Tryptophan hydroxylase 1 knockout and tryptophan hydroxylase 2 polymorphism: effects on hypoxic pulmonary hypertension in mice

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Am J Physiol Lung Cell Mol Physiol 293: L1045–L1052, 2007. First published August 3, 2007; doi:10.1152/ajplung.00082.2007. —Serotonin [5-hydroxytryptamine (5-HT)] biosynthesis depends on two rate-limiting tryptophan hydroxylases (Tph): Tph1, which is expressed in peripheral organs, and Tph2, which is expressed in neurons. Because 5-HT is involved in pulmonary hypertension (PH), we investigated whether genetic variations in Tph1 and/or Tph2 affected PH development in mice. To examine the functional impact of peripheral Tph1 deficiency on hypoxic PH, we used Tph1−/− mice characterized by very low 5-HT synthesis rates and contents in the gut and lung and increased 5-HT synthesis in the forebrain. With chronic hypoxia, 5-HT synthesis in the forebrain increased further. Hypoxic PH, right ventricular hypertrophy, and distal pulmonary artery muscularization were less severe (P < 0.001) than in wild-type controls. The Tph inhibitor p-chlorophenylalanine (100 mg·kg−1·day−1) further improved these parameters. We then investigated whether mouse strains harboring the C1473G polymorphism of the Tph2 gene showed different PH phenotypes during hypoxia. Forebrain Tph activity was greater and hypoxic PH was more severe in C57Bl/6 and 129X1/SvJ mice homozygous for the 1473C allele than in DBA/2 and BALB/cJ mice homozygous for the 1473G allele. Hypoxia increased 5-hydroxytryptophan accumulation but decreased 5-HT contents in the forebrain and lung, suggesting accelerated 5-HT turnover during hypoxia. These results provide evidence that dysregulation of 5-HT synthesis is closely linked to the hypoxic PH phenotype in mice and that Tph1 and Tph2 may contribute to PH development.

PULMONARY HYPERTENSION (PH) develops as a complication of various disease states or as a primary condition that has no detectable cause (13). Mutations in the bone morphogenetic protein receptor type 2 have been linked to familial cases of idiopathic PH (5, 21), but the molecular basis for common sporadic nonfamilial forms or associated forms is unknown (11). A major component of the pulmonary vascular remodeling process that leads to PH is proliferation of pulmonary artery smooth muscle cells (PASMCs), which might result from an inherent characteristic of PASMCs or a response to abnormal stimuli, such as upregulated growth factors.

Among growth factors implicated in PH progression, serotonin [5-hydroxytryptamine (5-HT)] is thought to play a prominent role (10, 12, 22, 25). PASMCs from patients with idiopathic PH showed excessive proliferation in response to 5-HT due to overexpression of the 5-HT transporter (5-HTT), which mediates the mitogenic effect of the indoleamine (10, 25). Transgenic animals overexpressing 5-HTT in smooth muscle spontaneously develop pulmonary vascular remodeling and PH (16). 5-HT receptors, including 5-HT1B, 5-HT2B, and 5-HT2A receptors, have also been reported to contribute to serotonergic effects on pulmonary vessels (22, 24). Whether, in addition to these alterations, changes in 5-HT synthesis further contribute to the pathogenesis of PH is not known. Increased peripheral bioavailability of 5-HT can lead to PH, for instance, in patients with 5-HT platelet storage abnormalities (18), as well as in individuals or experimental animals treated with the 5-HT-releasing drug dexfenfluramine (1, 22) and in Fawn-hooded rats, which have a platelet storage disease (27). Moreover, pharmacological increases in circulating 5-HT levels can induce PH and, more importantly, trigger the development of PH when bone morphogenetic protein receptor type 2 is dysfunctional (23). However, the functional impact of variations in 5-HT synthesis on PH development has not been specifically examined.

