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Abstract—One intrinsic abnormality of pulmonary artery smooth muscle cells (PA-SMCs) in human idiopathic pulmonary hypertension (iPH) is an exaggerated proliferative response to internalized serotonin (5-HT) caused by increased expression of the 5-HT transporter (5-HTT). To investigate whether 5-HTT overexpression in PA-SMCs is sufficient to produce PH, we generated transgenic mice overexpressing 5-HTT under the control of the SM22 promoter. Studies in SM22-LacZ$^+$ mice showed that the transgene was expressed predominantly in SMCs of pulmonary and systemic vessels. Compared with wild-type mice, SM22–5-HTT$^+$ mice exhibited a 3- to 4-fold increase in lung 5-HTT mRNA and protein, together with increased lung 5-HT uptake activity, but no changes in platelet 5-HTT activity or blood 5-HT levels. At 8 weeks of age, SM22–5-HTT$^+$ mice exhibited PH, with marked increases in right ventricular systolic pressure (RVSP), right ventricle/left ventricle+septum ratio, and muscularization of distal pulmonary vessels, but no changes in systemic arterial pressure. PH worsened with age. Except a marked decrease in Kv channels, no changes in the lung expression of mediators of pulmonary vascular remodeling were observed in SM22–5-HTT$^+$ mice. Compared with wild-type mice, SM22–5-HTT$^+$ mice showed depressed hypoxic pulmonary vasoconstriction contrasting with greater severity of hypoxia- or monocrotaline-induced PH. These results show that increased 5-HTT expression in PA-SMCs, to a level close to that found in human iPH, lead to PH in mice. They further support a central role for 5-HTT in the pathogenesis of PH, making 5-HTT a potential therapeutic target. (***Circ Res*. 2006;98:1323-1330.)**

Key Words: serotonin transporter ■ pulmonary hypertension ■ vascular smooth muscle ■ transgenic mice

Pulmonary hypertension (PH) occurs as a complication of various conditions or develops as a primary disease for which no underlying cause can be found. Medial hypertrophy and intimal thickening of pulmonary arteries are hallmark pathological features that ultimately lead to vessel obliteration.[1] Although hyperplasia of pulmonary-artery smooth muscle cells (PA-SMCs) is considered the main component of these changes, the nature of the primary defect responsible for triggering and maintaining PA-SMC proliferation in PH is poorly understood. In particular, to date, whether smooth muscle hyperplasia results from an inherent characteristic of PA-SMCs or from dysregulation of molecular events governing PA-SMC growth is still unknown.[2,3]

We recently reported that serotonin (5-hydroxytryptamine [5-HT]) and its plasmic membrane transporter (5-HTT) played a central role in the pathogenesis of PA-SMC proliferation in experimental and human PH, whether idiopathic (iPH) or associated with various diseases (aPH).[4–6] A key observation is that PA-SMCs from patients with iPH or aPH proliferate excessively when they are exposed to 5-HT.[6] In contrast, these cells proliferate to the same extent as PA-SMCs from control subjects when they are stimulated by various growth factors such as PDGF, EGF, FGF, IGF, or TGF-β.[5] This abnormal response to 5-HT is attributable to overexpression of 5-HTT, which leads to increased internalization of indoleamine, whose intracellularly mediated mitogenic action is therefore enhanced.[7,8] PA-SMCs from patients with iPH maintain increased 5-HTT expression when cultured, ie, removed from their in vivo environment, suggesting that increased 5-HTT expression by these cells may be a cardinal alteration in PH.[5,6] However, it is not known whether 5-HTT overexpression occurring selectively in PA-SMCs is sufficient to produce PH in vivo. This point is of particular importance because 5-HTT expression is controlled by many factors, including polymorphism of its gene promoter, hypoxia, inflammatory cytokines, and drugs known to increase

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the risk of iPH in humans.[5,8–10] Previous studies showed greater severity of hypoxic PH in transgenic mice that overexpress 5-HTT than in wild-type controls.[11] However, these transgenic animals also exhibit 5-HTT overexpression in various cell types in addition to PA-SMCs, including platelets, fibroblasts, endothelial cells, and neurons, which may lead to major changes in central and peripheral 5-HT bioavailibility. In a previous study of monocrotaline-induced PH in rats, we found that increased 5-HTT expression preceded the onset of PH, suggesting that 5-HTT overexpression occurred upstream to PH development in this model.[9] However, many biological abnormalities are associated with the onset of PH in monocrotaline-treated rats, which likely interfered with the specific action of 5-HTT in the process of pulmonary vascular remodeling.

In the present study, we generated mice that overexpressed 5-HTT under the control of the smooth muscle promoter SM22 (SM22–5-HTT$^+$ mice). The SM22 promoter was chosen because it drives preferential expression of transgenes in vascular smooth muscle (compared with visceral smooth muscle) and in arteries (compared with veins).[12] We addressed the following questions. (1) Do SM22–5-HTT$^+$ mice spontaneously develop PH in the absence of associated or environmental stimuli? (2) Does PH induced by 5-HTT overexpression occurring selectively in PA-SMCs share some of the pathological and biological abnormalities encountered in human PH? (3) Are SM22–5-HTT$^+$ mice more susceptible than paired wild-type mice to PH induced by various stimuli including exposure to hypoxia or treatment with the active monocrotaline derivative monocrotaline-pyrrole?

