Effect of Tryptophan Hydroxylase 1 Deficiency on the Development of Hypoxia-Induced Pulmonary Hypertension

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Abstract—Tryptophan hydroxylase 1 catalyzes the rate-limiting step in the synthesis of serotonin in the periphery. Recently, it has been shown that expression of the tryptophan hydroxylase 1 gene is increased in lungs and pulmonary endothelial cells from patients with idiopathic pulmonary arterial hypertension. Here we investigated the effect of genetic deletion of tryptophan hydroxylase 1 on hypoxia-induced pulmonary arterial hypertension in mice by measuring pulmonary hemodynamics and pulmonary vascular remodeling before and after 2 weeks of hypoxia. In wild-type mice, hypoxia increased right ventricular pressure and pulmonary vascular remodeling. These effects of hypoxia were attenuated in the tryptophan hydroxylase 1−/− mice. Hypoxia increased right ventricular hypertrophy in both wild-type and tryptophan hydroxylase 1−/− mice suggesting that in vivo peripheral serotonin has a differential effect on the pulmonary vasculature and right ventricular hypertrophy. Contractile responses to serotonin were increased in pulmonary arteries from tryptophan hydroxylase 1−/− mice. Hypoxia increased serotonin-mediated contraction in vessels from the wild-type mice, but this was not further increased by hypoxia in the tryptophan hydroxylase 1−/− mice. In conclusion, these results indicate that tryptophan hydroxylase 1 and peripheral serotonin play an essential role in the development of hypoxia-induced elevations in pulmonary pressures and hypoxia-induced pulmonary vascular remodeling. In addition, the results suggest that, in mice, serotonin has differential effects on the pulmonary vasculature and right ventricular hypertrophy. (Hypertension. 2007;49:1-5.)

Key Words: pulmonary circulation ■ serotonin ■ hypoxia ■ transgenic animals

Pulmonary arterial hypertension (PAH) is a progressive, often fatal disease characterized by an increase in pulmonary arterial pressure and pulmonary vascular remodeling. Recently, genetic factors have been associated with the development of PAH. Mutations in bone morphogenetic protein receptor II gene have been identified in >75% of patients with familial PAH.1,2 A genetic polymorphism in the serotonin (5-hydroxytryptamine [5-HT]) transporter (SERT) causing increased activity/expression of SERT has also been linked with PAH.3

Serotonin promotes pulmonary arterial smooth muscle cell proliferation, pulmonary arterial vasoconstriction, and local microthrombosis.4 Proliferation of pulmonary arterial smooth muscle cells is an important component of pulmonary arterial remodeling in PAH, which accounts for the increased thickness of the medial muscular coat in normally muscularized arteries and extension of muscle into smaller and more peripheral arteries. Elevated circulating levels of peripheral serotonin have been associated with the development of PAH clinically.5 It has also been shown that exogenously administered serotonin can potentiate the development of PAH in rats6 and can uncover a PAH phenotype in bone morphogenic protein receptor II mice.7 Moreover, mice overexpressing SERT (SERT+ mice) develop spontaneous PAH and are more susceptible to hypoxia-induced PAH, whereas mice deficient for the SERT are less susceptible.8–10 Blood serotonin levels are also elevated in mice after hypoxic exposure.11

Tryptophan hydroxylase (Tph) catalyzes the rate-limiting step in the synthesis of serotonin from tryptophan. By studying Tph1−/− mice, Walther and Bader12 demonstrated that there are 2 isoforms of Tph, now classified as Tph1 and Tph2. Tph2 is present exclusively in the brain but not the periphery. The classical Tph gene, now termed Tph1, is mainly expressed in the gut and mediates the generation of serotonin in the periphery.12 It has been shown recently that expression of the Tph1 gene is increased in lungs and pulmonary endothelial cells from patients with idiopathic PAH.13 To investigate the role of Tph1 in hypoxia-induced PAH, we have studied the development of PAH after 2 weeks of hypoxia in mice deficient in Tph1 (Tph1−/− mice). These mice lack serotonin synthesis in the periphery but have normal brain levels of serotonin.12,14

Methods

The investigation conforms with the United Kingdom Animal Procedures Act, 1986, and with the Guide for the Care and Use of...
Tph1\(^{-/-}\) Mouse

Transgenic mice that are deficient in Tph1 were generated by Bader and colleagues (MDC) as described previously\(^{12,14}\) and maintained on a C57BL/6 background. All of the experimental procedures were performed in accordance with the guidelines for the humane use of laboratory animals established at our institution.

Exposure to Hypoxia

Tph1\(^{-/-}\) and wild-type (WT) control mice were maintained in normoxic or hypobaric/hypoxic conditions for 2 weeks.\(^{15}\) The hypobaric chamber was depressurized over the course of 2 days to 550 mbar (equivalent to 10% O\(_2\)). Temperature was maintained at 21°C to 22°C, and the chamber was ventilated with air at \(\sim 45\) L/min.

