Let's Talk about Sex: A Novel Mechanism by Which Estrogen Receptor β Limits Hypoxia-Inducible Factor Expression in Pulmonary Endothelial Cells

Even before seminal studies on cattle demonstrated that hypoxic exposure at high altitude resulted in pulmonary hypertension and cor pulmonale, several observational studies and anecdotal reports showed that residence at high altitude caused significant cardiopulmonary changes (reviewed in Reference 1). In the decades since then, substantial effort has been brought to bear to identify the mechanisms involved in hypoxia-induced pulmonary hypertension (HPH). Studies using animals exposed to chronic hypoxia showed that males were more susceptible to hypoxia and pinpointed estrogens as a key reason for protection in females (2–4), although studies using mice overexpressing the serotonin transporter found that estrogen enhanced HPH (5). Other studies demonstrated a critical role for hypoxia-inducible factors (HIFs), transcription factors that regulate genes that contribute to adaption and maladaptation to hypoxia, in HPH (reviewed in Reference 1). Further evidence of a role for HIFs came from studies in populations residing at high altitude, which demonstrated that genetic mutations in HIF pathways were associated with cardiopulmonary protection (reviewed in Reference 6).

Although considerable evidence points to the role of sex hormones and HIFs in HPH, how these pathways might be linked is still a matter of some debate. Early studies indicated that the major estrogen sex hormone, estradiol (E2), reduced HIF signaling in hypoxic pulmonary arterial endothelial cells (PAECs) (4). More recently, the effect of E2 on bone morphogenetic protein signaling in PAECs was shown to be dependent on the level of oxygen and HIF activation, with E2 enhancing signaling under normoxic conditions but antagonizing signaling under hypoxic conditions (7).

It was against this background of oxygen-dependent E2 effects that Frump and colleagues (pp. 114–126) in this issue of the *Journal* pursued studies exploring the effects of hypoxia on estrogen receptors (8). In a comprehensive manner, the authors used gain- and loss-of-function approaches in both cultured cells and a murine model of HPH to define the relationship between the α and β subtypes of estrogen receptors (ERα and ERβ, respectively) and factors in the HIF pathway, including the α (oxygen-sensitive) subunits of HIF-1 and HIF-2 (HIF-1α and HIF-2α, respectively) and prolyl hydroxylase 2 (PHD2), a main factor in controlling degradation of HIFs. They found that hypoxic exposure increased ERβ expression specifically in lung tissue, with immunohistochemical staining revealing expression predominantly in the endothelium. The hypoxia-induced upregulation of ERβ was confirmed in cultured rat and human PAECs, and was mediated by HIF-1. Intriguingly, loss of ERβ, by silencing RNA in PAECs or genetic deletion in mice, caused a marked upregulation of HIF-2α during hypoxia. In cultured cells, both ERβ activation and hypoxia increased expression of PHD2, which functioned to suppress HIF-2α levels. Although the effect of hypoxia on ERβ protein expression was modest, the effect on PHD2 protein was striking, suggesting that in addition to increased ERβ protein levels, the observed translocation of ERβ to the nucleus was also an important factor in upregulating PHD2 expression.

These studies convincingly demonstrate that ERβ expression and nuclear translocation are increased by hypoxia in a HIF-1–dependent manner, and describe a novel negative-feedback mechanism by which ERβ subsequently upregulates PHD2 to limit HIF-2 activation in hypoxic PAECs. However, several important questions remain. For example, it is unclear why the effect of ERβ is apparently more specific for HIF-2α, as PHD2 should target both paralogs for degradation. These disparate results suggest that other, as yet unidentified factors are likely involved in the selective repression of HIF-2α. It is also unclear whether ERβ is a direct transcriptional target of HIF-1 or HIF-1–dependent intermediates are involved. The longer time course of the response may point to the latter as a more likely scenario.

The authors are to be commended for their efforts to correlate their *in vitro* results with an *in vivo* model of HPH. Results from mice deficient for ERβ corroborate the conclusion that this receptor was clearly responsible for at least part of the beneficial effects of E2 on hypoxia-induced remodeling and suppressed HIF-2 expression. Nonetheless, the *in vivo* studies also have several limitations. In particular, because normoxic controls were not included, it is not possible to determine the extent to which PHD2, HIF-1α, and HIF-2α expression was changed by hypoxia alone in this model. Similarly, ERα- and ERβ-deficient mice exposed to hypoxia in the absence of E2 were not evaluated. Surprisingly, in contrast to the *in vitro* results, E2 did not appear to have an effect on expression of either PHD2 or HIF-1 *in vivo*. There are several potential explanations for this discordance, including the timing of the measurements and the use of whole-lung homogenates to examine *in vivo* expression levels. The latter is of particular importance because the *in vitro* studies primarily used macrovascular ECs, which are known to exhibit a phenotype distinct from that of microvascular ECs (9), which constitute the bulk of the EC population in the lung and are where the majority of disease occurs. The authors also observed increased ERβ expression in hypoxic pulmonary arterial smooth muscle cells (PASMCs). Importantly, remodeling during HPH primarily results from PASMC proliferation, hypertrophy, and/or migration, and other reports have pointed to a role for HIFs in these responses (10, 11). Thus, from the present data it cannot be determined with certainty whether the target of E2/ERβ protection *in vivo* is

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PAECs, PASMCs, or a combination of the two. The fact that HIFs can be upregulated in PASMCs by PAEC-derived factors, such as endothelin-1 (12), provides a potential link between modulation of PAEC HIF levels by E2 and PASMC function and remodeling; however, further experiments will be needed to test this hypothesis.

Finally, a question remains as to how this information can be translated into therapy for patients. The data from the murine model would suggest that manipulating E2 might be beneficial, although the protocols used were preventative and it is unknown whether administering E2 after increased pressure and remodeling was already established would have reversed HPH. Additionally, hypoxia is typically not a feature of pulmonary arterial hypertension (PAH), the most severe form of pulmonary hypertension. It should be noted that elevated HIF levels have been reported in patients with PAH (13) and there is a clear sex bias, with PAH having a higher prevalence in females, although affected males appear to exhibit a more severe phenotype with worse survival (reviewed in Reference 14). This stands in contrast to HPH, which is more common in males. Recent studies reported that anastrozole treatment significantly reduced E2 levels in patients with PAH and improved 6-minute walk distance (15), suggesting that E2 exerts a detrimental effect in this population. These findings are consistent with increased E2 levels in the PAH rat model (SU5416/hypoxia), where reducing E2 corresponded to attenuated pulmonary hypertension (16). In contrast, other data from this model indicated that E2 promotes better cardiac function (17). In the current study by Frump and colleagues, hemodynamic parameters in the E2-treated hypoxic mice were not reported, and it is unclear what effect E2 or loss of ERβ might have had on right-ventricular function in these animals. Thus, additional investigation will be required to determine the extent to which E2 and ERβ modulate HIF expression and pulmonary vascular and right-ventricular responses in PAH.

As ever, sex and hormones remain a complicated topic. However, with respect to hypoxia and pulmonary hypertension, the study by Frump and colleagues sheds important new light on this complex issue by identifying a novel ERβ/HIF feedback pathway that affords protection against HPH. The next step will be to use this new information to begin developing therapies that can specifically target this pathway as a means to promote benefit in the pulmonary circulation while, if necessary, limiting any potential detrimental effects in the right ventricle. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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