Materials and Methods

Production of SM22–5-HTT$^+$ and SM22-LacZ$^+$ Transgenic Mice

Transgenic animals were produced as previously described.[12] The murine SM22 promoter (2191 bp starting 2126 bp before the start site) was used to drive expression of the human 5-HTT gene or the LacZ gene. All animal care and procedures were in accordance with institutional guidelines.

Immunoblotting and Immunostaining

The tissues were sonicated in PBS containing anti-proteases, and 150 μg of protein from each sample was used to detect 5-HTT protein as previously described.[4] The 5-HTT and β-galactosidase proteins were immunocytochemically labeled with polyclonal antibodies (Chemicon, Hampshire, UK) directed against 5-HTT and β-galactosidase (1:1000), respectively, as previously described.[4]

Lung and Platelet [^{3}H]5-HT Uptake

Lung tissues were homogenized in the uptake buffer (120 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 5.6 mmol/L glucose, 4 mmol/L Tris-HCl, 6.25 mmol/L HEPES, and 0.5 mmol/L ascorbic acid, pH 7.4). Platelet and lung [^{3}H]5-HT uptake were measured with and without fluoxetine (10^{-5} mmol/L), according to a previously described protocol.[4]

Blood 5-HT and 5-HIAA Concentrations

Serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were assayed in whole blood using electrochemical detection by high-performance liquid chromatography.[13]

Measurement of Systemic Arterial Pressure in Normoxic Mice

To measure systemic arterial pressure, animals were anesthetized with ketamine (60 mg/kg IP) and xylazine (10 mg/kg IP). The right carotid was exposed, and a polyethylene catheter was inserted into the carotid artery then connected to a pressure transducer.[4]

Assessment of PH

Mice were studied at 8, 20, and 55 weeks of age. Right ventricular systolic pressure (RVSP) and heart rate were recorded and the Fulton index (right ventricle/left ventricle plus septum [RV/LV+S]) was determined as previously described.[4] The lungs were removed, fixed, and processed for paraffin embedding. The percentage of muscularized vessels was calculated as previously described.[4] The cell proliferation rate in muscularized vessels was estimated by proliferating-cell nuclear antigen (PCNA) staining.[9]

Real-Time Quantitative PCR

Real-time quantitative PCR (RTQ-PCR) was used to measure lung levels of mRNAs encoding 5-HTT, bone morphogenetic protein receptor (BMPR)-II, BMPR-1A, BMPR-1B, BMP-2, BMP-4, endothelin-1 (ET-1), Tie-2 receptor, prostacyclin (PGI2) synthase, voltage-gated potassium channel (Kv)1.2, Kv1.5, Kv2.1, and Kv9.3, as previously described.[9]

Effects of 5-HTT Activation on Kv Channel Expression in Cultured Human PA-SMCs

The effects of 5-HT on Kv1.5 and Kv2.1 expression were studied on cultured human PA-SMCs collected as previously described.[5] Synchronized cells were exposed to 5-HT (10^{-6} mol/L) with or without fluoxetine (10^{-6} mol/L) in serum-free medium for 4 hours before RNA extraction.

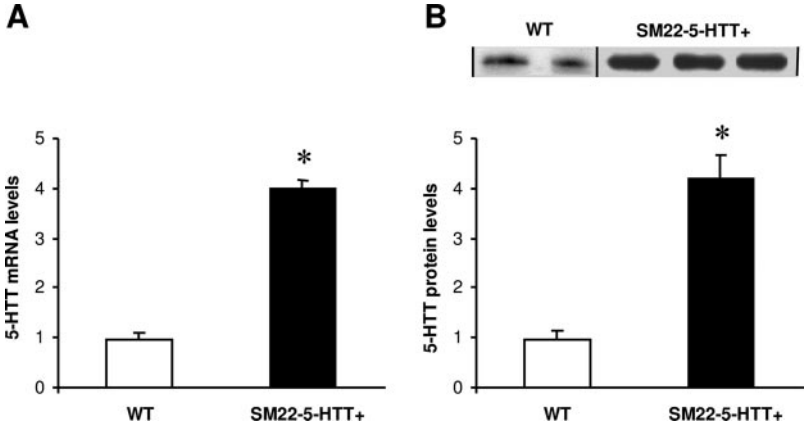

Figure 1. 5-HTT mRNA and protein in lung tissues from transgenic SM22–5-HTT$^+$ and wild-type (WT) mice. A, Quantification of products from RTQ-PCR obtained with primers recognizing both mouse and human 5-HTT mRNA. B, Western blot of 5-HTT protein in lungs from SM22–5-HTT$^+$ and wild-type mice. Relative protein levels were assessed by densitometric quantification of 5-HTT immunoblotting normalized against the β-actin level. The antibodies recognized human and mouse 5-HTT. Values are mean±SEM of 5 SM22–5-HTT$^+$ and 6 wild-type mice. *$P<0.01$ compared with values obtained in wild-type mice.

