Inhibition of gut- and lung-derived serotonin attenuates pulmonary hypertension in mice

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Inhibition of gut- and lung-derived serotonin attenuates pulmonary hypertension in mice. Am J Physiol Lung Cell Mol Physiol 303: L500–L508, 2012. First published July 13, 2012; doi:10.1152/ajplung.00049.2012.—Decreasing the bioavailability of serotonin (5-HT) by inhibiting its biosynthesis may represent a useful adjunctive treatment of pulmonary hypertension (PH). We assessed this hypothesis using LP533401, which inhibits the rate-limiting enzyme tryptophan hydroxylase 1 (Tph1) expressed in the gut and lung, without inhibiting Tph2 expressed in neurons. Mice treated repeatedly with LP533401 (30–250 mg/kg per day) exhibited marked 5-HT content reductions in the gut, lungs, and blood, but not in the brain. After a single LP533401 dose (250 mg/kg), lung and gut 5-HT contents decreased by 50%, whereas blood 5-HT levels remained unchanged, suggesting gut and lung 5-HT synthesis. Treatment with the 5-HT transporter (5-HTT) inhibitor citalopram decreased 5-HT contents in the blood and lungs but not in the gut. In transgenic SM22-5-HTT+ mice, which overexpress 5-HTT in smooth muscle cells and spontaneously develop PH, 250 mg/kg per day LP533401 or 10 mg/kg per day citalopram for 21 days markedly reduced lung and blood 5-HT levels, right ventricular (RV) systolic pressure, RV hypertrophy, distal pulmonary artery muscularization, and vascular Ki67-positive cells (P < 0.001). Combined treatment with both drugs was more effective in improving PH-related hemodynamic parameters than either drug alone. LP533401 or citalopram treatment partially prevented PH development in wild-type mice exposed to chronic hypoxia. Lung and blood 5-HT levels were lower in hypoxic than in normoxic mice and decreased further after LP533401 or citalopram treatment. These results provide proof of concept that inhibiting Tph1 may represent a new therapeutic strategy for human PH.

PULMONARY HYPERTENSION (PH) develops either as a complication of other diseases or as a primary disease for which no underlying cause can be found (10). Hyperplasia of pulmonary artery smooth muscle cells (PA-SMCs) is a hallmark pathological feature of all forms of PH and leads to structural remodeling and pulmonary vessel occlusion (27). Serotonin (5-HT) plays a key role in PA-SMC proliferation, both in animal models of experimentally induced PH and in primary and secondary human PH (8, 9). Convincing evidence indicates that 5-HT-related stimulation of PA-SMC proliferation requires 5-HT internalization through the 5-HT transporter (5-HTT) (8, 20–22) and activation of 5-HT2B receptors (19) in PA-SMCs. Accordingly, pharmacological blockade of 5-HTT or 5-HT2B receptors has been shown to prevent or reverse experimentally induced PH (14, 19).

Another anti-5-HT strategy consists of inhibiting 5-HT biosynthesis outside the central nervous system (18, 23, 30), which is thought to occur chiefly in the gut. This peripherally synthesized 5-HT is released into the bloodstream and immediately taken up and stored in the platelets (12). Alterations in these processes may be involved in the pathogenesis of PH in both humans and Fawn-hooded rats with 5-HT platelet storage abnormalities (17, 28). PH caused by chronic dexfenfluramine treatment has also been shown to involve alterations in peripheral 5-HT release and effects (1).

Recent data show that peripheral 5-HT synthesis occurs also in the lung endothelial cells, which express Tryptophan Hydroxylase (Tph1), and that increased 5-HT release from these cells may contribute to PH (6). Therefore, inhibiting 5-HT synthesis in both the gut and the lungs, to reduce delivery to the lung vasculature of 5-HT from platelets and pulmonary vascular endothelial cells, respectively, may hold promise for reversing or preventing PH.

