Evidence for a Control of Plasma Serotonin Levels by 5-Hydroxytryptamine$_{2B}$ Receptors in Mice$^S$


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ABSTRACT

A correlation between high plasma serotonin levels and total pulmonary resistance was reported in more than 80% of pulmonary hypertensive patients. When submitted to chronic hypoxia (10% O$_2$ for more than 3 weeks), wild-type mice develop lung vascular remodeling and pulmonary hypertension. We previously reported that, in contrast, the development of these hypoxia-dependent alterations is totally abolished in mice with genetically inactive 5-HT$_{2B}$ receptors, plasma serotonin levels were not modified by chronic hypoxia. We then confirmed that 5-HT$_{2B}$ receptors can control plasma serotonin levels by providing in vivo evidence that an acute agonist stimulation of 5-HT$_{2B}$ receptor triggers a transient increase in plasma serotonin that is serotonin transporter dependent and blocked by 5-HT$_{2B}$ receptor-selective antagonist or genetic ablation. Our data support the notion that a 5-HT$_{2B}$ receptor-dependent regulation of serotonin uptake is implicated in the control of plasma serotonin levels.

Primary pulmonary hypertension is a rare but fatal condition characterized by an elevation in pulmonary arterial pressure that is associated with pulmonary vasculature remodeling (Loscalzo, 2001). Several studies have suggested a role for serotonin (5-hydroxytryptamine (5-HT)) in pulmonary hypertension (for review, see Egermayer et al., 1999). A correlation between high plasma serotonin levels and total pulmonary resistance, first observed in a patient with a defect in platelet serotonin storage capacity (Hervé et al., 1990), was confirmed in more than 80% of pulmonary hypertensive patients (Hosoda, 1994; Hervé et al., 1995; Kérevue et al., 2000). In rats, roles for serotonin in the initiation and progression of monocrotaline-induced pulmonary hypertension have been suggested since plasma serotonin levels are raised, and pulmonary arteries show hyper-reactivity to serotonin. In these experiments, treatments with 5-HT$_R$ antagonists...
inhibited the development of pulmonary hypertension along with a decrease in the plasma serotonin concentrations and the number of proliferative cells, substantiating the pivotal role of plasma serotonin in the development of monocrotaline- or interleukin-6-induced pulmonary hypertension (Miyata et al., 2001).

The main peripheral sources of serotonin are as a neurotransmitter and local hormone in the gastrointestinal tract enterochromaffin cells and pulmonary neuroepithelial bodies. Blood serotonin, which is concentrated in the platelet-dense granules (>99%), is almost absent from plasma (nanomolar range) (Da Prada and Picotti, 1979). Defective platelet uptake (Awabdy et al., 2003) or release from activated platelets could generate increased plasma serotonin as in platelet storage diseases (Hervé et al., 1990). Furthermore, lungs have been reported to remove efficiently “free” serotonin from plasma (Gillis and Pitt, 1982). High levels of plasma serotonin could, thus, result from an impaired endothelial metabolism that would decrease 5-hydroxyindole-acetic acid (5-HIAA) levels and/or serotonin uptake. As well as being a pulmonary vasoconstrictor, plasma serotonin may exert a comitogenic influence on pulmonary vascular smooth muscle cells (Liu and Fanburg, 2005) and contribute to both hypoxia-induced acute vasoconstriction and chronic vascular remodeling through various serotonin receptors (5-HTRs) and the serotonin transporter (SERT).

When exposed to a few weeks of hypoxia (10% O2), wild-type mice develop pulmonary hypertension and lung vascular remodeling recapitulating at least in part the human pathology. Smooth muscle and endothelial cells from human and rodent pulmonary arteries express mRNAs for 5-HT1B, 5-HT2A, 5-HT7 and 5-HT2BRs and SERT (Ullmer et al., 2005). However, the link between 5-HT2BRs and pulmonary hypertension pathophysiology remains to be discovered. Since a correlation between plasma serotonin levels and total pulmonary resistance was consistently observed in pulmonary hypertensive patients, we asked whether serotonin receptors could participate in the control of plasma serotonin levels. In the present study, we are reporting that 5-HT2BRs are involved in a carrier-dependent control of plasma serotonin levels in mice.