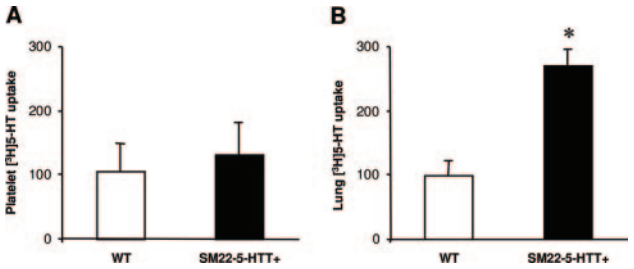

Figure 2. Relative values of [^{3}H]5-HT uptake by platelets (A) and lung homogenates (B) from transgenic SM22–5-HTT$^+$ and wild-type (WT) mice. Nonspecific uptake was determined in the presence of the specific 5-HTT inhibitor fluoxetine (10^{-5} mol/L). Each bar is the mean±SEM of 6 independent determinations. *$P<0.01$ for comparison with values obtained in wild-type mice.

Hemodynamic Response of Normoxic Mice to Acute Hypoxia

Right ventricular systolic pressure and heart rate were performed first while the animals were ventilated with room air then after 5 minutes of ventilation with a hypoxic gas mixture (8% O_2, 92% N_2).[4]

PH Induced by Exposure to Chronic Hypoxia or by Treatment with Monocrotaline Pyrrole

Mice aged 8 weeks received a single IV injection of the active monocrotaline derivative monocrotaline pyrrole (MCT-P) (5 mg/kg) or were exposed to chronic hypoxia (10% O_2, 90% N_2). Preliminary experiments revealed that intravenous administration of MCT-P induced PH within 15 days. PH and vascular remodeling were evaluated 2 weeks following hypoxia exposure or MCT-P injection.

Statistical Analyses

Data are presented as the means±SE. Statistical significance was tested using the nonparametric Mann–Whitney test or 2-way ANOVA followed by the Student–Newman–Keuls test.

Results

Validation of Transgene Function and Tissue Distribution

Lung transgene expression by 8-week-old transgenic SM22–5-HTT$^+$ mice was measured using RTQ-PCR and Western blot for 5-HTT mRNA and protein, respectively. SM22–5-HTT$^+$ mice showed a marked increase in lung 5-HTT expression compared with wild-type controls, with 3- to 4-fold increases in 5-HTT mRNA and protein levels (Figure 1). Accordingly, lung [^{3}H]5-HT uptake was 3 times higher in SM22–5-HTT$^+$ mice than in wild-type mice, whereas platelet [^{3}H]5-HT uptake did not differ between the 2 strains (Figure 2). To further validate the use of the SM22 promoter, we generated SM22-lacZ$^+$ mice. As shown in Figure 3A, SM22-lacZ$^+$ mice demonstrated β-galactosidase immunostaining in the media of pulmonary arteries but not in the bronchi. Most of the pulmonary vessels were stained, indicating diffuse transgene expression throughout the pulmonary vasculature. Immunohistochemical studies were also performed to visualize 5-HTT–like immunoreactivity in pulmonary vessels of transgenic SM22–5-HTT$^+$ and wild-type mice. As shown in Figure 3B, 5-HTT immunostaining was detected in pulmonary arteries from both SM22–5-HTT$^+$ and wild-type mice but with greater intensity in the transgenic mice.

Blood 5-HT and 5-HIAA Levels in Transgenic SM22–5-HTT$^+$ Mice and Wild-Type Mice

Whole-blood 5-HT levels did not significantly differ in SM22–5-HTT$^+$ mice and wild-type mice (21.0±1.5 and 20.8±1.6 μmol/L, respectively; means±SEM, n=6 in each group). Similarly, blood 5-HIAA levels were similar in

