Converging Evidence in Support of the Serotonin Hypothesis of Dexfenfluramine-Induced Pulmonary Hypertension With Novel Transgenic Mice

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Background—The incidence of pulmonary arterial hypertension secondary to the use of indirect serotonergic agonists such as aminorex and dexfenfluramine led to the “serotonin hypothesis” of pulmonary arterial hypertension; however, the role of serotonin in dexfenfluramine-induced pulmonary arterial hypertension remains controversial. Here, we used novel transgenic mice lacking peripheral serotonin (deficient in tryptophan hydroxylase-1; Tph1−/− mice) or overexpressing the gene for the human serotonin transporter (SERT; SERT+ mice) to investigate this further.

Methods and Results—Dexfenfluramine administration (5 mg · kg⁻¹ · d⁻¹ PO for 28 days) increased systolic right ventricular pressure and pulmonary vascular remodeling in wild-type mice but not in Tph1−/− mice, which suggests that dexfenfluramine-induced pulmonary arterial hypertension is dependent on serotonin synthesis. Dexfenfluramine was also administered to normoxic SERT+ mice and SERT+ mice exposed to chronic hypoxia. Dexfenfluramine and SERT overexpression had additive effects in increasing pulmonary vascular remodeling; however, in hypoxic SERT+ mice, dexfenfluramine reduced both systolic right ventricular pressure and pulmonary vascular remodeling. Pulmonary arterial fibroblasts from SERT+ mice, but not wild-type mice, proliferated in response to hypoxia. Dexfenfluramine inhibited hypoxia-induced proliferation of pulmonary arterial fibroblasts derived from SERT− mice in a manner dependent on SERT activity. Dexfenfluramine also inhibited the hypoxia-mediated increase in phosphorylation of p38 mitogen-activated protein kinase in SERT+ pulmonary arterial fibroblasts.

Conclusions—The results suggest that peripheral serotonin is critical for the development of dexfenfluramine-induced pulmonary arterial hypertension and that dexfenfluramine and SERT overexpression have additive effects on pulmonary vascular remodeling. We propose that dexfenfluramine can also inhibit hypoxia-induced pulmonary vascular remodeling via SERT activity and inhibition of hypoxia-induced p38 mitogen-activated protein kinase. (Circulation. 2008;117: 2928-2937.)

Key Words: hypertension, pulmonary ■ hypoxia ■ dexfenfluramine ■ serotonin

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by both constriction and remodeling of the pulmonary arteries. The original “serotonin hypothesis of PAH” was derived from the observation that obese patients using appetite suppressants such as aminorex and dexfenfluramine were at increased risk of developing PAH.1 Dexfenfluramine is a substrate for the serotonin transporter (SERT) and can increase extracellular levels of serotonin (5-hydroxytryptamine [5-HT]).2 The serotonin hypothesis of anorexigen-induced PAH is, however, controversial. For example, dexfenfluramine has not only been shown to exacerbate PAH but also to protect against both hypoxia- and monocrotaline-induced PAH.4,5 Moreover, dexfenfluramine has direct contractile and proliferative effects on the pulmonary vasculature.6–10 An understanding of the pharmacology of dexfenfluramine is essential and important for the identification of other drugs that may also be risk factors for PAH. This will prevent a repetition of the catastrophic cases of PAH associated with the indirect serotonergic agents in the past. This is of current importance in light of recent reports on the increased abuse of amphetamine-like stimulants, which have also been associated with the development of PAH.11 To test the serotonin hypothesis of dexfenfluramine directly for the first time, we studied the effects...
of dexfenfluramine on the development of PAH in mice deficient in tryptophan hydroxylase 1 (TPH1), which is the rate-limiting enzyme responsible for the peripheral synthesis of serotonin (Tph1<sup>−/−</sup> mice).<sup>12</sup>

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**Clinical Perspective p 2937**

Only a small proportion of patients who historically had taken dexfenfluramine developed PAH,<sup>1</sup> which suggests genetic susceptibility or the involvement of additional risk factors. A genetic polymorphism in SERT that causes increased activity/expression of SERT has been linked to PAH in humans,<sup>13</sup> and mice overexpressing SERT (SERT<sup>+</sup> mice) develop elevated pulmonary pressures and are more susceptible to hypoxia-induced PAH.<sup>14</sup> Because SERT is a likely risk factor for PAH, and dexfenfluramine is a SERT substrate, we have also investigated the interactions between SERT overexpression and dexfenfluramine both in vivo and in vitro.