The critical step in 5-HT biosynthesis is catalyzed by the rate-limiting enzyme tryptophan hydroxylase (Tph). Tph activity, therefore, serves as a marker for 5-HT synthesis. Tph, which was initially thought to derive from a single gene, exists as two isoforms, Tph1 and Tph2, which are encoded by separate genes. Tph1 is expressed mainly in the gut and pineal gland (4). The recently identified Tph2 gene appears to be exclusively expressed in neurons and is known as the neuronal isoform (30). Thus 5-HT synthesis is thought to be controlled chiefly by Tph2 in the central nervous system and by Tph1 in peripheral organs (3, 4, 26, 30). Although peripherally produced 5-HT is synthesized chiefly by the enterochromaffin cells in the gut (15), small amounts of 5-HT may also be formed in other organs. In recent studies, we found that human pulmonary vascular endothelial cells produced 5-HT and expressed Tph1 and that 5-HT synthesis and Tph1 expression were increased in cells from patients with idiopathic PH compared with cells from controls (8).
The objective of the present study was to investigate whether alterations in Tph1 and Tph2 activities contribute to the extent of pulmonary vascular remodeling and to the development of \( \text{PH} \) in vivo. We first examined the functional impact of peripheral Tph1 deficiency on hypoxic \( \text{PH} \) and the effect of treatment with the powerful Tph inhibitor \( \text{p-chlorophenylalanine (PCPA)} \) \((20)\) in Tph1\(^{-/-}\) mice. We reasoned that an effect of PCPA on \( \text{PH} \) development in Tph1\(^{-/-}\) mice would support a role for Tph2 activity. Moreover, brain 5-HT synthesis has been shown to be influenced by a single-nucleotide polymorphism (C1473G) in the Tph2 gene in mice \((31)\). C57Bl/6 and 129X1/SvJ mice are homozygous for the 1473C allele, whereas DBA/2 and BALB/cJ mice are homozygous for the 1473G allele \((31)\). We therefore examined Tph1 and Tph2 expression and activity in various organs of these mouse strains exposed to normoxia and hypoxia, and we looked for differences in hypoxic \( \text{PH} \) severity and in effects of PCPA across mouse strains.

**MATERIALS AND METHODS**

**Mice.** C57Bl/6, 129X1/SvJ, DBA/2, and BALB/cJ mice were purchased from Charles River \((l’Arbresle, France)\). Mice lacking Tph1 \((\text{Tph1}^{-/-/})\) were generated by homologous recombination and maintained on a pure 129X1/SvJ genetic background \((3)\). The effects of chronic hypoxia were studied by exposure of mice to 10\% \( \text{O}_2 \) for 20 consecutive days, with or without concomitant administration of the Tph inhibitor PCPA at 100 mg kg\(^{-1}\) day\(^{-1}\) \text{ip}, a dose that has been shown to be effective in previous studies \((2, 20)\); control mice were kept in room air. The procedures were approved by the Institutional Animal Care and Use Committee of Hôpital Henri Mondor.

**Assessment of \( \text{PH} \).** Mice were anesthetized and ventilated with room air. Right ventricular (RV) systolic pressure (RVSP) was recorded. 5-Hydroxytryptophan (5-HTP) accumulation rates and serotonin [5-hydroxytryptamine (5-HT)] levels in forebrain, gut, lung, and blood of normoxic wild-type \([\text{Tph1}^{+/+}]\) and tryptophan hydroxylase 1-knockout \([\text{Tph1}^{-/-/-}]\) mutant mice. Top: 5-HTP accumulation 30 min after administration of the aromatic \( \text{l}-\)amino acid decarboxylase inhibitor NSD-1015 \((100 \text{mg/kg ip)}\). Bottom: 5-HT levels. Tph1\(^{-/-/-}\) mice exhibited marked decreases in 5-HTP accumulation rates and 5-HT tissue levels compared with Tph1\(^{+/+}\) mice, except in forebrain, where 5-HTP accumulation was increased. Values are means \( \pm \text{SE} \) \((n = 5)\); \(* P < 0.05; ** P < 0.001 \) vs. corresponding Tph1\(^{+/+}\).