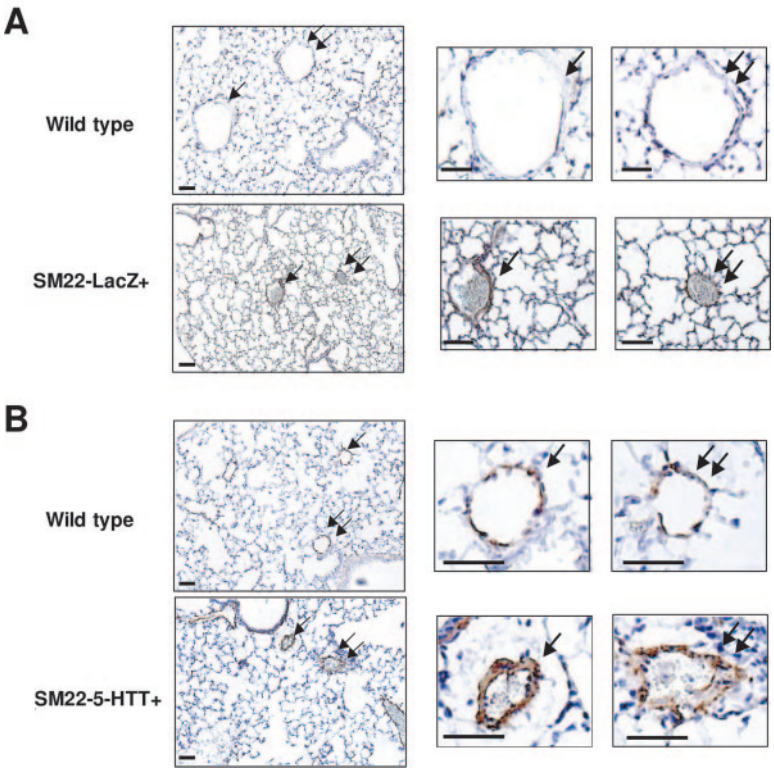

Figure 3. A, Immunohistochemical localization of β-galactosidase in lungs from transgenic SM22-LacZ$^+$ and wild-type mice. Paraffin sections of lung tissues were used for immunohistochemical analysis with anti–β-galactosidase antibodies. Immunoreactivity was detected with 3,3′-diaminobenzidine (brown staining) and nuclei were counterstained with hematoxylin (blue staining). Dense immunostaining is visible in the smooth muscle cells from a pulmonary vessel of a transgenic SM22-LacZ$^+$ mouse, without staining in the bronchi. B, Immunohistochemical localization of 5-HTT in lungs from transgenic SM22–5-HTT$^+$ and wild-type mice. 5-HTT immunostaining is visible in the media of pulmonary vessels and appears stronger in the medial layer of remodeled pulmonary arteries from SM22–5-HTT$^+$ mice. Scale bar=50 μm.

transgenic (0.612±0.040 μmol/L, n=5) and wild-type (0.716±0.048 μmol/L, n=5) mice.

Development of PH and Vascular Remodeling in SM22–5-HTT+ Mice

SM22–5-HTT+ mice (8-weeks old) did not differ from wild-type mice with respect to body weight (23±1 g versus 22±2 g, *P*=NS), systemic arterial pressure (93±5 mm Hg versus 91±3 mm Hg, *P*=NS), or heart rate (360±14 bpm versus 342±11 bpm, n=6 in each group, *P*=NS). In contrast, RVSP and RV/LV+S weight ratio were significantly higher in SM22–5-HTT+ mice than in wild-type controls, indicating the presence of sustained PH in transgenic mice (Figure 4). SM22–5-HTT+ mice experienced PH progression over time, with higher RVSP values at 55 weeks than at 20 weeks and at 20 weeks than at 8 weeks (Figure 4A). Concomitantly, SM22–5-HTT+ mice developed marked pulmonary vascular remodeling, as shown by increased density of muscularized pulmonary vessels in SM22–5-HTT+ mice at any age compared with wild-type mice (Figure 4C). As illustrated in Figure 4, muscularization was more prominent in 20-week-old mice than in 8-week-old mice and was greatest in 55-week-old mice. In SM22–5-HTT+ mice, PCNA labeling showed proliferation of smooth muscle cells in distal pulmonary vessel walls (Figure 4D). PCNA-positive cell counts were 60±6% and 55±12% in muscularized pulmonary vessels from SM22–5-HTT+ mice aged 20 and 55 weeks, respectively, compared with 6±2% in vessels from wild-type mice (*P*<0.01).

Lung Molecular Alterations Induced by 5-HTT Overexpression in SM22–5-HTT+ Mice

We investigated whether development of PH in SM22–5-HTT+ mice was associated with changes in the expression of genes involved in pulmonary vascular remodeling. Using RTQ-PCR, we found that lung levels of mRNAs encoding ET-1, Tie2 receptor, prostacyclin-synthase, BMPR-2, BMPR-1A, BMPR-1B, BMP-2, BMP-4, Kv1.2, and Kv9.3 were not altered in SM22–5-HTT+ mice compared with wild-type controls (Figure 5). In contrast, both Kv1.5 and Kv2.1 mRNA levels were markedly decreased in lungs from SM22–5-HTT+ mice compared with controls at 20 weeks of age (Figure 5C). Because Kv1.5 and Kv2.1 channels are predominantly expressed by SMCs,[14] we performed additional in vitro studies on human PA-SMCs to examine the effect of 5-HT on the expression of these channels. As shown in Figure 6, incubation of PA-SMCs with 5-HT (10^{-6} mol/L) for 4 hours dramatically reduced Kv1.5 mRNA levels. This effect was abolished by the selective 5-HTT inhibitor fluoxetine (10^{-6} mol/L).

Hemodynamic Responses to Acute Hypoxia

The effect of an acute hypoxic challenge on RVSP was examined in 20-week-old normoxic mice (Figure 7). Exposure to 8% O_2 elicited a marked increase in RVSP in wild-type mice (from 18.2±0.6 to 23.1±0.7 mm Hg; *P*<0.01) but not in SM22–5-HTT+ mice (from 28.1±0.7 to 28.6±0.6 mm Hg). Heart rates were similar in transgenic and wild-type mice.