**Methods**

The investigation conforms with the United Kingdom Animal Procedures Act (1986) and with the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

**The Tph1<sup>−/−</sup> Mouse**

Transgenic mice (female, 2 to 3 months old) deficient in Tph1 (Tph1<sup>−/−</sup> mice) were developed by Bader and colleagues on a C57BL/6 background as described previously.<sup>12</sup>

**The SERT<sup>+</sup> Mouse**

Transgenic mice (female, 5 months old) that overexpress the gene for human SERT (SERT<sup>+</sup> mice) were developed from the C57BL/6×CBA background strain by Harmar and colleagues as described previously.<sup>15</sup>

**Exposure to Hypoxia**

SERT<sup>+</sup> mice and their wild-type (WT) controls were exposed to 14 days of hypoxia as described previously<sup>16</sup> and compared with normoxic age-matched controls.

**Dexfenfluramine Study**

Mice received dexfenfluramine (Sigma, Gillingham, United Kingdom), at a dose of 5 mg · kg<sup>−1</sup> · day<sup>−1</sup> by oral gavage for 28 days. Control groups received the vehicle, distilled H<sub>2</sub>O (dH<sub>2</sub>O). In those mice also exposed to hypoxia, dexfenfluramine was administered for 14 days before hypoxia exposure and during the 14 days of hypoxic exposure.

**Assessment of PHT**

**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.

**Lung Histology**

One sagittal section was obtained from the left lung. Sections were stained with elastica-Van Gieson stain and assessed microscopically for muscularization of small pulmonary arteries (<30 µm external diameter) as described previously.<sup>16</sup> Lung sections from 4 to 6 mice from each group were studied. Approximately 150 arteries from each lung section were assessed.

**In Vivo Hemodynamic Measurements**

Pressure and heart rate measurements were measured and analyzed as described previously.<sup>16</sup>

**Primary Culture of Mouse Pulmonary Artery Fibroblasts**

First- and second-generation pulmonary arteries were obtained from at least 4 mice per group, and pulmonary artery fibroblasts (PAFs) were cultured as described previously.<sup>17</sup> Cells expressed S100A4/Mts1 but not α-smooth muscle actin and thus were identified as fibroblasts.

**[^H]Thymidine Incorporation**

PAFs were grown to 60% confluence in 24-well plates and subjected to quiescence for 24 hours, and drugs were added as described below. PAFs were exposed to dexfenfluramine (300 nmol/L to 10 µmol/L) under normoxic or hypoxic (5% O<sub>2</sub>) conditions for 24 hours. The following inhibitors were added to PAFs, either alone or in combination with dexfenfluramine (3 µmol/L) for 24 hours: SB224289 (5-HT<sub>1B</sub> receptor antagonist; 300 nmol/L), ketanserin (5-HT<sub>2A</sub> receptor antagonist; 30 nmol/L), SB204741 (5-HT<sub>3A</sub> receptor antagonist; 300 nmol/L), citalopram (SERT inhibitor; 100 nmol/L), SB203580 (p38 mitogen-activated protein [MAP] kinase inhibitor; 5 µmol/L), and U0126 (MAP kinase/extracellular signal-regulated kinase [Erk] inhibitor; 1 µmol/L). Inhibitors were added at least 30 minutes before addition of dexfenfluramine or exposure to hypoxia.[^H]Thymidine incorporation was used as a measure of DNA replication and thus cell proliferation, as described previously.<sup>17</sup>

**Western Blot Analysis**

After treatment with dexfenfluramine (3 µmol/L) under either normoxic or hypoxic conditions, Western blot analyses were performed on PAFs from WT and SERT<sup>+</sup> mice as described previously<sup>17</sup> with the following modifications. Primary antibodies were diluted in 5% BSA in Tris-buffered saline with 0.1% Tween 20, applied overnight at 4°C.

**Myography**

All pharmacological experiments were done in third-generation, endothelium-intact, intralobar pulmonary resistance arteries (PRAs; ~200 to 300 µm internal diameter) with wire myography as described previously.<sup>16</sup> Contractile responses to serotonin (0.1 nmol/L to 100 µmol/L) were studied in PRAs removed from both vehicle- and dexfenfluramine-dosed mice. The effects of the SERT inhibitor citalopram (100 nmol/L) were also studied in PRAs from WT and SERT<sup>+</sup> mice.