**Fig. 1.** 5-HT accumulation rates and serotonin levels in various tissues of normoxic wild-type \([\text{Tph1}^{+/+}]\) and tryptophan hydroxylase 1-knockout \([\text{Tph1}^{-/-/-}]\) mutants. Top: 5-HT accumulation 30 min after administration of the aromatic \( \text{l}-\)amino acid decarboxylase inhibitor NSD-1015 \((100 \text{mg/kg ip)}\). Bottom: 5-HT levels. Tph1\(^{-/-/-}\) mice exhibited marked decreases in 5-HTP accumulation rates and 5-HT tissue levels compared with Tph1\(^{+/+}\) mice, except in forebrain, where 5-HTP accumulation was increased. Values are means \( \pm \text{SE} \) \((n = 5)\); \(* P < 0.05; ** P < 0.001 \) vs. corresponding Tph1\(^{+/+}\).

**Fig. 2.** 5-HTP accumulation rates and 5-HT levels in forebrain, gut, lung, and blood of normoxic mice harboring the C1473G polymorphism of the Tph2 gene. Top: 5-HTP accumulation 30 min after administration of NSD-1015 \((100 \text{mg/kg ip)}\). Bottom: 5-HT levels. 5-HTP accumulation in forebrain was higher in 129X1/SvJ and C57Bl/6 mice homozygous for the 1473C allele of the Tph2 gene than in DBA/2 and BALB/cJ mice homozygous for the 1473C allele \((31)\). We therefore examined Tph1 and Tph2 expression and activity in various organs of these mouse strains exposed to normoxia and hypoxia, and we looked for differences in hypoxic \( \text{PH} \) severity and in effects of PCPA across mouse strains.

**Fig. 3.** 5-HT accumulation rates and 5-HT levels in various tissues of normoxic mice harboring the C1473G polymorphism of the Tph2 gene. Top: 5-HTP accumulation 30 min after administration of NSD-1015 \((100 \text{mg/kg ip)}\). Bottom: 5-HT levels. 5-HTP accumulation in forebrain was higher in 129X1/SvJ and C57Bl/6 mice homozygous for the 1473C allele of the Tph2 gene than in DBA/2 and BALB/cJ mice homozygous for the 1473C allele \((31)\). We therefore examined Tph1 and Tph2 expression and activity in various organs of these mouse strains exposed to normoxia and hypoxia, and we looked for differences in hypoxic \( \text{PH} \) severity and in effects of PCPA across mouse strains.

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corded immediately after an injection of pentobarbital sodium (40 mg/kg ip), the thorax was opened, the lungs and heart were removed, and the RV and left ventricle (LV) + septum (LV + S) were weighed for determination of the RV hypertrophy index (RVHI). The percentage of muscularized pulmonary vessels was determined in lung sections stained with hematoxylin-phloxin-saffron and orcein-picro-indigo-carmine (8).

Assessment of 5-HT synthesis and 5-HT. For monoamine analysis, animals were decapitated, and blood from trunk vessels was collected in chilled EDTA tubes (K3-EDTA, CML, Nemours, France). Mouse organs were dissected, and monoamines were extracted and analyzed for levels of 5-HT and 5-hydroxytryptophan (5-HTP) using high-performance liquid chromatography coupled with electrochemical detection, as previously described (17). For assessment of the 5-HT synthesis rate in vivo, mice were treated with the aromatic L-amino acid decarboxylase inhibitor NSD-1015 (3-hydroxybenzylhydrazine dihydrochloride, 100 mg/kg ip) and decapitated 30 min later (17, 28). Organs were then dissected for determination of 5-HTP and 5-HT levels. Absolute values were calculated from 5-HTP and 5-HT peak areas, with reference to authentic standards.

Measurement of Tph1 and Tph2 mRNAs. Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Cergy-Pontoise, France) and estimated from optical density measurements (ratio of absorbance at 260 nm to absorbance at 280 nm). Then cDNA was synthesized using random hexamer primers and the SuperScript II RT system (Invitrogen). Gene expression was analyzed using quantitative RT-PCR for mouse Tph1 and Tph2 genes with the TaqMan PCR technique. Predeveloped assay reagents, including primers and probes for the target genes, and endogenous control were supplied by Applied Biosystems (Courtaboeuf, France). For each sample, 25 ng were used, amplification was performed in duplicate, and the threshold cycle (Ct) was determined. Signal detection and result analysis were performed using Prism 7000 sequence detection software (Applied Biosystems). Relative quantification was achieved using the comparative Ct method (2ΔΔCt) by normalization for 18S ribosomal RNA.