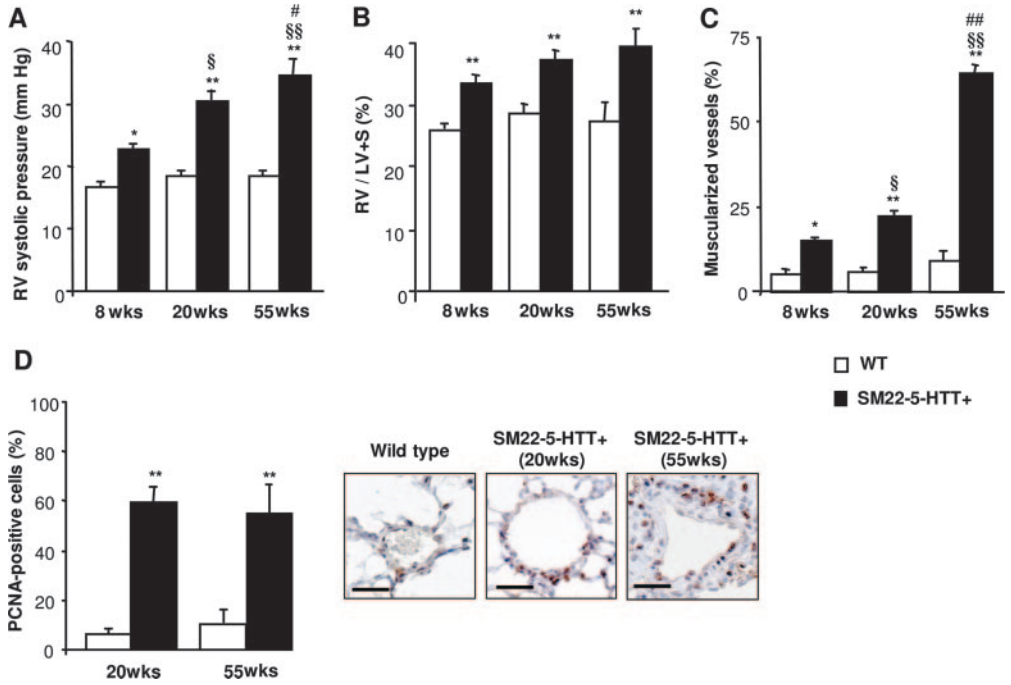

Figure 4. Development of PH and vascular remodeling in SM22–5-HTT+ vs wild-type mice at 8 and 20 weeks of age under normoxic conditions. A, RV systolic pressure. B, Fulton index: RV/LV+S. C, Percentage of muscularized vessels. D, In situ PA-SMC proliferation in muscularized vessels of SM22–5-HTT+ and wild-type (WT) mice at 20 weeks of age, shown by immunohistochemistry for PCNA. To quantify VSMC proliferation, we counted PCNA-positive cells in the media of muscularized vessels and expressed the result as the percentage of the total smooth muscle cell count. Representative pictures of PCNA staining. Scale bar=50 μm. Values are means±SEM (n=10 in each group). **P*<0.05 and ***P*<0.01 compared with values in wild-type mice; §*P*<0.05 compared with corresponding values in 8-week-old mice; #*P*<0.05 compared with corresponding values in 20-week-old mice (A and C).

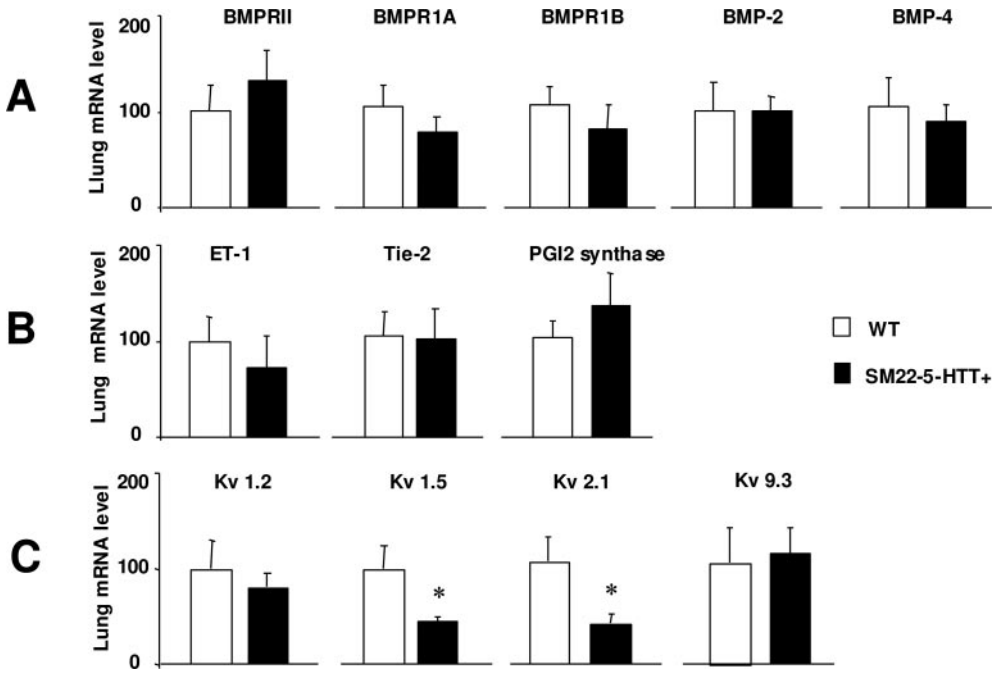

Figure 5. Relative lung levels of mRNAs encoding BMPR2, BMPR1A, BMPR1B, BMP-2, BMP-4 (A); endothelin (ET-1), Tie-2 receptor, and prostacyclin (PGI2) synthase (B); and voltage-dependent potassium channels Kv1.2, Kv1.5, Kv2.1, and Kv9.3 (C) in wild-type (WT) and SM22–5-HTT$^+$ mice at 20 weeks of age under normoxic conditions. Values are means±SEM. *$P<0.05$ and **$P<0.01$ compared with respective values in wild-type mice.

