A Role For Prolyl Hydroxylase Domain (phd) Proteins In Respiratory Control

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Rationale

Acute ventilatory responses to hypoxia are mediated primarily by the carotid bodies. There is increasing evidence for a role of the hypoxia-inducible factor (HIF) family of transcription factors in regulating cardiorespiratory responses to hypoxia. Prolyl hydroxylase domain (PHD) proteins act as oxygen sensors in the HIF pathway, regulating the stability of HIFα according to oxygen availability. Of the three PHD isoforms identified to date, PHD2 is of particular importance in cardiovascular control. We hypothesized that mice deficient in PHD2 would have enhanced ventilatory responses to hypoxia, and that this may be due to an effect on carotid body structure and/or function.

Methods

Homozygous PHD2 inactivation leads to in utero death, but PHD2 heterozygotes ($PHD2^{+/-}$) are viable and healthy. Seven littermate pairs of $PHD2^{+/-}$ and wild-type C57BL/6 mice (age 66±14 days, mean±SD) were exposed to 5 min hypoxia (10% oxygen), with and without added carbon dioxide (3%). Minute ventilation in awake, unrestrained mice was measured using whole body plethysmography. In three pairs of mice, the carotid bodies were subsequently excised and sections stained using antibodies against tyrosine hydroxylase (TH), to allow estimation of carotid body volume and chemosensory (type 1) cell density.

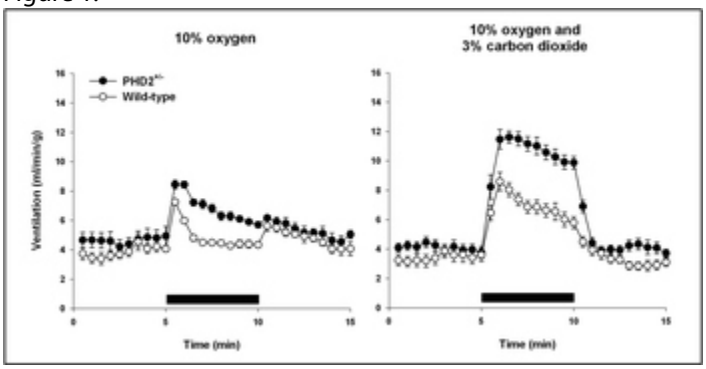
Results

Ventilatory responses to hypoxia were enhanced in $PHD2^{+/-}$ mice (Fig 1). In response to hypoxia alone, the acute hypoxic ventilatory response (AHVR), defined as the difference between minute ventilation in the two minutes before and after the onset of hypoxia, was 111% greater in $PHD2^{+/-}$ mice, compared with wild-type littermates ($p<0.05$, Student's T-test). During hypoxia with added carbon dioxide, AHVR was 66% greater in $PHD2^{+/-}$ mice, compared with wild-types ($p<0.01$). Mean carotid body volume was 76% greater in $PHD2^{+/-}$ mice than in wild-type mice ($p<0.01$, Fig 2), but the density of TH-positive cells within the carotid body was similar for both genotypes ($p>0.4$).

Conclusions

We have demonstrated an enhanced ventilatory response to hypoxia in $PHD2^{+/-}$ mice, in association with carotid body hyperplasia. Similar ventilatory phenotypes are seen in wild-type mice or humans after exposure to prolonged hypoxia, and are reported in patients with constitutive HIF upregulation. Our findings further implicate the PHD-HIF axis in respiratory control, and may have implications for patients with chronic hypoxemia and/or disordered chemical control of breathing.

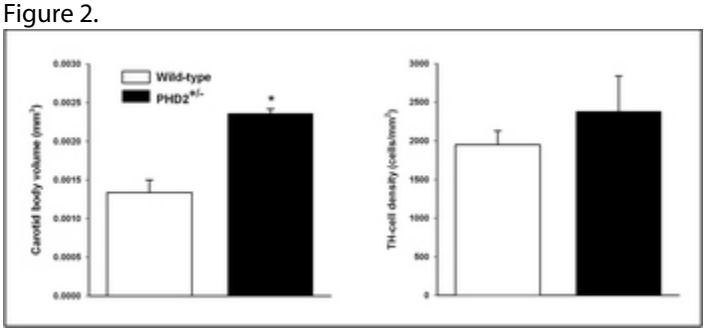
Figure 1.



Minute ventilation in PHD2+/- mice and wild-type littermates (n=7 pairs, mean±SEM). Animals breathed air for the first and final 5 min of the study. The black bar indicates 10% oxygen (left panel) or 10% oxygen, 3% carbon dioxide (right panel). For each mouse, exposures

were repeated on two different days and the mean calculated.

Figure 2.



Mean total volume and TH-cell density in carotid bodies from PHD2+/- mice and wild-type littermates (n=3 pairs, mean±SEM). Carotid bodies were substantially larger in the PHD2+/- animals, compared with wild-types (left panel,* $p<0.01$), but there was no significant difference between genotypes with respect to TH-positive cell density (right panel).

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