Statistical analyses. Values are mean ± SE. The differences in 5-HTP and 5-HT levels between wild-type and Tph1+/− mice were analyzed using the nonparametric Mann-Whitney test. One-way ANOVA was used to assess 5-HTP and 5-HT levels in mice with different Tph2 genotypes. For data from Tph1+/+ and Tph1−/− mice, as well as wild-type mice with different Tph2 genotypes, we performed two-way ANOVA to assess the effects of normoxia, hypoxia, or hypoxia + PCPA on RVSP, RVHI, and the percentage of muscularized pulmonary arteries. When ANOVA showed an interaction between exposure conditions and genotype, mouse groups were further compared using an unpaired nonparametric test.

RESULTS

5-HT synthesis rates and tissue contents in Tph1−/− vs. Tph1+/+ normoxic mice. As expected, Tph1 knockout resulted in a dramatic reduction in 5-HT synthesis rates and/or tissue contents in the gut and lung compared with wild-type animals generated on a 129X1/SvJ background (Fig. 1). 5-HTP accumulation after administration of NSD-1015, an inhibitor of aromatic L-amino acid decarboxylase, was greater in the forebrain than in the gut or lung in wild-type mice. In Tph1−/− mice, 5-HTP accumulation was markedly decreased in the gut, lung, and blood compared with wild-type mice, but not in the forebrain, where a modest elevation was observed, in contrast to a slight decrease in 5-HT contents compared with wild-type controls (Fig. 1). Gut, lung, and whole blood 5-HT levels were dramatically reduced in Tph1−/− mice compared with wild-type controls (Fig. 1).

5-HT synthesis rates and tissue contents in normoxic mice harboring the C1473G polymorphism of the Tph2 gene. 5-HTP accumulation in the forebrain was higher in 129X1/SvJ and C57Bl/6 mice homozygous for the 1473C allele of the Tph2 gene than in DBA/2 and BALB/cJ mice homozygous for the 1473G allele (Fig. 2). In the gut and lung, 5-HTP levels were higher in 129X1/SvJ mice than in the other three strains, which exhibited similar 5-HTP accumulation in these peripheral tissues. 5-HT contents in the forebrain and gut were higher in 129X1/SvJ mice than in the other strains, whereas 5-HT levels in the lung and blood showed no significant differences across strains.

Development of PH and vascular remodeling in Tph1−/− vs. Tph1+/+ mice and effects of PCPA treatment. Under normoxia, body weight, RV weight-to-body weight ratio, and heart rate were similar in Tph1−/− and wild-type mice (Fig. 3, Table 1). After 20 days of exposure to hypoxia, RVSP and the RV/LV + S index were significantly lower in Tph1−/− mice than in...
RVSP, RV/LV difference in PH severity across mouse strains. However, the DBA/2 and BALB/cJ mice; as a result, PCPA abolished the reduction was larger in the 129X1/SvJ and C57Bl/6 than in the DBA/2 and BALB/cJ mice. PCPA reduced PH severity in all four mouse strains, but no significant differences were noted between values of all three parameters indicated greater severity of PH in the gut. No significant differences were noted between norms in Tph1 mice, increased 5-HTP accumulation was noted only in the forebrain. This can be exclusively attributed to the response of Tph2 activity to hypoxia. Tph1 and Tph2 mRNA in wild-type 129X1/SvJ and BALB/cJ mice under normoxia and hypoxia. As expected, no Tph1 mRNA was detected in organs from Tph1 mice. In 129X1/SvJ wild-type mice, Tph1 mRNA was measured in the forebrain, brain stem, lung, and gut; levels were the highest in the gut. No Tph1 mRNA was detected in the liver. Hypoxia did not alter Tph1 mRNA levels in any of these organs: 8.5 ± 1.7 and 6.9 ± 1.1 (relative units) in the forebrain (P not significant [NS]), 1.9 ± 0.4 and 2.5 ± 0.3 in the brain stem (P = NS), 3.0 ± 1.1 and 2.8 ± 0.3 in the lung (P = NS), and 28.2 ± 2.7 and 26.2 ± 4.9 in the gut (P = NS) in hypoxia and normoxia, respectively. Similar values were measured in BALB/cJ mice, in which hypoxia was also found to not affect Tph1 mRNA. Relative Tph2 mRNA in the brain stem was 4.3 ± 0.5 and 5.3 ± 1.3 during normoxia and hypoxia.