PH in SM22–5-HTT$^+$ and Wild-Type Mice After Exposure to Chronic Hypoxia or Treatment With Monocrotaline-Pyrrole

These experiments were performed to investigate whether PH induced by chronic hypoxia or MCT-P was more severe in SM22–5-HTT$^+$ mice than in wild-type mice. Mice aged 8 weeks were used for these studies. Fifteen days after exposure to 10% O_2 or treatment with MCT-P, both SM22–5-HTT$^+$ and wild-type mice exhibited increases in RVSP (Figure 8A), Fulton index (Figure 8B), and muscularization of pulmonary vessels (Figure 8C) compared with preexposure values. However, the RVSP increase and degree of RV hypertrophy and distal pulmonary vessel muscularization were more severe in SM22–5-HTT$^+$ mice than in wild-type mice following administration of MCT-P or exposure to hypoxia (Figure 8).

Discussion

Our results show that transgenic mice with 5-HTT overexpression occurring selectively in SMCs spontaneously develop PH. Because 5-HTT overexpression in PA-SMCs is a characteristic of human iPH or aPH, these data indicate that 5-HT internalization by PA-SMCs plays a prominent role in the pathogenesis of human PH. One major point is that PH in SM22–5-HTT$^+$ mice developed despite unaltered 5-HT bioavailibility, solely as a consequence of increased 5-HTT protein expression in SMCs. We found that genes belonging to the BMP pathway and genes expressed by endothelial cells, whose expression is usually altered in human or experimental PH, remained unaltered in the lungs of SM22–5-HTT$^+$ mice. In contrast, the expression of Kv channels which are predominantly expressed by PA-SMCs, was lower in SM22–5-HTT$^+$ mice than in wild-type mice. Compared with wild-type mice, SM22–5-HTT$^+$ mice showed depressed hypoxic pulmonary vasoconstriction but developed more severe hypoxia- or monocrotaline-induced PH.

The main question addressed in this study was whether induction in mice of an intrinsic abnormality constantly found in PA-SMCs from patients with various forms of PH was sufficient to induce PH in the absence of other stimuli. The use of the SM22 promoter to drive 5-HTT expression

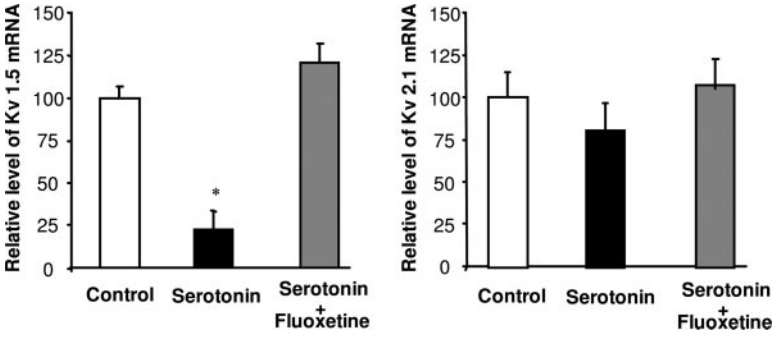

Figure 6. Effects of 5-HT (10^{-6} mol/L) on Kv1.5 and Kv2.1 mRNA levels in cultured human pulmonary artery smooth muscle cells. The response to 5-HT was also measured in the presence of 10^{-5} mol/L fluoxetine. Values are means±SEM of 6 independent experiments. *$P<0.01$ compared with control values in nonstimulated, basal condition.

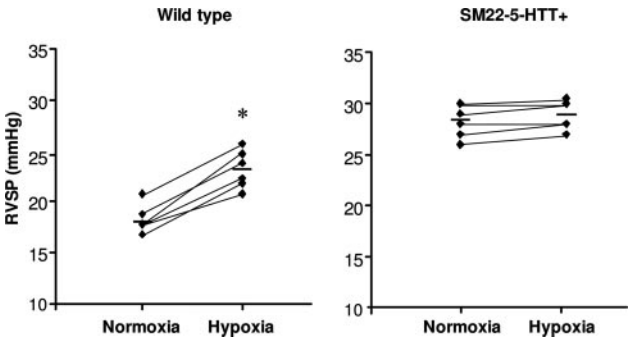

Figure 7. Individual and mean (horizontal line) RSVP in normoxic wild-type and SM22–5-HTT$^+$ mice while ventilated with room air (normoxia) and after 5 minutes of ventilation with a hypoxic gas mixture (hypoxia). Baseline RVSP during normoxic ventilation was higher in SM22–5-HTT$^+$ than in wild-type mice. RSVP increased in wild-type mice but not SM22–5-HTT$^+$ mice. *$P<0.01$ for comparison with values recorded during normoxia.

selectively in SMCs caused a 3- to 4-fold increase in lung 5-HTT protein levels. SM22–5-HTT$^+$ mice studied at 8 weeks of age developed PH, as reflected by significant increases in RVSP, Fulton index, and muscularization of distal pulmonary vessels. Increased PA-SMC proliferation appeared to be the main factor in pulmonary vascular remodeling in SM22–5-HTT$^+$ mice, as reflected by the increased PCNA labeling of pulmonary vessels. PH worsened with age, as a result of progressive pulmonary vessel remodeling. The level of increased 5-HTT expression in SM22–5-HTT$^+$ mice was very close to that found in humans with PH. 5-HTT expression in PA-SMCs from patients with iPH or aPH is usually 2- to 4-fold that of control subjects, depending on the type of PH and on the individual genotype.[5,6] The fact that SM22–5-HTT$^+$ mice spontaneously developed PH therefore indicates that vascular 5-HTT overexpression per se is sufficient to induce PA-SMC hyperplasia and subsequent development of PH.