The critical step in 5-HT biosynthesis is catalyzed by the rate-limiting enzyme Tph, which exists as two isofoms, Tph1 and Tph2, encoded by separate genes. Tph1 is expressed mainly in the gut and pineal gland (4), whereas Tph2 is expressed only in neurons (29). In previous studies, we showed that Tph1 played a greater role than Tph2 in hypoxia-induced PH in mice (18). Thus selective Tph1 inhibition may hold promise for limiting the effects of 5-HT on PH progression. A new Tph inhibitor, LP533401 (2S)-2-amino-3-(4-(2-amino-6-(2,2,2-trifluoro-1-(3′-fluorobiphenyl-4-yl)ethoxy)pyrimidin-4-yl)phenyl)propanoic acid, was recently shown to block Tph1 in vivo without affecting Tph2 activity, as it cannot cross the blood-brain barrier (23, 30). When evaluated as a treatment for irritable bowel syndrome, LP533401 in a dose of 100 mg/kg body wt induced no overt deleterious effects (23, 30).

We reasoned that LP533401 may have therapeutic potential in PH when used either alone or in combination with a 5-HT transporter inhibitor. To assess this possibility, we first examined the consequences of treatment with LP533401 and/or the 5-HTT inhibitor citalopram on 5-HT levels in various organs (gut, lung, and brain) and blood of mice. We then compared the effects of chronic treatment with LP533401, citalopram, or both on 5-HT levels in the gut, lungs, and blood, as well as on PH-related parameters in mice overexpressing 5-HTT in
smooth muscle cells (SM22-5-HTT+ mice), which spontaneously develop severe PH. Finally, we investigated whether these drug regimens prevented PH in mice exposed to chronic hypoxia.

MATERIALS AND METHODS

Adult male mice (C57Bl/6j) were used in conformity with institutional guidelines that complied with national and international laws and policies. All animal experiments were approved by the Institutional Animal Care and Use Committee of the French National Institute of Health and Medical Research (INSERM)-Unit 955, Créteil, France.

Exposure to chronic hypoxia. Male mice (10–15 wk of age) with a mean weight of 25 g were exposed to chronic hypoxia (9% O2) in a ventilated chamber (Biospherix, New York, NY). The hypoxic environment was established by flushing the chamber with a mixture of room air and nitrogen then recirculating the gas mixture. The chamber was opened every other day for 1 h to clean the cages, administer the drugs, and replenish the food and water supplies. Normoxic mice were kept in the same room, with the same light-dark cycle.

Production of SM22-5-HTT+ mice. Transgenic mice overexpressing 5-HTT in smooth muscle cells under the control of the SM22 promoter (SM22-5-HTT+) were produced and bred on a C57Bl/6j background, as previously described (13). SM22-5-HTT+ mice are fertile and have a normal life span and normal growth (13). Only male mice were used for the experiments at 18 wk of age.

Treatments. To assess the effects of Tph inhibition on 5-HT levels in the brain (brainstem), gut, lungs, and blood, we treated normoxia-exposed mice with LP533401 (Dalton Pharma Services, Toronto, Ontario, Canada) given daily by gavage in three doses (30, 100, or 250 mg/kg per day), in polyethylene glycol with 5% dextrose (40:60 ratio) as the vehicle. For comparative studies, mice were randomly allocated to normoxia or hypoxia exposure; both groups were treated once daily with 250 mg/kg LP533401, 10 mg/kg citalopram, both, or the vehicle.

Assessment of PH. Mice were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) intraperitoneally. A 26-gauge needle connected to a pressure transducer was inserted into the right ventricle and right ventricular systolic pressure (RVSP), and heart rate was recorded immediately (18). If the heart rate fell below 300 bpm, systemic drugs, and replenish the food and water supplies. Normoxic mice were kept in the same room, with the same light-dark cycle.

Measurement of Tph1 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 gene with the TaqMan PCR technique. Predeveloped assay reagents, including primers and probes for the target genes, and endogenous control were supplied by Applied Biosystems (Courtabeuf, France). For each sample, 25 ng were used, amplification was performed in triplicate, 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 (2−ΔΔCt) by normalization for 18S ribosomal RNA.

RESULTS

Effects of treatment with the Tph inhibitor LP533401 on gut, lung, blood, and brain 5-HT contents in normoxic mice. Basal 5-HT contents varied across sites: values were highest in the duodenum, intermediate in the lungs and blood, and lowest in the brain (Fig. 1A). To examine the effects of increasing LP533401 doses on 5-HT levels, we treated mice with 30, 100, or 250 mg/kg per day of LP533401 for 4 days, and we measured 5-HT levels 12 h after the last administration. 5-HT levels decreased in a dose-dependent manner, to 60% in the duodenum and 40–50% in the lungs and blood (Fig. 1A). No changes were detected in brain 5-HT content (Fig. 1A), as expected with a drug that does not cross the blood-brain barrier (23).