Assessment of PAH

Measurement of Right Ventricular Hypertrophy

Right ventricular hypertrophy (RVH) was assessed by measuring the right ventricular free wall (RV) and left ventricle together with the septum (LV+S) separately. The ratio RV/LV+S was calculated. RV and LV+S weights per gram of body weight were also calculated.

Lung Histology

Three sagittal sections were obtained from left lungs. Sections were stained with ElasticaVanGieson stain and microscopically assessed for muscularization of pulmonary arteries (<80 \(\mu\)m external diameter) as described previously.\(^{15}\) Lung sections from 4 to 6 mice from each group were studied.

Statistical Analysis

Intergroup statistical comparisons were made by 1-way ANOVA. When significance was attained (\(P<0.05\)), differences were established using the Tukey’s multiple comparison test. Other analyses were carried out using a Student \(t\) test. Data are expressed as mean±SEM.

Results

Hypoxia-Induced PAH

Hypoxia induced increases in RVPs by \(\approx 50\%\) and also induced RVH and pulmonary vascular remodeling in WT mice (Figure 1). Mean SAP was slightly elevated (\(P<0.05\)), whereas mRVP was slightly decreased (\(P<0.05\)) in normoxic Tph1\(^{-/-}\) mice compared with WT animals (Figure 1). Tph1 knockout itself had no effect on the percentage of remodeled vessels in control mice. Hypoxia-induced increases in pulmonary vascular remodeling and RVPs were ablated in Tph1\(^{-/-}\) mice (Figure 1), providing direct evidence for the first time that peripheral Tph1 plays a crucial role in hypoxia-induced PAH. RV/LV+S and RV/body weight was elevated in Tph1\(^{-/-}\) mice (\(P<0.001\)), and RVH was still evident in the Tph1\(^{-/-}\) mice under both normoxic and hypoxic conditions (Figure 1). HR was unaffected by either Tph1 knockout or hypoxia (data not shown).

Contractile Responses to Serotonin in Pulmonary Resistance Arteries From Tph1\(^{-/-}\) Mice

5-HT induced a marked contractile response in the mice arteries from all of the groups (Figure 2). Contractile responses to serotonin were increased in vessels from Tph1\(^{-/-}\) mice (Figure 2). The maximum response was increased by \(\approx 30\%\), and the potency also increased (pEC\(_{50}\) WT: 6.4±0.2; \(n=6\) and Tph1\(^{-/-}\): 7.0±0.14; \(n=8\); \(P<0.01\)). After hypoxia, responses to serotonin were increased in the WT mice, with
the maximum response increasing by ~45% (pEC50 hypoxic WT: 7.4±0.1; n=6; P<0.01 versus normoxic). The contractile responses to serotonin were not affected further by hypoxia in the Tph1+/− vessels (pEC50 hypoxic Tph1+/−): 7.4±0.2; n=7; Figure 2). These effects on responses to serotonin were not because of changes in contractility per se, because the contractile response to 50 mmol/L KCl was not significantly different between groups: 2.16±0.20 mN (WT normoxic), 2.30±1.10 mN (Tph1+/− normoxic), 2.26±0.29 mN (WT hypoxic), and 2.15±0.23 mN (Tph1+/− hypoxic).

Discussion

Hypoxia-induced elevations in RVP and pulmonary vascular remodeling were markedly inhibited in the Tph1+/− mice. This provides direct evidence, for the first time, that Tph1 and peripheral serotonin, not brain serotonin, are critical to the development of hypoxia-induced PAH. This is unlikely to be because of inhibition of the hypoxic pulmonary vasoconstriction, because there is little evidence to support a role for serotonin in this.16 It is likely to be because of the mitogenic effects of serotonin promoting pulmonary vascular remodeling, as our results show that hypoxia-induced pulmonary vascular remodeling was inhibited in hypoxic Tph1-deficient mice, whereas pulmonary vascular reactivity to either serotonin or KCl was not compromised in hypoxic Tph1+/− mice compared with their WT controls. mRVP was slightly decreased in the normoxic Tph1+/− mice. This suggests that, in mice, peripheral serotonin normally has a pressor influence in the pulmonary circulation. Certainly, we and others have demonstrated that exogenously administered serotonin exerts both pressor and mitogenic effects on the pulmonary circulation.3,4,17,18 Tph1 is expressed in lung pulmonary neuroendocrine cells, and both hypoxia and mechanical stretch have been shown to induce increased Tph1 expression and serotonin release in rabbit lung.19 In light of these observations, our results suggest that, in mice, chronic hypoxia itself may induce Tph1 synthesis and subsequent serotonin release, which acts as a mitogen in pulmonary arteries, contributing to the pulmonary vascular remodeling and subsequent onset of pulmonary hypertension. The elevation of pulmonary vascular tone that accompanies PAH is likely to induce further stretch-induced Tph1 expression and serotonin release. Others have demonstrated inhibition of hypoxia- and monocrotaline-induced PAH using approaches that nonspecifically inhibit both Tph1 and Tph2 such as p-chlorophenylalanine.20,21 Here, we have identified Tph1 as the enzyme that plays the pivotal role in hypoxia-induced PAH, and this may, therefore, be a novel therapeutic target for PAH.