The 5-HTT protein is known to be responsible for the internalization and storage of 5-HT in platelets and neurons. Consequently, changes in 5-HTT expression might be expected to induce major changes in circulating 5-HT levels and/or 5-HT bioavailability in the central nervous system and in peripheral tissues. Although in previous studies, transgenic mice with 5-HTT overexpression driven by the human 5-HTT gene promoter were more susceptible to hypoxic PH than wild-type mice, these transgenic mice probably had additional alterations in 5-HT bioavailibility.[11] In our study, these alterations were limited by the use of a promoter that was selective for SMCs. As a consequence, neither platelet 5-HT uptake nor blood levels of 5-HT or its metabolite 5-HIAA differed significantly between transgenic and wild-type mice. Thus, another striking finding from our study is that 5-HTT protein overexpression in SMCs led to pulmonary vascular remodeling and PH despite the absence of associated changes in indoleamine bioavailability.

It is noteworthy that in SM22-LacZ$^+$ mice the SM22 promoter was found to direct lung β-galactosidase expression chiefly in the media of pulmonary arteries, inducing only faint β-galactosidase immunostaining in bronchial SMCs. This observation is consistent with previous studies showing that the SM22 promoter drives preferential expression of transgenes in vascular smooth muscle, as opposed to visceral smooth muscle.[12] In SM22–5-HTT$^+$ mice, the 5-HTT expression increase occurred in the media of all types of vessels, including pulmonary and systemic arteries. However, vascular remodeling developed only in the pulmonary circulation of the transgenic mice, which exhibited no structural changes in the carotid arteries or differences in blood pressure compared with wild-type mice. The differential effects of 5-HTT overexpression in pulmonary versus systemic vessels are consistent with our previous findings that the mitogenic effect of 5-HT on SMCs is mediated by 5-HTT only in PA-SMCs.[8] These findings are of clinical relevance because they suggest that conditions associated with increased 5-HTT expression in a given individual might predominantly affect pulmonary vessels. SM22 is expressed in the embryonic pulmonary vasculature, raising the possibility that pulmonary vascular remodeling may have developed in SM22–5-HTT$^+$ as a result of a congenital defect in the pulmonary vasculature. However, examination of pulmonary vessels from neo-

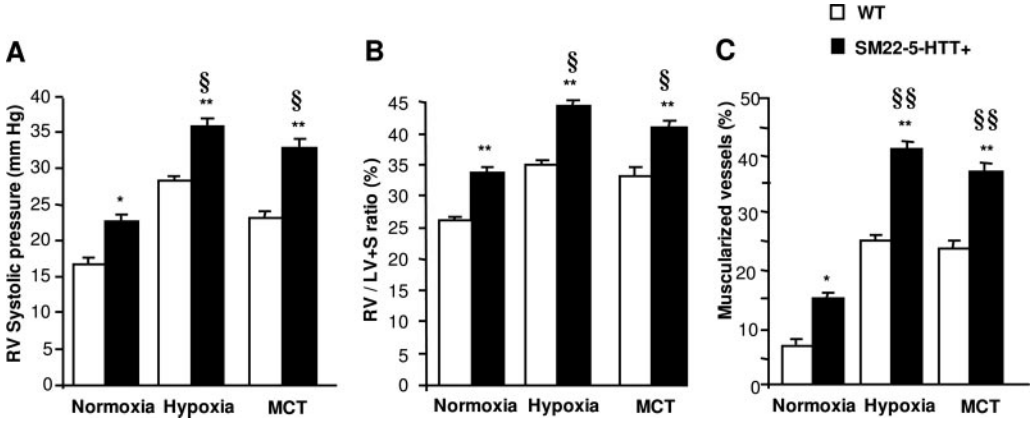

Figure 8. Development of PH in SM22–5-HTT$^+$ and wild-type mice exposed to chronic hypoxia or treated with the active monocrotaline derivative monocrotaline-pyrrole (MCT-P). RV systolic pressure (A), Fulton index (RV/LV+S) (B), and percentage of muscularized pulmonary vessels (C) in SM22–5-HTT$^+$ and wild-type mice at 8 weeks of age after a 2-week exposure to 10% O_2 or after treatment with MCT-P (5 mg/kg IV). Values are means±SEM (n=10 in each group). *$P<0.05$ and **$P<0.01$ compared with corresponding values in wild-type mice; §$P<0.05$ and §§$P<0.01$ compared with corresponding values in normoxic mice.

natal animals failed to show marked differences between SM22–5-HTT+ and wild-type mice.