To assess the time course of 5-HT levels after LP533401 treatment, we measured 5-HT levels at different times after a single 250 mg/kg dose (Fig. 1B). Duodenum and lung 5-HT contents decreased similarly, with the maximal reduction averaging 50% at 24 h in both organs (Fig. 1B). No change occurred in blood within the first 24 h following LP533401 administration, consistent with the absence of 5-HT synthesis in circulating blood cells (Fig. 1B). Mice given 15 days of LP533401 in a dose of 250 mg/kg per day showed similar changes in gut, lung, and blood 5-HT contents compared with mice treated for 3 days under normoxic conditions (data not shown).

Effects of Tph inhibitor LP533401, 5-HTT inhibitor citalopram, or their combination in mouse models of pulmonary hypertension. SM22-5-HTT+ transgenic mice overexpressed 5-HTT. In normoxia, SM22-5-HTT+ mice exhibited significant increases in the ratio of RV weight over body weight (RVwt/BW), RVSP, RV/LV+S, and distal pulmonary vessel muscularization compared with wild-type control mice (Table 1, Fig. 2). PH reversal was similar after 3 wk of LP533401 (250 mg/kg daily) vs. citalopram (10 mg/kg daily), with significant reductions in RVwt/BW, RVSP, RV/LV+S, pulmonary vessel muscularization, and Ki67-stained dividing vascular cells compared with vehicle-treated SM22-5-HTT+ mice (Table 1, Fig. 2). The 3-wk treatment duration was chosen based on previous studies in this model (15). As shown in Fig. 2, combined LP533401 and citalopram treatment resulted in significantly larger reductions in RVSP and RV/LV+S than those induced by...
either drug alone. In contrast, pulmonary vessel muscularization and vascular cell proliferation were not further decreased by the drug combination compared with LP533401 or citalopram alone (Fig. 2). Combined treatment produced a limited but significant increase in body weight that was not seen with either drug alone (Table 1).

At baseline, 5-HT levels in the lungs, duodenum, and blood did not differ significantly between SM22-5-HTT+ mutants and paired wild-type normoxic mice (Fig. 3). LP533401 in a dose of 250 mg/kg per day for 3 wk decreased the 5-HT levels to 50–70% of those measured in the same tissues in vehicle-treated mice (Fig. 3). Daily citalopram treatment (10 mg/kg) for 3 wk decreased lung and blood 5-HT levels to a greater extent (70%) than did chronic LP533401 administration but failed to affect gut 5-HT levels. As illustrated in Fig. 3B, combined treatment with both drugs did not significantly alter

Table 1. Body weight, heart rate, and ratios of right or left ventricle weight over body weight in C57Bl/6j and SM22-5-HTT+ mice after a 21-day treatment with LP533401 (250 mg/kg daily, per os), citalopram (10 mg/kg daily ip), combination of both drugs, or vehicle

<table>
<thead>
<tr>
<th></th>
<th>C57Bl/6j</th>
<th>SM22-5HTT+</th>
<th>Vehicle</th>
<th>LP533401</th>
<th>Citalopram</th>
<th>Combination</th>
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<tr>
<td>Body Weight, g</td>
<td>26.74 ± 0.33</td>
<td>22.22 ± 0.51</td>
<td>21.75 ± 0.58</td>
<td>23.40 ± 0.83</td>
<td>24.51 ± 0.77*</td>
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<tr>
<td>Heart Rate, beats/min</td>
<td>398 ± 14</td>
<td>340 ± 22</td>
<td>310 ± 7</td>
<td>393 ± 17</td>
<td>338 ± 26</td>
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<td>RV wt/ body wt, mg/g</td>
<td>0.69 ± 0.03</td>
<td>1.05 ± 0.02</td>
<td>0.90 ± 0.03*</td>
<td>0.95 ± 0.06*</td>
<td>0.86 ± 0.05*</td>
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<tr>
<td>LV wt/ body wt, mg/g</td>
<td>3.53 ± 0.10</td>
<td>3.72 ± 0.13</td>
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<td>3.85 ± 0.20</td>
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<tr>
<td>RV/LV+5 wt</td>
<td>19.5 ± 0.8</td>
<td>28.5 ± 0.6</td>
<td>24.6 ± 0.7†</td>
<td>24.6 ± 1.0†</td>
<td>21.4 ± 1.02‡§</td>
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Values are means ± SE of 6 independent determinations in each group; RV, right ventricle; LV, left ventricle. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. respective values in vehicle-treated SM22-serotonin transporter (5-HTT)+ mice. §P < 0.05 for comparison with LP533401 treatment alone; *P < 0.05 for comparison with citalopram treatment alone.
Fig. 2. Right ventricular systolic pressure (RVSP) (A), Fulton index [right ventricle/left ventricle + interventricular septum (RV/LV+IVS)] (B), and percentage of muscularized distal pulmonary arteries (C) in wild-type (WT) C57Bl/6j mice (n = 6) and transgenic SM22-5-HT transporter (5-HTT)+ mice (n = 6 in each group) after 21 days of daily treatment with the tryptophan hydroxylase (Tph) inhibitor LP533401 (250 mg/kg per day), 5-HTT inhibitor citalopram (10 mg/kg per day), both, or vehicle. Values are means ± S. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with corresponding values in vehicle-treated SM22-5-HTT+ mice. #P < 0.05 for the intergroup comparison.