**Statistical Analysis**

Data were analyzed with a 2-way ANOVA followed by Bonferroni post hoc test. Student’s t test was used to compare normoxic and hypoxic values in the Table. Data are expressed as mean±SEM. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**Effects of Dexfenfluramine on the Development of PAH in Tph1<sup>−/−</sup> Mice**

TPH catalyzes the rate-limiting step in the synthesis of serotonin from tryptophan. There are 2 isoforms of TPH. TPH1 is mainly expressed in the gut and mediates the generation of serotonin in the periphery, whereas TPH2 is present exclusively in the brain.<sup>12</sup> To determine the role of peripheral serotonin in dexfenfluramine-induced PAH in vivo, we investigated the effects of dexfenfluramine ingestion in mice genetically deficient for TPH1 (Tph1<sup>−/−</sup> mice).<sup>12</sup>
Table. pEC<sub>50</sub> and E<sub>max</sub> Values for Serotonin-Induced Vasoconstriction in WT and SERT<sup>+</sup> Mouse PRAs

<table>
<thead>
<tr>
<th></th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>E&lt;sub&gt;max&lt;/sub&gt;</th>
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<tr>
<td>SERT&lt;sup&gt;+&lt;/sup&gt; Dfen</td>
<td>5.44±0.17†</td>
<td>83.8±6.6</td>
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<td>Hypoxic</td>
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<tr>
<td>WT vehicle</td>
<td>6.84±0.13</td>
<td>130.8±9.6</td>
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<tr>
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<td>6.71±0.07**</td>
<td>103.4±5.7</td>
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pEC<sub>50</sub> indicates negative logarithm of EC50; E<sub>max</sub>, maximal effect; Dfen, dexfenfluramine; and n, number of mice.

Effects of dexfenfluramine (chronically: 5 mg · kg⁻¹ · d⁻¹) and citalopram (acutely: 100 nmol/L). Data are shown as mean±SEM.

*p<0.001 vs WT vehicle; †p<0.05 and ‡p<0.001 vs WT Dfen; §p<0.05, ‖p<0.01, ¶p<0.001 vs normoxic data; #p<0.001 vs WT; **p<0.001 vs SERT<sup>+</sup>.

Dexfenfluramine increased systolic right ventricular pressure (sRVP) and induced pulmonary vascular remodeling in WT mice but not Tph1<sup>−/−</sup> mice (Figures 1A and 1B), which indicates an important role for peripheral serotonin in dexfenfluramine-induced PAH. Dexfenfluramine had no effect on RVH in WT mice (Figure 1C). The effects of dexfenfluramine were specific to the pulmonary vasculature, because neither heart rate nor systemic blood pressure was affected by dexfenfluramine (data not shown).

**Effects of Dexfenfluramine on the Development of PAH in SERT<sup>+</sup> Mice**

To investigate any influence the overexpression of SERT may have on the effects of dexfenfluramine on the development of PAH, we studied dexfenfluramine-induced PAH in mice overexpressing SERT (SERT<sup>+</sup> mice) and WT controls. In WT mice, as described above, dexfenfluramine increased sRVP and pulmonary vascular remodeling under normoxic conditions. Dexfenfluramine had no additive effect, however, on hypoxia-induced increases in sRVP or pulmonary vascular remodeling in WT mice (Figures 2A and 2B). Normoxic SERT<sup>+</sup> mice displayed elevated sRVP, and dexfenfluramine had no additional effect on this. SERT<sup>+</sup> mice also exhibited pulmonary vascular remodeling, and the effects of dexfenfluramine on pulmonary vascular remodeling were additive to this such that the highest degree of remodeling was observed in mice overexpressing SERT and treated with dexfenfluramine. Surprisingly, therefore, dexfenfluramine reduced both sRVP and pulmonary vascular remodeling in SERT<sup>+</sup> mice exposed to hypoxia (Figures 2A and 2B). Despite dexfenfluramine-induced changes in sRVP and pulmonary vascular remodeling, dexfenfluramine did not affect RVH in any of the groups of mice (Figure 2C). The effects of dexfenfluramine were specific to the pulmonary vasculature, because systemic arterial pressure and heart rate were not affected (data not shown).