Investigating SM22–5-HTT+ mice has shed light on relationships between molecular pathways known to affect pulmonary vascular remodeling. First, BMPRII has been identified as the gene causing familial PH, and BMPRII+/− mice were recently shown to be more susceptible than control mice to PH induced by inflammatory stimuli.[15–17] BMPRII+/− mice, however, do not develop PH spontaneously.[17] We investigated the BMP pathways in SM22–5-HTT+ mice by measuring lung expression of BMPRII, BMPRIa, BMPRIb, BMP-2, and BMP-4. No alterations in lung levels of mRNAs encoding these molecules were found, suggesting that the BMPRII pathway was not involved in PH in SM22–5-HTT+ mice. Second, we investigated whether endothelial cell alterations might have contributed to PH in SM22–5-HTT+ mice. A consistent feature of various types of experimental or human PH is increased lung expression of ET-1, which may be associated with downregulation of prostacyclin synthase and activation of Tie2 receptors.[18–21] In lungs from SM22–5-HTT+ mice, expression of ET-1, Tie2 receptor, and prostacyclin synthase was not significantly different from that in wild-type animals, suggesting that PH in these transgenic mice developed without a major contribution from endothelial cells. Third, key actors of the contractile and proliferative status of PA-SMCs are voltage-gated K channels (Kv) including Kv1.2, Kv1.5, Kv2.1, and Kv9.3.[14] It is now well established that blockade or downregulation of Kv channels, notably Kv1.5 and Kv2.1, causes membrane depolarization and stimulates PA-SMC proliferation.[22,23] Inhibition of 1 or several of these channels by acute hypoxia also contributes to initiate hypoxic pulmonary vasoconstriction.[22,24] Because reduced expression of these channels is a characteristic feature of human idiopathic PH and of hypoxia-induced PH,[22,25,26] 1 current hypothesis is that downregulation of Kv channels contributes importantly to the pathogenesis of various types of experimental and human PH. In our study, lung expression of both Kv1.5 and Kv2.1 channels was markedly decreased in SM22–5-HTT+ mice. Interestingly, treatment of human PA-SMCs by 5-HT in vitro induced a similar marked reduction in the expression of Kv1.5 channels, indicating that downregulation of this channel was not related to the remodeling process per se, but rather appeared to be a downstream effect of 5-HT internalization by these cells. Therefore, a direct link between 5-HTT and Kv channels might explain why these membrane-bound proteins show opposite variations in PH of various origins. The molecular link between 5-HTT and Kv channels remains to be identified, although downstream effects of 5-HTT activation may involve NADPH oxidase activity,[27] which has been shown to alter Kv channel expression and/or activity.[28,29] One may suggest that some of the cellular effects of 5-HTT overexpression in SM22–5-HTT+ mice were mediated by downregulation of Kv channels. Although such a hypothesis is beyond the scope of our study, some of the functional abnormalities in SM22–5-HTT+ mice were probably related to changes in Kv channels. Indeed, it is now well established that 1 direct consequence of Kv channel downregulation is a reduction in the extent of hypoxic pulmonary vasoconstriction.[22] Interestingly, in SM22–5-HTT+ mice, the pulmonary pressure response to hypoxia was completely abrogated, indicating that these mice are unresponsive to acute hypoxic vasoconstriction. These results are consistent with those obtained during chronic hypoxia, where increased PA-SMC proliferation coexists with blunted hypoxic vasoconstriction because PA-SMCs are in a depolarized state. Moreover, these results agree with our previous finding that hypoxic pulmonary vasoconstriction in 5-HTT knock-out mice was, on the contrary, potentiated.[4]

Although SM22–5-HTT+ mice did not develop hypoxic pulmonary vasoconstriction, they experienced PH of greater severity compared with wild-type controls after exposure to chronic hypoxia or the monocrotaline pyrrole derivative. In previous studies in both rats and mice, we found that these 2 types of PH were associated with 5-HTT overexpression and that deleting the 5-HTT gene or pharmacologically inhibiting 5-HTT activity protected against hypoxia- or monocrotaline-induced PH.[4,9] 5-HTT+ mice did not appear more sensitive to chronic hypoxia or to monocrotaline than wild-type mice, indicating that 5-HTT overexpression did not act synergistically with hypoxia or monocrotaline to cause PH. These results are consistent with the fact that 5-HTT transgene expression, in contrast to the 5-HTT gene, is unresponsive to external stimuli such as those induced by hypoxia or monocrotaline.[8,9] Our results are, therefore, consistent with the proposal that the level of 5-HTT expression is an important modifier of the PH phenotype, whatever the cause of PH. In line with this conclusion, we recently provided evidence that the severity of PH in patients with chronic obstructive lung disease is closely related to polymorphism of the 5-HTT gene promoter.[30] In particular, PH was particularly severe in patients who carried the biallelic L form of the 5-HTT gene promoter. Polymorphism of the 5-HTT gene promoter, however, cannot explain the high expression of 5-HTT in PA-SMCs from patients with either iPH or aPH. Whether increased 5-HTT expression occurs as a primary defect or is secondary to other abnormalities in human PH remains an unsolved question. In any case, our results in mice demonstrate clearly that 5-HTT overexpression is a key factor during PH development. SM22–5-HTT+ mice should now be considered a useful in vivo model for investigating how environmental and/or genetic factors modify their PH phenotype.

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