D: number of proliferating vascular cells expressed as the percentage of Ki67-labeled cells over the total number of cells counted in the media of at least 20 muscularized vessels per mice. Lung sections showing smooth muscle cell proliferation (Ki67 immunostaining) in pulmonary vessels of a WT or SM22-5-HTT+ mouse under each treatment condition. Bar = 25 μm. Brown staining indicates Ki-67-positive cells (red arrowheads). *P < 0.05, **P < 0.01, and ***P < 0.001 compared with corresponding values in vehicle-treated SM22-5-HTT+ mice. #P < 0.05 for the intergroup comparison.
5-HT levels in the lung and blood as seen with citalopram alone.

Chronically hypoxic mice exposed to 9% oxygen for 15 days developed PH with marked increases in RV/BW (Table 2), RVSP, RV/LV+S, distal pulmonary vessel muscularization, and Ki67-stained dividing vascular cells compared with control normoxic mice (Fig. 4). LP533401 and citalopram produced similar attenuations in PH development, with significant decreases in RV/BW (Table 2), RVSP, RV/LV+S, muscularized pulmonary vessels, and Ki67 immunostaining (Fig. 4) compared with chronically hypoxic mice treated with the vehicle. Combined LP533401 and citalopram treatment did not exert larger effects on the PH-related parameters than either drug alone (Table 2, Fig. 4).

Under normoxia, 15 days of LP533401 treatment had no significant effects on BW, RV/BW, LV+S/BW, or heart rate (Table 2). Furthermore, no changes in pulmonary hemodynamics were noted in normoxic mice treated with LP533401 and/or citalopram compared with vehicle-treated mice (data not shown). In contrast, BW decreased in hypoxic mice given LP533401 alone or combined with citalopram (Table 2).

The data in Fig. 5 show that exposure to chronic hypoxia was associated with marked decrease in lung and blood 5-HT levels, whereas duodenum 5-HT levels remained unchanged. LP533401 250 mg/kg per day for 15 days further decreased the lung and blood 5-HT levels and lowered the duodenum 5-HT levels (Fig. 5). Chronic citalopram treatment (10 mg/kg per day) diminished the lung and blood 5-HT levels to a greater extent than did LP533401; however, in contrast to LP533401, citalopram failed to significantly modify duodenum 5-HT contents (Fig. 5). With combined LP533401 and citalopram treatment, the decreases in lung and blood 5-HT levels were similar to those produced by citalopram alone and the decrease in duodenum 5-HT levels was similar to that seen with LP533401 alone (Fig. 5).

**Lung Tph1 mRNA levels in wild-type and SM22-5-HTT+ mice.** Lung Tph1 mRNA levels were not altered during exposure to hypoxia in wild-type mice (0.48 ± 0.1 in hypoxia vs. 0.53 ± 0.1 in normoxia, NS) and were not affected by chronic treatment with LP533401, citalopram, or their combination. In contrast, lung Tph1 mRNA levels were increased in SM22-5-HTT+ mice compared with normoxic wild-type mice (0.96 ± 0.06 vs. 0.53 ± 0.1, respectively) and were not affected by chronic treatment with LP533401, citalopram, or their combination.