Materials and Methods

Reagents. LY-266097 and RS-127445 were kindly provided by Eli Lilly & Co. (Indianapolis, IN). BW-723C86 and all other chemicals were reagent grade, purchased from usual commercial sources. The radioactive compound [3H]LY-266097 (925 GBq/mmol) was provided by Dr. J. Würch (Roche, Basel, Switzerland), whereas [3H]serotonin binoxalate (745 GBq/mmol) and [3H]NADH (2.28 TBq/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA).

Hypoxia. The 5-HT2BR mutant mice (∼/) were generated in the 129PAS pure genetic background that was used as controls (∼/−) (Nebigil et al., 2000). Mice 6 weeks of age (20–25 g) were randomly divided into groups of 50% male and 50% female mice, maintained either in room air (21% O2) or in normobaric chambers (21–23°C) with hypoxic air (10% O2, 90% N2, 2 or 5 weeks; Launay et al., 2002). Normoxic mice were kept in the same room with the same 12/12-h light/dark cycle. Classically, groups of 10 mice were treated with a vehicle, a highly selective 5-HT2BR (full neutral) antagonist RS-127445 at 1 mg/kg/day or dexfenfluramine at the “therapeutic” dose of 2.5 mg/kg/day delivered by miniosmotic pumps (ALZET; DURECT Corporation, Cupertino, CA) from the beginning of the hypoxic treatments. All animal care and procedures were in accordance with institutional guidelines and European regulations.

Cardiovascular Evaluations. Mice were anesthetized with i.p. injection of ketamine hydrochloride (60 mg/kg) and xylazine (8 mg/kg). Right ventricular systolic pressure (RVSP) was measured by insertion into the right ventricle of a 26-gauge needle connected to a pressure transducer. After a blood sample was collected by cardiac puncture for measurements, the pulmonary artery was cannulated through an incision in the right ventricle and perfused with Earle’s balanced salt solution (37°C, 20 cm of H2O pressure).

Lung Parameters. Lung vascular bed around pulmonary arteries was collected and prepared for culture (Launay et al., 2002). The culture was maintained in Dulbecco’s modified Eagle’s medium supplemented with serotonin-depleted serum (10%) for 24 h, washed with Hanks’ balanced salt solution, and grown for 24 h in serum-free medium [Dulbecco’s modified Eagle’s medium/F-12 (1:1) with 5 μg/ml insulin, 5 μg/ml transferrin, 30 nM selenium, 20 nM progesterone, and 100 μM putrescine] before serotonin uptake was performed.

To measure serotonin uptake, after incubation for 1 min with 25, 50, and 100 nM [3H]serotonin binoxalate, cells were lysed by addition of 0.1 N NaOH and radioactivity was counted using liquid scintillation spectrometry. Specific serotonin uptake was assessed as the difference between total uptake and uptake in the presence of 1 μM paroxetine. Due to a lack of materials, only three concentrations of [3H]serotonin were used, leading only to an estimate of the initial rates (1 min uptake) of specific serotonin uptake versus serotonin concentration. After a nonlinear regression analysis according to a hyperbolic model (EZ-FIT program; Department of Medical Products, du Pont de Nemours, Glendon, PA), we determined the Michaelis-Menten constant (Km) and initial rate (Vmax) of serotonin uptake into lung vascular cells.

