EDITORIAL

Endothelins and pulmonary hypertension, what directions for the near future?

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Among the various vasoactive molecules or growth factors that have been tentatively implicated in pulmonary hypertension (PH), endothelin (ET) is particularly important because the potential therapeutic efficacy of ET-receptor antagonists is being investigated in patients with PH [1]. ET was discovered in 1988 and found to be the most potent vasoconstrictor ever known [2]. The discovery that ET was abundantly expressed in the lung directed attention toward its potential role in the initiation or progression of PH. Beneficial effects of chronic treatment with specific ET-receptor antagonists in experimental PH were first reported in 1994–1995 [3, 4]. ET-receptor antagonists now hold considerable promise for the treatment of human PH [1], illustrating how fast basic research has moved to clinical use in this specific area.

Experimental studies of the expression and effects of lung ET have contributed substantially to the current fund of knowledge about the pathophysiological role of ET in PH and are in good agreement with those obtained in human PH. The current understanding of the ET system in the pathogenesis of PH can be summarized as follows: 1) the ET system seems activated in all forms of human PH [5] and in all animal models of PH [6, 7]; 2) ET may contribute to the development of PH through its potent vasoconstricting properties, or its promitogenic properties [8–10]; 3) ET-receptor blockade protects against PH and, more convincingly, reverses established sustained experimental PH [3, 4, 11–14]. Each of these effects has been documented by a large number of studies. However, several questions of crucial importance when considering ET as a therapeutic target remain unanswered. 1) Why is the lung ET system activated during PH? 2) Why is lung ET well tolerated in the normal lung but not during PH, with possible explanations involving changes in ET-receptor function or distribution? 3) Which ET-receptor subtypes should be preferentially targeted during PH? and should selective or nonselective ET-receptor antagonists be used in patients with PH? Two of these important questions are addressed in the paper by TAKAHASHI *et al.* [15], which is best discussed separately.

Expression and distribution of endothelin receptors in the pulmonary vasculature during pulmonary hypertension

The interpretation of the role of ET in the pulmonary circulation is complicated by the fact that ET may work through two receptor subtypes, ETA and ETB [16, 17], with opposite effects. ETA is present on vascular smooth muscle cells and mediates vasoconstriction and proliferation [8, 9]. ETB is found predominantly on endothelial cells, where it promotes vasodilation [18] by releasing nitric oxide (NO), prostacyclin or other endothelium-dependent vasodilators [19, 20]. The ETB receptor has also been shown to indirectly modulate ET-1 synthesis through a negative feedback loop involving NO and to participate in the clearance of circulating ET-1 [21]. In normal mammals, administration of ET induces ETB receptor-dependent pulmonary vasodilation, even in doses that cause systemic vasoconstriction [22]. Thus, differences in ETB receptor distribution across vascular beds may explain why ET induces dilation of the pulmonary circulation and constriction of the systemic circulation. Accordingly, prolonged ETB receptor blockade has been shown to cause PH in the sheep foetus [23], and selective ETB receptor blockade to have adverse haemodynamic effects in animals with PH secondary to congestive heart failure [24]. Moreover, in patients with chronic heart failure, acute ETA receptor blockade causes selective pulmonary vasodilation [25] with a reduction in plasma ET-1. These observations suggest that ETB receptors may mediate dilation of the normal pulmonary vascular bed and may attenuate pulmonary vasoconstriction associated with various diseases. However, the ETB receptor is present both on the endothelium and on the smooth muscle, where it mediates opposite effects [26]. Thus, increasing local ET concentrations within the pulmonary vascular wall converts the vasodilating effect to a vasoconstricting effect [19, 27].

Evidence of major changes in vasoreactivity to ET-1 has additionally been obtained in experimental models of PH. Exposure of rats to chronic hypoxia abolishes the vasodilator and enhances the vasoconstrictor effects of ET-1 [19]. Alterations in endothelial function may explain these findings, since impaired ETB-mediated vasodilation, whether related [20] or unrelated to nitric oxide synthesis [19], has been reported during chronic hypoxia. Consistent with this

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finding, improvement of endothelial function reduces ET-induced vasoconstriction [28].

Changes in the location and distribution of ET-receptor subtypes may be pivotal in causing pulmonary vasoreactivity alterations and favouring ET-induced pulmonary vascular remodelling. In their study published in this issue of the *European Respiratory Journal*, TAKAHASHI et al. [15] found that in normal rats, the ETA receptor was located in the media of the pulmonary arteries and veins and predominated in proximal segments such as the elastic arteries and large muscular arteries. In a previous paper, the same group showed that ETB receptors in the media predominated in the distal segments of pulmonary arteries, whereas those in the intima were found mainly in proximal segments [29]. An important finding from these studies is that immunoreactivity for ETA receptors increased in the media of distal vessels after exposure to chronic hypoxia, suggesting that the shift in expression from proximal to distal arteries after exposure to hypoxia may play an important role in vascular remodelling. The combination of loss of ETB-induced vasodilation and increased ETA-mediated sensitivity to ET in distal segments may well contribute to the development of the structural changes associated with PH. Conversely, increased ETA receptor-expression in remodelled pulmonary arteries may be viewed as a process secondary to smooth muscle hyperplasia in distal vessels.

Which endothelin receptors should be blocked in pulmonary hypertension?