Table 1. Body weight, heart weight, and heart rate in wild-type and Tph1−/− mice after 20 days of normoxia or hypoxia with or without PCPA

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia</th>
<th>Hypoxia + PCPA</th>
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<tr>
<td></td>
<td>Wild-type (n = 4)</td>
<td>Tph1−/− (n = 4)</td>
<td>Wild-type (n = 5)</td>
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<tr>
<td>Final body wt, g</td>
<td>26.4 ± 3.3</td>
<td>27.3 ± 3.0</td>
<td>28.0 ± 2.1</td>
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<tr>
<td>RV wt/body wt, mg/g</td>
<td>1.10 ± 0.07</td>
<td>0.86 ± 0.09</td>
<td>1.65 ± 0.06</td>
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<tr>
<td>LV wt/body wt, mg/g</td>
<td>4.0 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>395 ± 40</td>
<td>394 ± 19</td>
<td>340 ± 16</td>
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</table>

Values are means ± SE; n, number of mice. PCPA, p-chlorophenylalanine; RV, right ventricle; LV, left ventricle. *P < 0.05 vs. corresponding wild-type values. †P < 0.01 vs. corresponding normoxia values.

Table 2. Body weight, heart weight, and heart rate in 129X1/SvJ, C57Bl/6, DBA/2, and BALB/cJ mice after 20 days of normoxia or hypoxia with or without PCPA

<table>
<thead>
<tr>
<th></th>
<th>129X1/SvJ</th>
<th>C57Bl/6</th>
<th>DBA/2</th>
<th>BALB/cJ</th>
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<tr>
<td></td>
<td>Final body wt, g</td>
<td>22.4 ± 0.5</td>
<td>22.4 ± 0.3</td>
<td>24.4 ± 0.5</td>
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<td></td>
<td>RV wt/body wt, mg/g</td>
<td>1.10 ± 0.01</td>
<td>0.96 ± 0.02</td>
<td>0.82 ± 0.03</td>
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<td></td>
<td>LV wt/body wt, mg/g</td>
<td>4.3 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.1</td>
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<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>404 ± 6</td>
<td>392 ± 8</td>
<td>360 ± 9</td>
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<td></td>
<td>Final body wt, g</td>
<td>22.3 ± 0.6</td>
<td>20.8 ± 0.3</td>
<td>21.8 ± 0.7</td>
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<td>RV wt/body wt, mg/g</td>
<td>2.15 ± 0.10</td>
<td>2.07 ± 0.05</td>
<td>1.82 ± 0.03</td>
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<tr>
<td></td>
<td>LV wt/body wt, mg/g</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>348 ± 7</td>
<td>395 ± 23</td>
<td>334 ± 15</td>
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<tr>
<td></td>
<td>Final body wt, g</td>
<td>20.0 ± 0.5</td>
<td>18.7 ± 0.3</td>
<td>19.4 ± 0.8</td>
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<td></td>
<td>RV wt/body wt, mg/g</td>
<td>1.42 ± 0.05</td>
<td>1.51 ± 0.10</td>
<td>1.23 ± 0.07</td>
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<tr>
<td></td>
<td>LV wt/body wt, mg/g</td>
<td>3.8 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>3.4 ± 0.2</td>
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<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>344 ± 6</td>
<td>335 ± 11</td>
<td>340 ± 10</td>
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</table>

Values are means ± SE (n = 8). *P < 0.05 vs. 129X1/SvJ. †P < 0.05 vs. 129X1/SvJ. ‡P < 0.05 vs. corresponding normoxia values. §P < 0.05 vs. corresponding normoxia values.
respectively. No Tph2 mRNA could be quantified in the other tissues, except for a discrete quantitative RT-PCR signal for Tph2 mRNA in the forebrain and gut, but only after 36–37 cycles. In the lung, no signal was detected up to 40 cycles. No significant differences in brain stem Tph2 mRNA were observed across mouse strains or between normoxia and hypoxia.