Dosage of the 5-HT2BR expression was performed by binding experiments using a selective tritiated radioligand (LY-266097). Briefly, cell membranes were prepared by four cycles of homogenization (Brinkmann P10 disruptor; Brinkmann Instruments, Westbury, NY) and centrifugation (48,000g for 15 min). The assay was...
established to achieve steady-state conditions and to optimize specific binding (Kellermann et al., 1996). Fifty micrograms of membrane proteins was incubated with 1 nM [3H]LY-266097 at 4°C for 60 min. Nonspecific binding was determined 1 μM RS-127445. Assays were terminated by vacuum filtration through glass fiber filters (GF/B), which had been pretreated with 0.1% polyethyleneimine. Total and bound radioactivity was determined by liquid scintillation counting. Greater than 80% specific binding was achieved in these assays.

For elastolytic activities, conditioned media (380 μl) were incubated at 37°C with 200 μg [3H]elastin produced by radiolabeling of purified insoluble elastin from bovine nuchal ligament (Elastin Products Company, Inc., Owensville, MO) with [3H]NaBH₄ as previously described (Takahashi et al., 1973). After 24 h, the radioactivity in 250 μl of the supernatant was determined by liquid scintillation spectrometry as a measure of elastolysis. To control for nonenzymatic degradation, radiolabeled elastin was incubated with medium from tissue-free cultures. Elastase activity was related to a standard curve generated with human leukocyte elastase (0.075 to 5.0 ng) (Elastin Products Company, Inc.).

**Blood Parameters.** Plasma 3,4-dihydroxyphenylglycol (DHPG) and 5-HIAA levels were measured by high-performance liquid chromatography, and serotonin in both plasma and whole blood was measured by radioenzymology (Berlin et al., 1995; Kerever et al., 2000).

**Acute Injections.** Acute i.p. injections of the preferential 5-HT₂BR agonist BW-723C86 (10 mg/kg), selective 5-HT₂BR antagonist RS-127445 (1 mg/kg), dexfenfluramine (10 mg/kg), or nordexfenfluramine (10 mg/kg) were performed on 5-HT₂BR+/− and −/− mice before blood was collected by cardiac puncture for measurements over a 30-min time period. Pretreatment with paroxetine was performed by i.p. injection twice a day of the selective SERT blocker paroxetine (1 mg/kg) over 2 days, reducing platelet serotonin uptake by more than 90%.

**Statistics.** The reported data represent the mean of individual values ± S.E.M. (n = number of individuals at the end of treatments as indicated in the text). Comparisons were performed using the nonparametric Kruskal-Wallis test. Significance was set at P < 0.0001. This level of significance also applies to correlation between individual values, assessed by Kendall rank coefficients. For the group comparisons, statistical comparisons were made by one-way analysis of variance. Difference between groups was established using the Bonferroni-Dunn multiple comparison tests (P < 0.0001).

**Results**

**The Hypoxia-Dependent Increase in Plasma Serotonin Levels Is 5-HT₂BR-Controlled.** We previously reported that 5 weeks of hypoxia increased RVSP, media wall thickness, pulmonary elastase activity, expression of 5-HT₂BRs, and thymidine incorporation in lungs of wild-type but not of 5-HT₂BR−/− or 5-HT₂BR-antagonist, RS-127445-treated −/− mice (Launay et al., 2002). A progressive increase in RVSP was detected in −/− mice treated from 2 to 5 weeks under hypoxia (10% O₂) (Fig. 1A). We tested serotonin levels in blood samples of the hypoxia-treated mice after acute or 2 or 5 weeks of hypoxia. Strikingly, as the expression of 5-HT₂BRs in lungs (Fig. 1B), plasma serotonin levels (Fig. 1C) were significantly increased in −/− mice after exposure to 2 weeks of chronic hypoxia, before significant changes in remodeling factors could be evidenced (e.g., elastase activity: Fig. 1D) but not in hypoxic 5-HT₂BR−/− mice. No change in whole-blood serotonin could be evidenced.