**Effects of Dexfenfluramine and Hypoxia in PAFs**

Serotonin can cause proliferation of pulmonary arterial smooth muscle cells and PAFs by entering the cell via the SERT and activating either the Erk1/Erk2 MAP kinase pathway or the p38 MAP kinase pathway. To investigate why dexfenfluramine has differential actions under normoxic and hypoxic conditions, we chose to study PAFs derived from both WT and SERT<sup>+</sup> mice. We chose PAFs over pulmonary arterial smooth muscle cells because (1) SERT is strongly overexpressed on the adventitial layer of pulmonary arteries from SERT<sup>+</sup> mice and (2) we needed to study a cell type that had a robust proliferative response to experimental hypoxia. PAF proliferation is considered to be an important contributor to the pathobiology of pulmonary hypertension. Indeed, acute hypoxia causes PAF proliferation in...
rat, human, and bovine models in vitro, and human\textsuperscript{22} and bovine\textsuperscript{21} PAFs proliferate in response to hypoxia in the absence of growth factors. In our experience, mouse pulmonary arterial smooth muscle cells do not proliferate in response to hypoxia. Hence, we studied the effects of dexfenfluramine on proliferation and activation of Erk1/Erk2 and p38 MAP kinase pathways in PAFs derived from both WT and SERT\textsuperscript{2/SERT} mice. Hypoxia increased the serum-induced proliferative response in WT PAFs but did not in itself exert a proliferative effect. Dexfenfluramine had no effect on proliferation of WT PAFs under either normoxic or hypoxic conditions (Figure 3A). Interestingly, SERT\textsuperscript{2/SERT} PAFs proliferated in response to hypoxia, and dexfenfluramine inhibited this proliferation in a concentration-dependent manner. Data are shown as mean±SEM. *P<0.001 vs serum normoxic; †P<0.001 vs control hypoxic.

We then examined the mechanism by which dexfenfluramine inhibits hypoxia-induced proliferation of SERT\textsuperscript{2/SERT} PAFs. Because dexfenfluramine is both an SERT substrate and a 5-HT\textsubscript{2} receptor agonist,\textsuperscript{23} the effects of the SERT inhibitor citalopram and the 5-HT\textsubscript{2B} receptor antagonist SB204741 on the response to dexfenfluramine were studied. However, because antagonism of the 5-HT\textsubscript{2A} receptor also inhibited hypoxic proliferation of SERT\textsuperscript{2/SERT} PAFs, this was not studied in conjunction with dexfenfluramine. The inhibitory effect of dexfenfluramine on hypoxia-induced proliferation of SERT\textsuperscript{2/SERT} PAFs was abolished in the presence of citalopram but was not affected by antagonism of the 5-HT\textsubscript{3A} receptor (Figure 5). The effects of dexfenfluramine on phosphorylation of Erk1/Erk2 and p38 MAP kinase were then determined. Hypoxia caused a profound increase in phosphorylation of p38 MAP kinase in SERT\textsuperscript{2/SERT} but not WT PAFs. Dexfenfluramine inhibited this hypoxia-induced phosphorylation of p38 MAP kinase (Figure 6A). Phosphorylation of Erk1/Erk2 MAP kinase was not}

![Figure 2](image-url)

**Figure 2.** Effects of dexfenfluramine (Dfen) ingestion and overexpression of SERT on sRVP (A), pulmonary vascular remodeling (B), and RVH (C) as assessed by RV/LV+S in both normoxic and hypoxic mice. Data are shown as mean±SEM. *P<0.05, **P<0.01, ***P<0.001 vs normoxic WT vehicle; †P<0.05, ††P<0.001 vs hypoxic WT vehicle; ‡P<0.01, ‡‡P<0.001 vs hypoxic SERT\textsuperscript{2} vehicle; and §P<0.01 vs normoxic WT dexfenfluramine, normoxic SERT\textsuperscript{2} vehicle.

![Figure 3](image-url)

**Figure 3.** Effects of dexfenfluramine (Dfen) on proliferation of PAFs from WT (A) and SERT\textsuperscript{2/SERT} (B) mice. A, Dexfenfluramine has no effect on proliferation of WT PAFs. B, PAFs from SERT\textsuperscript{2/SERT} mice proliferate in response to hypoxia, and dexfenfluramine inhibits this proliferation in a concentration-dependent manner. Data are shown as mean±SEM. *P<0.001 vs serum normoxic; †P<0.001 vs control hypoxic.
affected by hypoxia or dexfenfluramine in either WT or SERT+ PAFs (Figure 6B).