**DISCUSSION**

We show here that treatment of mice with the selective Tph1 inhibitor LP533401 leads to sustained 5-HT level decreases in the gut, lungs, and blood without affecting brain 5-HT levels. The parallel reductions in gut (duodenum) and lung 5-HT levels in mice given a single LP533401 dose, together with the

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**Table 2. Body weight, heart rate, and ratios of RV or LV weight over body weight in C57Bl/6J mice after a 14-day treatment with LP533401 (250 mg/kg daily, per os), citalopram (10 mg/kg daily ip), combination of both drugs, or vehicle under normoxic or hypoxic conditions**

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<th>Normoxic</th>
<th>Hypoxic</th>
<th>Hypoxic</th>
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<tr>
<td></td>
<td>Control</td>
<td>LP533401</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Body Weight, g</td>
<td>27.68 ± 0.12</td>
<td>26.29 ± 0.59</td>
<td>25.66 ± 0.89</td>
</tr>
<tr>
<td>Heart Rate, beats/min</td>
<td>351 ± 21</td>
<td>416 ± 25</td>
<td>350 ± 18</td>
</tr>
<tr>
<td>RV wt/ body wt, mg/g</td>
<td>0.61 ± 0.01</td>
<td>0.66 ± 0.01</td>
<td>1.17 ± 0.01</td>
</tr>
<tr>
<td>LV wt/body wt, mg/g</td>
<td>3.17 ± 0.08</td>
<td>3.24 ± 0.03</td>
<td>3.51 ± 0.02</td>
</tr>
<tr>
<td>RT/LV+S wt</td>
<td>19.4 ± 0.82</td>
<td>20.2 ± 0.64</td>
<td>33.4 ± 0.76</td>
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Values are means ± SE of 6 independent determinations in each group. *P < 0.05 vs. respective values in vehicle-treated hypoxic mice.
unchanged blood 5-HT levels, are consistent with our hypothesis of substantial intrapulmonary 5-HT synthesis in addition to 5-HT synthesis in the gut. Chronic LP533401 treatment was associated with partial PH reversal in SM22-5-HTT/H11001 mice and with PH attenuation in chronically hypoxic mice. Until now, the only molecules known to affect the serotonergic system in PH were drugs modulating 5-HT effects on target cells, notably through inhibition of the 5-HTT or blockade of 5-HT receptors expressed by PA-SMCs (5-HT1B, 5-HT2A, and 5-HT2B) (5, 7, 19, 24). Because the rate of 5-HT
synthesis is controlled at the critical step of tryptophan conversion to 5-hydroxytryptophan by the rate-limiting enzyme Tph, we and others previously investigated Tph as a candidate gene for PH (18, 26). We have reported that Tph1<sup>−/−</sup> mice, which exhibit reduced 5-HT synthesis rates and contents in the gut and lungs, also develop less severe hypoxic PH than do paired wild-type mice (18). 5-HT production outside the central nervous system may occur chiefly in the enterochromaffin cells (12), from which 5-HT is released into the bloodstream and almost completely taken up by circulating platelets. Thus the platelet 5-HT content depends heavily on the rate of 5-HT synthesis by enterochromaffin cells, and Tph1 deficiency leads to a major reduction in platelet 5-HT content (3). However, in previous studies, we also showed that 5-HT was produced in the human lung by pulmonary endothelial cells expressing Tph1 (6). Both Tph1 expression and the 5-HT synthesis rate were increased in cells from patients with idiopathic PH, supporting a contribution of increased 5-HT availability near the PA-SMCs to the pulmonary vascular remodeling that characterizes PH. Studies in mice corroborated these findings by showing that Tph1 mRNA and Tph activity were measurable in wild-type lungs although in small amounts compared with those found in the gut (18).

In the present study, we used a selective Tph1 inhibitor to diminish lung 5-HT bioavailability without affecting 5-HT functions in the central nervous system (23, 30). As expected, LP533401 administration to wild-type normoxic mice was associated with dose-dependent decreases in lung, blood, and gut 5-HT levels, with no change in brain 5-HT contents. Studies of the effects of a single LP533401 administration over time showed early decreases in gut and lung 5-HT contents but no change in blood 5-HT contents up to 24 h following the dose. The early decrease in 5-HT levels in the gut and lungs is consistent with immediate inhibition of Tph activity in these organs, whose 5-HT contents depend chiefly on local 5-HT synthesis. In contrast, blood 5-HT levels that are linked to the storage ability of circulating platelets need more time to decrease after Tph activity inhibition. We found a significant blood 5-HT decrease 4 days after LP533401 treatment initiation, as expected given the persistent decrease in 5-HT originating from the gut and slow turnover of platelets in blood. Thus two conclusions can be drawn from these results: 1) 5-HT is synthesized locally in the lungs via a pathway that can be pharmacologically inhibited by short-term treatment with a selective Tph1 inhibitor; and 2) partial inhibition of 5-HT synthesis in the gut is associated with a delayed decrease in blood 5-HT content.