ET receptor antagonists hold promise as new therapeutic tools for PH. However, it remains unclear which receptors should be blocked. For the reasons mentioned above, (*i.e.* absence of ETB-mediated vasodilation during PH and redistribution of ETA receptors along the pulmonary vascular tree), targeting both ETA and ETB receptors might theoretically prove more effective than selectively antagonizing ETA receptors. Blocking all ET-1-induced effects, conversely, might compromise treatment efficacy by removing the potentially protective effect of the endothelial ETB receptors. Moreover, nonselective receptor antagonists may reduce ETB-mediated clearance of ET, increasing the circulating levels of ET-1, whereas selective ETA-receptor antagonists may produce the opposite changes [30]. This issue remains unresolved. Chronic treatment with the ETA receptor antagonist, BQ-123, or the ETA and ETB receptor antagonist, bosentan, attenuates the development of hypoxic PH [3, 4]. Furthermore, when BQ-123 or bosentan treatment is started after two weeks of hypoxia and continued for four weeks, there is significant reversal of pulmonary hypertension, right heart hypertrophy and pulmonary vascular remodelling, despite continuing exposure to hypoxia [11]. Beneficial effects of BQ-123 have also been shown in neonatal PH caused by ligation of the ductus arteriosus in foetal lambs [23], suggesting that endogenous ET-1 may contribute to the pulmonary vascular remodelling of neonatal PH and that failure of the pulmonary circulation to

adapt at birth may be mainly ascribable to ETA-receptor activation. A single study has compared the efficacy of a selective *versus* a nonselective antagonist of the ETA and ETB receptors. In this study, conducted in rats with monocrotaline-induced PH, the two treatment strategies produced similar benefits. However, the RV hypertrophy diminished only in the animals receiving the nonselective antagonist [12]. Unfortunately, the extent of vascular remodelling was not specifically examined in this study. Therefore, it is still not known whether mixed nonselective ETA/ETB receptor antagonists are preferable over selective ETA receptor antagonists in PH, or whether the severity or cause of PH influences the relative benefits of these two strategies. Further studies are needed, especially in human PH, to assess both the distribution and sublocalization of ETA and ETB receptors within the remodelled pulmonary vascular bed.

Why is endothelin synthesis increased in pulmonary arteries during pulmonary hypertension?

One important finding of the study by TAKAHASHI et al. [15] is that ET expression is increased in remodelled pulmonary vessels of rats during the development of PH induced by chronic exposure to hypoxia. As mentioned earlier, ET-1 synthesis seems to be increased in all forms of PH, in both experimental animals [31] and humans [5]. Therefore, the choice of the best therapeutic strategy targeting ET depends on the mechanism that accounts for the effect. One possibility is that ET synthesis may occur as a secondary process during PH development, contributing to worsen the PH. This possibility does not fit in well with the considerable efficacy of ET receptor blockade in reducing or reversing PH. Another possibility involves increased ET synthesis as one of the primary steps in the pathogenesis of PH. Data obtained in hypoxic PH lend support to this hypothesis. Indeed, the classical understanding of chronic hypoxic PH is based on the well-established concept that vascular remodelling is a response to sustained pulmonary vasoconstriction and increased pulmonary artery pressure, which increase shear stress, presumably triggering hypertrophy and proliferation of the vascular smooth muscle cells [32]. Although this concept is still valid in many aspects, it may not fully explain the pathophysiology of hypoxic PH. In a recent study, mice lacking the serotonin transporter gene had less hypoxia-induced pulmonary vascular remodelling despite enhanced hypoxic pulmonary vasoconstriction [33]. Therefore, although precapillary vasoconstriction may contribute to pulmonary arterial muscularization, it cannot be considered as the only trigger of this response [32]. This suggests that some of the vasoconstricting substances released in hypoxic lung tissue, endothelin and serotonin in particular, may serve as growth factors for vascular smooth muscle cells or may fulfil other functions independent from their effects on vascular tone and from the extent of pulmonary vasoconstriction.

The other mechanism that has been suggested during the last few years as possibly involved in

hypoxia-induced pulmonary vascular remodelling, is a direct effect of hypoxia on the expression of specific genes involved in smooth muscle cell proliferation. In a recent study, mice partially deficient in hypoxia-inducible factor-1 (HIF-1), an essential mediator of transcriptional responses to decreased oxygen availability, had less severe hypoxic PH with reduced muscularization of distal pulmonary vessels [34]. This is evidence that specific genes expressed under the control of HIF-1 during exposure to hypoxia are involved in pulmonary vascular smooth muscle cell proliferation. Among vasoactive molecules or growth factors that have been implicated in PH, inducible nitric oxide synthase, haemoxygenase-1, vascular endothelial growth factor (VEGF), VEGF receptors, serotonin transporter [35], and ET-1 are expressed by hypoxia-inducible genes that contain functionally important HIF-1 binding sites [36]. Therefore, ET may induce vascular remodelling after being activated in response to hypoxia through specific mechanisms. If this is the case, increased ET release in the hypoxic lung may be a key determinant of vascular remodelling acting through both vasoconstriction and growth promotion. Why ET is released in abundance in nonhypoxic PH, however, remains a mystery. The conditions that govern ET synthesis by endothelial or smooth muscle cells seem incompletely understood. Reduced NO formation or NO bioavailability may influence ET production. Furthermore, ET production may occur when smooth muscle cells shift from a nonsecreting to a secreting phenotype.

Alterations in endothelin synthesis in human pulmonary hypertension may result from direct or indirect genetic mechanisms involving alterations in intracellular signalling pathways. Again, further experimental studies should be done, but human studies are warranted.

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