Effects of Dexfenfluramine and Hypoxia on Contractile Responses

Because we have established that dexfenfluramine induces PAH via a peripheral serotinergic mechanism, and serotonin is a pulmonary vasoconstrictor, we determined the effects of dexfenfluramine ingestion on pulmonary vascular reactivity to serotonin. Dexfenfluramine had no effect on pulmonary vascular reactivity to serotonin in PRAs from WT or SERT+/H11001 mice, under either normoxic (Figure 7A; Table) or hypoxic (Figure 7B; Table) conditions. The potency of serotonin was markedly reduced in PRAs from normoxic SERT+ mice compared with PRAs from normoxic WT mice, and this was reversed on preincubation with the SERT inhibitor citalopram (100 nmol/L). However, there was no difference in potency of serotonin in PRAs from hypoxic WT and hypoxic SERT+ mice, both of which displayed an elevated maximum response to serotonin compared with their normoxic controls (Figure 7B; Table).

Discussion

The appetite-suppressant effect of fenfluramine derivatives is thought to be dependent on increased indoleamine release, inhibition of SERT activity, and subsequent 5-HT receptor stimulation. Because neurons, platelets, and pulmonary endothelial and smooth muscle cells share the same SERT encoded by a single gene, one school of thought is that it is this action of dexfenfluramine that promotes PAH. Indeed, increased serotonin plasma levels have been observed during treatment with fenfluramine derivatives. In addition, the dexfenfluramine metabolite nordexfenfluramine is an agonist of the 5-HT2A, 5-HT2B, and 5-HT2C receptors. However, dexfenfluramine has also been reported to have nonserotinergic and direct effects on pulmonary arteries, including inhibition of potassium channels, increased intracellular calcium, vasoconstriction (albeit with very low potency), and mitogenic effects.

Here, we investigated the role of peripheral serotonin in dexfenfluramine-induced PAH directly by studying the effects of dexfenfluramine in Tph1−/− mice. We show that dexfenfluramine induced PAH in WT mice but not in Tph1−/− mice. Hence, the present study is the first to definitively show that dexfenfluramine mediates the development of PAH via a peripheral serotinergic mechanism rather than via nonserotinergic effects. This provides further evidence that peripheral serotonin plays a causative role in the development of PAH. Indeed, we recently showed that hypoxia-induced PAH is severely ablated in Tph1−/− mice, which indicates that de novo synthesis of peripheral serotonin is essential for the development of hypoxia-induced PAH. Moreover, in rodents, serotonin infusion can potentiate the development of hypoxia-induced PAH, and inhibition of serotonin receptors or the SERT has been shown to inhibit PAH secondary to hypoxia and monocrotaline-injection.

With respect to the mechanism by which serotonin may influence the development of PAH, it promotes pulmonary artery vasoconstriction, local microthrombosis, and proliferation of pulmonary arterial smooth muscle cells and...
Serotonin-induced proliferation of pulmonary vascular cells has been shown to be mediated via SERT, and a genetic polymorphism that causes increased activity/expression of SERT has been linked to PAH. The SERT polymorphism is associated with exaggerated PAH in patients with chronic obstructive lung disease and with an increased risk of developing PAH at high altitudes. Although distribution of the SERT polymorphism does not differ between patient and control groups, PAH patients with the SERT polymorphism may present at an earlier age than those without it. Because dexfenfluramine is a SERT substrate, we were interested in investigating whether overexpression of SERT predisposed the body to dexfenfluramine-induced PAH.

Figure 6. PAFs from both WT and SERT+ mice were treated with or without 3 μmol/L dexfenfluramine (Dfen) and exposed to normoxia (N) or hypoxia (H) for 24 hours before preparation of soluble cell extracts. Samples were then fractionated by SDS-PAGE for immunoblotting with the indicated antibodies. Quantitation of data from multiple experiments is also shown. A, Hypoxic exposure induces an increase in phosphorylated (phospho) p38 MAP kinase in PAFs from SERT+ mice but not WT mice. This is abolished in the presence of 3 μmol/L dexfenfluramine. B, Phosphorylation of Erk1/Erk2 MAP kinase was not affected by hypoxia or dexfenfluramine in either WT or SERT+ PAFs. Quantitative data are shown as mean±SEM. *P<0.01 vs hypoxic control. Fold Inc. indicates fold increase.
We showed that under normoxic conditions, the degree of pulmonary vascular remodeling is highest in SERT mice exposed to dexfenfluramine, because of the additive effect of SERT overexpression and dexfenfluramine. This is not surprising, because dexfenfluramine acts through SERT to mediate release of serotonin, and serotonin promotes proliferation of PAFs and pulmonary arterial smooth muscle cells through an SERT-mediated mechanism. It may be expected, however, that the dexfenfluramine-induced remodeling observed in SERT mice would have been accompanied by a commensurate elevation in right ventricular pressure (RVP); however, the RVP in these mice is already elevated, probably to the maximum extent. Indeed, despite a vast increase in pulmonary vascular remodeling, SERT mice do not display increased RVP in response to hypoxia. Certainly, the present study group and others have rarely observed increases in sRVP in mice beyond 50 mm Hg.