We compared the effects of Tph1 inhibition on peripheral 5-HT levels to those of citalopram, a selective 5-HTT inhibitor (2). Chronic citalopram treatment markedly decreased blood 5-HT levels; in contrast, gut 5-HT contents were unchanged, indicating that citalopram did not affect 5-HT synthesis in this organ. Platelet 5-HTT blockade by citalopram prevents the platelets from taking up and storing 5-HT, thereby inevitably resulting in platelet 5-HT depletion because platelets cannot synthesize 5-HT (2). Citalopram blocks not only 5-HT uptake by platelets but also 5-HT internalization by PA-SMCs (7). Conceivably, citalopram may increase lung 5-HT bioavailability (by inhibiting platelet 5-HT uptake) on the one hand while protecting against potential growth-promoting effects of 5-HT in the lung vasculature (by inhibiting 5-HT internalization by PA-SMCs) on the other. With respect to the potential harmful effects of free plasma 5-HT on the pulmonary vasculature, Tph1 inhibitor therapy used alone or with citalopram might be of interest, as a means of both diminishing the gut output of 5-HT and decreasing the production of 5-HT in the lungs.

To compare the effects of LP533401 on pulmonary hypertension with those of citalopram, we first examined the ability of both drugs to reverse PH in SM22-5-HTT<sup>+</sup> mice exhibiting similar lung, gut, and blood 5-HT levels as those found in wild-type normoxic mice. In SM22-5-HTT<sup>+</sup> mice, the spontaneous development of persistent PH is related to enhanced 5-HT effects on target PA-SMCs via the effector molecule 5-HTT (13). We studied these mice at 2–3 mo of age, when...
several cellular abnormalities participate in the pulmonary vascular remodeling process, in addition to the increased 5-HTT activity in PA-SMCs. Previous studies have documented other complex molecular abnormalities in this model, as in other types of PH (15). In the present study, we measured increased Tph1 expression in SM22-5-HTT+ mice but not in chronically hypoxic mice. However, inhibiting 5-HTT by citalopram or reducing lung and blood 5-HT levels via Tph1 inhibition by LP533401 led to similar degrees of PH reversal. Moreover, combining LP533401 and citalopram slightly increased the degree of PH reversal. These results show that, in this particular model, a selective Tph1 inhibitor can partially reverse PH. Moreover, they emphasize the usefulness of combining a selective Tph1 inhibitor with a drug that targets 5-HT effects at the cellular level.

We also compared the effects of the Tph1 inhibitor LP533401 to those of citalopram in mice exposed to chronic hypoxia. Exposure to chronic hypoxia markedly reduced lung 5-HT contents and blood 5-HT levels but had no effect on gut 5-HT contents. Previous findings by our group showed that these changes occurred concomitantly with an increase in lung 5-HT synthesis despite no change in Tph1 expression (18). We have also reported that hypoxia increases 5-HTT expression and activity, and other studies have established that hypoxia increases monoamine oxidase activity. Thus complex hypoxia-induced alterations probably contribute to the observed changes in blood and lung 5-HT levels in mice exposed to hypoxia. These alterations may also explain why treatment with either the Tph1 inhibitor or citalopram was less efficient in the hypoxic model than in SM22-5-HTT+ mice. Moreover, in the hypoxic model, combining the two drugs failed to magnify the effects, in contrast to our findings in SM22-5-HTT+ mice. Nevertheless, the Tph1 inhibitor LP533401 partially prevented PH development in hypoxic mice, indicating that, even when blood 5-HT levels are decreased, inhibiting 5-HT synthesis is effective in attenuating PH and the associated pulmonary vascular remodeling process.

In conclusion, the Tph1 inhibitor LP533401, which is suitable for oral once-daily administration, decreases lung 5-HT contents and attenuates experimental PH. Whether such a strategy is useful for treating pulmonary hypertension will require further studies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