Curiously, mSAP was slightly elevated in the Tph1+/− mice. The systemic cardiovascular system is under both central and peripheral serotonergic control,22 but as the Tph1+/− mice have normal brain serotonin, the effect on blood pressure must be via a lack of peripheral serotonin. Tph1+/− mice have been shown previously to have functional cardiac alterations that can progress to heart failure.23 Serotonin can also directly mediate systemic arterial vasconstriction and relaxation via receptors located on the vascular endothelium and smooth muscle. For example, various 5-HT1 receptors and the 5-HT2B receptor mediate vasodilation via endothelial release of NO, whereas the 5-HT2A receptor mediates vasoconstriction.4 Because the Tph1+/− mice exhibit higher than normal mSAP, this may, therefore, be because of serotonin normally exerting an antihypertensive influence via such vasodilator effects and/or via depressed cardiac output. Others have shown that the contractile response to serotonin in the isolated aorta is not affected in Tph1+/− mice,24 and so a direct effect on cardiac function may be suggested. We did observe an increase in LV+S weights in the Tph1+/− mice under both hypoxic and normoxic conditions. The degree of left ventricular hypertrophy in the normoxic Tph1+/− mice was very small, however, as was the increase in mSAP, and there was no other overt evidence of heart failure. Clinically, patients with left ventricular dysfunction often present with PAH, and we have demonstrated previously that a rabbit model of mild left ventricular function has associated PAH.25 The mild left ventricular hypertrophy observed in the Tph1+/− mice is, therefore, unlikely to account for the ablation of hypoxia-induced PAH that we observed in this study.

Exaggerated serotonin-induced vasoconstriction may contribute to hypoxia-induced PAH. We have shown previously that contractile responses to serotonin are elevated in pulmonary arteries from hypoxic rats. This is in part because of an increase in 5HT1B receptor activation.26 Here we show that constriction to serotonin is markedly elevated in hypoxic WT mice, an effect that we have also reported previously.15 We have suggested previously that such an increased response to serotonin in mice may be because of increased 5HT1B and 5HT2A receptor activity.15 In normoxic Tph1+/− mice, there was increased vasconstriction in response to serotonin. This cannot be because of an increase in serotonin-induced stimulation via serotonin synthesis. Hence, this is likely to be because of compensatory increases in 5HT1B and/or 5HT2A receptor signaling in the face of decreased local serotonin. Exposure to chronic hypoxia did not increase the contractile response to serotonin further in Tph1+/− mice, presumably because these already exhibited markedly elevated contractile responses to serotonin. These results suggest that the ablation
of PAH in the Tph1−/− mice was not because of inhibition of vascular reactivity but because of the inhibition of pulmonary remodeling. In light of recent observations that expression of the Tph1 gene is increased in lungs and pulmonary endothelial cells from patients with idiopathic PAH,13 these results suggest that peripheral serotonin and Tph1 may play an important role in the development of PAH, both experimentally and clinically, and may present a novel therapeutic target.

Despite having vastly reduced increases in RVP and pulmonary vascular remodeling in response to hypoxia, Tph1−/− mice still develop RVH. This suggests that RVH in mice occurs as the result of a direct effect of hypoxia on the right ventricle, which is independent of an increase in RVP and does not require peripheral serotonin. However, as discussed above, there are cardiac functional abnormalities in Tph1−/− mice, which can lead to heart failure,23 despite there being no cardiac structural defects. Hence, peripheral serotonin may normally have a regulatory role in cardiac function. One suggestion is that, in the Tph1−/− mice, a drop in circulating serotonin may lead to a decrease or lack of reflex stimuli by sensory nerves resulting in loss of contractility.23 Here we also show that Tph1−/− mice exhibit slight RVH even before hypoxic exposure, suggesting that serotonin may normally protect against RVH. Indeed, serotonin protects murine ventricular cardiomyocytes against serum deprivation–induced apoptosis, suggesting a role for serotonin as a survival factor of cardiomyocytes.27 If serotonin does play a dual role, inducing elevated pulmonary pressures and pulmonary vascular remodeling while protecting right ventricular myocyte function, this may well explain the dissociation of RVH from RVP that we see in mice where the serotonin system is altered. For example, we have reported previously that SERT+ mice show elevated RVP in the absence of RVH.10

In conclusion, our results indicate that Tph 1 (peripheral) serotonin plays an essential role in the development of hypoxia-induced elevations in pulmonary pressures and hypoxia-induced pulmonary vascular remodeling. The results also suggest that, in hypoxia-induced PAH, serotonin has differential effects on the pulmonary vasculature and RVH.

**Disclosures**

None.

**References**


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