Under hypoxic conditions, dexfenfluramine reduced sRVP and pulmonary vascular remodeling in SERT mice. Protective effects of dexfenfluramine against both hypoxia- and monocrotaline-induced PAH have been reported by others, but no mechanism has been proposed. We investigated this further by examining the effects of hypoxia and dexfenfluramine on proliferation of PAFs isolated from SERT and WT mice. The first interesting observation was that hypoxia induced proliferation, but only in cells derived from SERT mice. This process was dependent on the 5-HT2A receptor and the p38 MAP kinase pathway and may explain the exaggerated hypoxia-induced pulmonary vascular remodeling observed in SERT mice. SERT and the 5-HT2A receptor have previously been shown to coregulate hypoxia-induced proliferation of rat PAFs. The p38 MAP kinase pathway has been associated with hypoxia-induced proliferation of PAFs from various species, including humans. Moreover, p38 MAP kinase can regulate SERT activity. Basal SERT phosphorylation is sensitive to p38 MAP kinase inhibitors, and serotonin uptake is reduced by both p38 MAP kinase inhibitors and small interfering RNA suppression of p38 MAP kinase in rat brain synaptosomes and HEK-293 cells. Further, p38 MAP kinase stimulation has been shown to increase serotonin uptake. Because p38 MAP kinase and SERT interact, increased activation/expression of SERT may lead to increased hypoxia-induced phosphorylation of p38 MAP kinase, consistent with our observations. Moreover, hypoxia-induced activation of p38 MAP kinase may further influence SERT activity as described above.

Consistent with our in vivo results, dexfenfluramine demonstrated a protective effect by inhibiting hypoxia-induced proliferation in SERT PAFs. The inhibitory action of dexfenfluramine on hypoxia-induced proliferation of SERT PAFs was mediated via uptake by SERT, because it could be blocked by the SERT inhibitor citalopram. Furthermore, dexfenfluramine inhibited hypoxia-induced phosphorylation of p38 MAP kinase in SERT PAFs. Multiple upstream mechanisms (eg, stress factors, cytokines, MAP kinase kinase kinases, and MAP kinase kinases) and downstream mechanisms (eg, MAP kinase phosphatases), as well as scaffolding protein activities, act cooperatively to regulate the activity of...
p38 MAP kinase. Hypoxia and dexfenfluramine could interact at any one of these multiple “checkpoints” to increase or attenuate the phosphorylation of p38 MAP kinase. Because activation of p38 MAP kinase is essential for hypoxia-induced proliferation of SERT+ PAFs, inhibition of this protein may provide the mechanism by which dexfenfluramine protects against hypoxia-induced proliferation of SERT+ PAFs. Moreover, because p38 MAP kinase has also been linked to vascular proliferation associated with monocrotaline-induced PAH, and SERT is overexpressed in pulmonary arteries from monocrotaline-treated rats, the results of the present study provide a potential mechanism for the protective effects of dexfenfluramine on PAH secondary to monocrotaline injection.

Under normoxic conditions, serotonin has been shown to signal through the GTPase-coupled protein Rac-1, inducing sequential activations of NADPH oxidase, reactive oxygen species, and Erk1/Erk2 MAP kinase. However, there is evidence that proliferation is induced via differential pathways under normoxic and hypoxic conditions. For example, serotonin can stimulate activation of p38 MAP kinase in PAFs from chronic hypoxic but not normoxic rats. Moreover, bovine PAFs proliferate to hypoxia via a different mechanism than normoxic serum-induced proliferation. This is entirely consistent with the results we show here in that dexfenfluramine promoted PAH under normoxic conditions but inhibited hypoxia-induced activation of p38 MAP kinase and thereby protected against hypoxia-induced PAH in the SERT+ mice.