**DISCUSSION**

We found that the severity of hypoxic PH in mice was directly linked to the rate of 5-HT synthesis catalyzed by the Tph enzyme isoforms Tph1 and Tph2. Thus, in mice lacking the Tph1 gene and, therefore, exhibiting marked reductions in 5-HT synthesis rates and contents in peripheral organs, hypoxic PH was far less severe than in wild-type mice. Treatment with the Tph inhibitor PCPA further reduced PH severity in Tph1/H11002/H11002 mice, with normalization of RVSP and pulmonary artery muscularization, suggesting persistence of some peripheral Tph activity in Tph1/H11002/H11002 mice. Evidence for involvement of Tph2 was obtained by showing that mice harboring a polymorphism of the Tph2 gene and showing differences in brain 5-HT synthesis rates according to their genotype also showed differences in the severity of hypoxic PH. The variations in PH severity were abolished by PCPA, indicating that neuronal Tph2 activity contributes to the severity of hypoxic PH. In addition, hypoxia led to an increase in 5-HTP accumulation in the forebrain and lung that was not associated with increased 5-HT contents, suggesting accelerated 5-HT turnover.

The only molecules known to implicate the serotonergic system in PH were those that modulate the effects of 5-HT on target cells, namely, 5-HTT and 5-HT receptors (5-HT1B, 5-HT2A, and 5-HT2B) expressed by PASMC (9, 10, 12, 22, 24). Although the 5-HT synthesis rate is controlled at the critical step of tryptophan conversion to 5-HTP by the rate-limiting enzyme Tph, Tph has not been investigated as a candidate gene for PH. 5-HT production outside the central nervous system occurs chiefly in the enterochromaffin cells, from which the indoleamine is released into the bloodstream and taken up by circulating platelets (14, 15). Thus one hypothesis is that only 5-HT released from platelets acts on pulmonary vessels and that lung 5-HT bioavailability is independent of the rate of 5-HT synthesis. However, measurements of circulating free 5-HT levels in humans or experimental animals with PH failed to confirm this hypothesis. Moreover, in recent studies, we
showed that 5-HT was produced in the human lung by pulmonary endothelial cells expressing Tph1 (6). Tph1 expression and the 5-HT synthesis rate were increased in cells from patients with idiopathic PH, supporting a contribution to pulmonary vascular remodeling of increased 5-HT availability in the immediate vicinity of PASMCs (6). Our findings in mice are consistent with these earlier results obtained in human tissues. Tph1 mRNA and 5-HTP accumulation were detected in the lungs of wild-type mice; however, the amounts were small compared with those found in the gut. Tph2 mRNA was not detected in the lung, suggesting that 5-HTP accumulation measured in this organ was related to Tph1 only. Indeed, in Tph1−/− mice, 5-HTP accumulation rates and 5-HT contents were markedly reduced in the gut and lung. Hypoxia-induced PH was less severe in Tph1−/− mice, which exhibited less RV hypertrophy and pulmonary artery muscularization than wild-type controls. Treatment with the Tph inhibitor PCPA induced an additional protective effect on all PH parameters; as a result, RVSP and pulmonary artery muscularization in hypoxic PCPA-treated Tph1−/− mice did not differ from those in wild-type normoxic mice. Hypoxia-induced PH was less severe in Tph1−/− mice, which exhibited less RV hypertrophy and pulmonary artery muscularization than wild-type controls. Treatment with the Tph inhibitor PCPA induced an additional protective effect on all PH parameters; as a result, RVSP and pulmonary artery muscularization in hypoxic PCPA-treated Tph1−/− mice did not differ from those in wild-type normoxic mice. Our findings that significant levels of peripheral 5-HT were detected in Tph1−/− mice and that PCPA reduced PH severity in these mice strongly suggest a role for Tph2 in hypoxia-induced pulmonary vascular remodeling. Both Tph isoforms may exist in peripheral organs. In the gut, for instance, Tph1 is found in the enterochromaffin cells and Tph2 in the enteric nervous system (14), indicating that neuronal Tph2 may be present in nerve endings at the periphery. Nevertheless, in our study, the quantitative RT-PCR signal for Tph2 was detected in the gut only after 36–37 cycles and was not detected in the lung. It is therefore likely that Tph2 contributed to lung 5-HT bioavailability via the release of the indoleamine from myenteric neurons.

