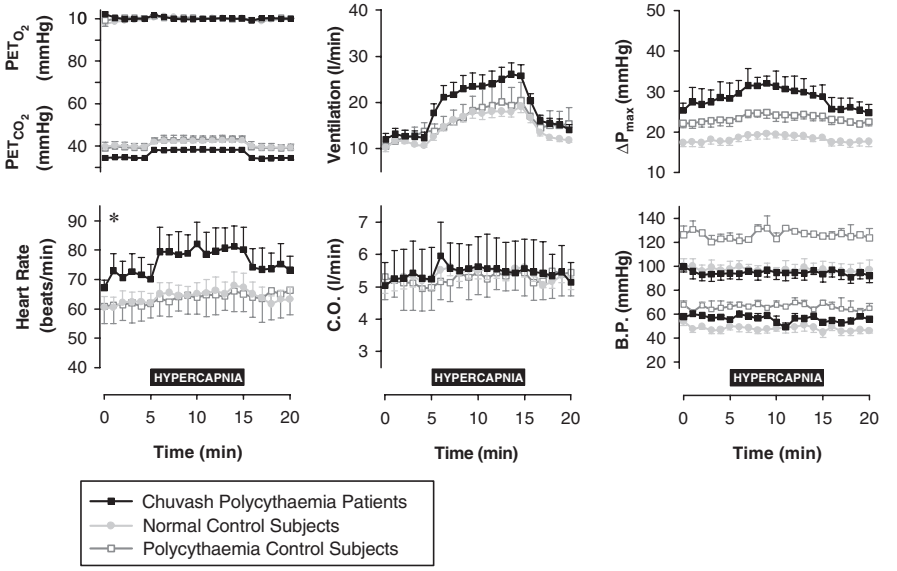

Fig. 1 Hypercapnia Protocol: End-tidal Gas Control and Physiological Responses. The baseline air-breathing $P_{ET_{CO2}}$ was lower in the CP patient group. ΔP_{max} is a standard echocardiographic index of pulmonary vascular tone. C.O., cardiac output. B.P., blood pressure. Systolic (upper plot) and diastolic blood pressure (lower plot) are shown. Values are mean ± SEM. Asterisk (*) indicates a significantly greater response in the CP patient group than in the normal control group ($p < 0.05$)

3.3 Hypercapnia Protocols

The changes in ventilation, ΔP_{max} and cardiac output stimulated by hypercapnia did not differ significantly between the groups (Fig. 1). However, hypercapnia provoked a 3.7-fold greater rise in heart rate in CP patients than in normal controls ($p < 0.05$).

3.4 Polycythaemia Control Subjects

The polycythaemia control subjects had normal pulmonary function tests (Table 1). The major physiological abnormalities present in the CP patients were not present in the polycythaemia control subjects (Fig. 1; repeated measures ANOVA).

4 Discussion

It is striking that CP patients had elevated basal ventilation (as evidenced by arterial hypocapnia whilst breathing air) and baseline pulmonary arterial hypertension, as well as displaying extremely high ventilatory and pulmonary vascular sensitivities

to acute hypoxia, as together these are characteristic features of acclimatisation to the hypoxia of high altitude. As discussed previously (Smith *et al*. 2006), these findings strongly implicate the VHL-HIF system in human acclimatisation to hypoxia and establish an important role for VHL in regulating cardiopulmonary control in hypoxia-naïve individuals.

CP is inherited recessively and thus VHL signalling is impaired from conception. It is therefore remarkable that CP patients do not resemble individuals born and raised at high altitude, in whom physiological responses to hypoxia are blunted (reviewed by Rupert and Hochachka 2001), but rather resemble individuals who are acutely acclimatised to hypoxia. Furthermore, whereas high altitude natives tend to develop large lungs (Rupert and Hochachka 2001), the pulmonary function test results reported in this study suggest the opposite is true of CP patients. This aspect of the CP phenotype may reflect a lung-specific role of HIF in foetal development, in which HIF is known to perform critically important functions (Semenza 2004).

Involvement of VHL-HIF in cardiopulmonary control is consistent with the nature of some of the genes HIF is known to regulate including tyrosine hydroxylase, endothelin-1, endothelial nitric-oxide synthase, $\alpha_{1\beta}$-adrenergic receptor, adrenomedullin, heme oxygenase-1 and atrial natriuretic peptide (Schofield and Ratcliffe 2004). It is interesting that CP patients had increased heart rate sensitivity to both hypoxia and hypercapnia. One theoretical explanation is that pathological up-regulation of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, contributes to a state of heightened sympathetic activation.

In conclusion, our findings demonstrate that a subtle disorder of HIF degradation profoundly alters human cardiopulmonary physiology. Thus, it appears that the VHL-HIF transcriptional signalling pathway, which is so central to intracellular oxygen sensing, also plays a major role in calibrating the organ systems upon which cellular oxygen delivery ultimately depends. This has important implications for nascent therapies directed towards manipulation of the VHL-HIF pathway as a treatment for cancer and ischaemic/hypoxic vascular disease.

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References

Ang, S.O., Chen, H., Hirota, K., Gordeuk, V.R., Jelinek, J., Guan, Y., Liu, E., Sergueeva, A.I., Miasnikova, G.Y., Mole, D., Maxwell, P.H., Stockton, D.W., Semenza, G.L. and Prchal, J.T. (2002) Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. Nat. Genet. 32, 614–621.

Jaakkola, P., Mole, D.R., Tian, Y.M., Wilson, M.I., Gielbert, J., Gaskell, S.J., Kriegsheim, A.V., Hebestreit, H.F., Mukherji, M., Schofield, C.J., Maxwell, P.H., Pugh, C.W. and Ratcliffe, P.J. (2001) Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 292, 468–472.

Maxwell, P.H., Wiesener, M.S., Chang, G.W., Clifford, S.C., Vaux, E.C., Cockman, M.E., Wykoff, C.C., Pugh, C.W., Maher, E.R. and Ratcliffe, P.J. (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399, 271–275.

Robbins, P.A., Swanson, G.D. and Howson, M.G. (1982) A prediction-correction scheme for forcing alveolar gases along certain time courses. J. Appl. Physiol. 52, 1353–1357.

Rupert, J.L. and Hochachka, P.W. (2001) The evidence for hereditary factors contributing to high altitude adaptation in Andean natives: a review. High Alt. Med. Biol. 2(2), 235–256.

Schofield, C.J. and Ratcliffe, P.J. (2004) Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol. 5, 343–354.

Semenza, G.L. (2004) Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology (Bethesda) 19, 176–182.

Smith, T.G., Brooks, J.T., Balanos, G.M., Lappin, T.R., Layton, D.M., Leedham, D.L., Liu, C., Maxwell, P.H., McMullin, M.F., McNamara, C.J., Percy, M.J., Pugh, C.W., Ratcliffe, P.J., Talbot, N.P., Treacy, M. and Robbins, P.A. (2006) Mutation of von Hippel–Lindau tumour suppressor and human cardiopulmonary physiology. PLoS Med. 3(7), e290.