Because dexfenfluramine has been shown to influence vascular tone in the pulmonary circulation, we examined the effect of dexfenfluramine on serotonin-induced contraction in PRAs taken from both normoxic and hypoxic mice. Chronic dexfenfluramine administration had no effect on serotonin-induced vasoconstriction in PRAs. This observation suggests that increased contractile activity in response to serotonin did not play a role in the dexfenfluramine-induced increase in RVP. Instead, increased RVP in response to dexfenfluramine is likely due to dexfenfluramine-induced pulmonary vascular remodeling. Interestingly, we actually observed a decrease in the potency of serotonin in the SERT+ mouse PRAs. Because this was reversed by the SERT inhibitor citalopram, it was likely due to increased SERT activity removing serotonin from its receptor sites. Consistent with this hypothesis, we have previously shown that SERT inhibitors can potentiate responses to serotonin in fawn-hooded rat pulmonary arteries, in which there is also overexpression of SERT.

Despite having vastly increased RVP in response to dexfenfluramine, WT mice did not develop RVH. We have previously reported (and confirmed in the present study) that SERT+ mice show elevated RVP in the absence of RVH. On the other hand, Tph1−/− mice are protected against hypoxia-induced increases in RVP but still develop RVH. This suggests that in mice, hypoxia can directly induce RVH, and this is not dependent on peripheral serotonin or an increase in RVP. Because Tph1−/− mice exhibited slight RVH even before hypoxic exposure, serotonin may normally protect against RVH. Serotonin may protect murine ventricular cardiomyocytes against serum deprivation–induced apoptosis, which suggests a role for serotonin as a survival factor of cardiomyocytes. The role of apoptosis in nonproliferative cells such as myocytes is controversial, although compelling evidence exists that terminally differentiated cardiomyocytes can undergo apoptosis. Apoptosis of cardiac myocytes is considered a feature of both ischemic and nonischemic cardiomyopathies and ventricular remodeling. If serotonin does play a dual role, inducing elevated pulmonary pressures and pulmonary vascular remodeling while protecting against right ventricular hypertrophy, this may well explain the dissociation of RVH from RVP that we see in mice in which the serotonin system is altered.

In conclusion, we have confirmed for the first time, in vivo, that the mechanism by which dexfenfluramine mediates PAH is dependent on peripheral serotonin synthesis. Increased pulmonary vascular remodeling secondary to increased activity/expression of SERT is additive to dexfenfluramine-induced remodeling in vivo, and it is possible that SERT overexpression may be a risk factor for dexfenfluramine-induced PAH. We also propose that dexfenfluramine can have protective effects on pulmonary vascular remodeling via inhibition of p38 MAP kinase. These results have wider implications, because other SERT substrates, such as methamphetamine and amphetamine, may also be linked to cases of PAH.

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Disclosures

None.

References


The serotonin hypothesis of pulmonary arterial hypertension (PAH) arose in the 1960s because of the incidence of PAH associated with the intake of the anorexigenic drug aminorex, which was withdrawn from the market. Dexfenfluramine was used as an anorexigen in the 1980s. Like aminorex, it is a serotonin transporter substrate and indirect serotonin agonist. It also was subsequently associated with an increased risk of developing PAH. An understanding of the pharmacology of dexfenfluramine is essential and important to identify other drugs that may also be risk factors for PAH. There has been much controversy, however, about the mechanism by which dexfenfluramine mediated PAH. It has both serotonergic and nonserotonergic effects and has been reported to both exacerbate and protect against PAH in hypoxic animal models of the disease. Here, we show for the first time that dexfenfluramine-induced PAH is dependent on peripheral serotonin synthesis rather than its nonserotonergic effects. We also report that overexpression of the serotonin transporter may be a risk factor for dexfenfluramine-induced PAH. Intriguingly, we report that dexfenfluramine can also inhibit hypoxia-induced phosphorylation of p38 mitogen-activated protein kinase, thus providing a mechanism by which dexfenfluramine can protect against hypoxia-induced PAH in animal models. These results have wider implications in light of recent reports on the increased abuse of other serotonin transporter substrates and indirect serotonin agonists such as methamphetamine and amphetamine, which may also be risk factors for the development of PAH.
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