A single-nucleotide polymorphism C1473G in the Tph2 gene was recently identified in mice (31). C57Bl/6 and 129X1/SvJ mice, which are homozygous for the 1473C allele, exhibit higher forebrain Tph activity than DBA/2 and BALB/cJ mice, which are homozygous for the 1473G allele. Our comparison between the four mouse strains confirmed these findings by showing the highest 5-HTP synthesis rates in the forebrain in C57Bl/6 and 129X1/SvJ mice. 5-HTP accumulation rates in the gut and lungs were higher in the 129X1/SvJ mice than in the other three strains, but no differences in 5-HTP levels in other organs were found. However, hypoxia-induced PH was more severe in 129X1/SvJ and C57Bl/6 than in DBA/2 and BALB/cJ mice, supporting a close association between PH severity and the Tph2 genotype. Moreover, although PCPA reduced PH severity in all four mouse strains, as previously shown in rats (19), the reduction was greater in the C57Bl/6 and 129X1/SvJ than in the DBA/2 and BALB/cJ mice; as a result, PCPA abolished the difference in PH severity across mouse strains. These results support the idea that Tph2 activity may also contribute to control the development of pulmonary vascular remodeling. Both Tph isoforms may exist in peripheral organs. In the gut, for instance, Tph1 is found in the enterochromaffin cells and Tph2 in the enteric nervous system (14), indicating that neuronal Tph2 may be present in nerve endings at the periphery. Nevertheless, in our study, the quantitative RT-PCR signal for Tph2 was detected in the gut only after 36–37 cycles and was not detected in the lung. It is therefore likely that Tph2 contributed to lung 5-HT bioavailability via the release of the indoleamine from myenteric neurons.

An important finding from our study is that hypoxia led to increased 5-HT accumulation in the forebrain and lungs in all mouse strains, despite no changes in Tph1 or Tph2 mRNA levels. Although mRNA levels are not necessarily reflective of protein expression, this finding suggests that the hypoxia-induced increase in 5-HT synthesis resulted from enhanced enzyme activity and not from increased gene expression.
terestingly, hypoxia increased the rate of 5-HTP accumulation in the forebrain of Tph1−/− mice, as well as in the lungs of wild-type mice, suggesting that the activities of Tph2 and Tph1 were stimulated by hypoxia. Importantly, hypoxia not only increased 5-HT synthesis but also markedly reduced lung 5-HT contents and blood 5-HT levels, a combination suggesting a sharp increase in the indoleamine turnover rate during hypoxia. Consistent with this finding, PH severity in the four mouse strains failed to correlate with 5-HT levels in the forebrain or periphery, probably because local indoleamine turnover was the key factor in PH development. We showed previously that hypoxia increases 5-HTT expression and activity (7), and evidence is available establishing that hypoxia decreases monoamine oxidase activity (29). Because 5-HTT mediates the mitogenic effect of 5-HT in human and rodent pulmonary vascular smooth muscle cells, hypoxia-induced acceleration of 5-HT turnover may constitute a strong driving force behind pulmonary vascular remodeling. Thus experimental hypoxia PH is characterized by at least two molecular alterations of the 5-HT pathway: increased indoleamine production and enhanced indoleamine effects on target cells via effector molecules such as 5-HTT and, possibly, some of the 5-HT receptors.

In previous studies, we found that idiopathic PH was characterized by overexpression of Tph1 by pulmonary endothelial cells and 5-HTT by PASMCs and that both abnormalities interacted directly to induce hyperplasia of the smooth muscle cells (6). In experimental studies, we obtained evidence that the levels of 5-HTT expression or activity were important determinants of the pulmonary vascular remodeling process (16). The new data reported here extend these findings by showing that dysregulation of 5-HT synthesis in vivo is closely linked to vascular smooth muscle cells in pulmonary hypertension. Thus experimental hypoxic vascular smooth muscle hyperplasia.

References