This increase in plasma serotonin levels indeed persisted after 5 weeks of hypoxia when the pulmonary hypertensive pathology was fully established (Fig. 2, A–C) but not in...
hypoxic 5-HT$_{2B}$R$^{-/-}$ or 5-HT$_{2B}$R-antagonist, RS-127445-treated $^{+/+}$ mice. Furthermore, the increase in plasma serotonin levels was potentiated in the presence of dexfenfluramine in $^{+/+}$ mice but not in hypoxic 5-HT$_{2B}$R$^{-/-}$ or RS-127445-treated $^{+/+}$ mice (Fig. 2C). No significant reduction of whole-blood serotonin could be observed in hypoxic $^{-/-}$ and $^{+/+}$ mice. In contrast and as expected, in hypoxic and dexfenfluramine-exposed $^{-/-}$ and $^{+/+}$ mice, a significant reduction of whole-blood serotonin was observed, thus independently of 5-HT$_{2B}$Rs (Fig. 2B). Following the increase in plasma serotonin levels, the serotonin main catabolite 5-HIAA was also increased in chronic hypoxia dexfenfluramine-treated $^{+/+}$ mice (Fig. 2D). Although not reaching statistical significance ($P > 0.05$), a trend toward a decreased serotonin degradation as expressed by an increased ratio between plasma levels of serotonin and 5-HIAA was observed in both hypoxic 5-HT$_{2B}$R$^{-/-}$ and $^{+/+}$ mice. These first observations suggest a role of 5-HT$_{2B}$Rs in plasma serotonin regulation that precedes other pulmonary hypertension parameters.

The Hypoxia-Dependent Increase in Plasma Serotonin Levels Is Correlated with RVSP and 5-HT$_{2B}$R Expression. When comparing the plasma serotonin levels and the others parameters affected by hypoxia, we observed significant ($P < 0.0001$) correlations between all the individual plasma serotonin values and RVSP values (Fig. 3A), lung 5-HT$_{2B}$R expression site numbers (Fig. 3B), or plasma 5-HIAA values (Fig. 3C). Moreover, the ratios of serotonin/5-HIAA were also significantly correlated with plasma DHPG values, confirming that these ratios reflect monoamine oxidase A (MAOA) activity (Fig. 3E). However, the correlation was not significant between individual plasma and whole-blood serotonin levels (Fig. 3D). These results further substantiate the evidence that 5-HT$_{2B}$Rs are required for the regulation of plasma serotonin levels, independently of platelets, which store most of the whole-blood serotonin.

5-HT$_{2B}$Rs Affect Lung Serotonin Uptake Activity. We determined the parameters of serotonin uptake activity in mouse lung vascular cultures after different treatments. Significant variations in the lung uptake activity were detected that were entirely depending on changes in estimated SERT initial rate of uptake ($V_i$). Chronic hypoxia decreased the lung serotonin uptake activity in $^{+/+}$ mice (Table 1) but not its $K_m$ (reflecting SERT affinity for serotonin). As expected, chronic hypoxia in the presence of dexfenfluramine nearly completely blocked the lung serotonin uptake activity in both $^{+/+}$ and 5-HT$_{2B}$R$^{-/-}$ mice (Table 1). A significant increase (27%) in basal normoxic serotonin uptake $V_i$ was observed in 5-HT$_{2B}$R$^{-/-}$ compared with $^{+/+}$ mice, with no effect of hypoxia (Table 1). No change in expression levels of SERT could be detected in any genotype or treatment (189 ± 5 fmol/mg protein) as assessed by paroxetine binding experiments (not illustrated). These results show that 5-HT$_{2B}$Rs are required to regulate hypoxic and basal normoxic lung serotonin uptake activity.

Direct in Vivo Activation of 5-HT$_{2B}$Rs Results in an Increase of Plasma Serotonin Levels. To test whether
normoxic mice respond to activation of 5-HT<sub>2B</sub>Rs, we acutely injected 5-HT<sub>2B</sub>R agonists and assessed the serotonin plasma levels. Over a 30-min time period, plasma levels were extremely stable after vehicle injection in mice. A significant increase in plasma serotonin levels over this control value was observed at 10 min after i.p. injection of the 5-HT<sub>2B</sub>R preferential agonist BW-723C86 (10 mg/kg) (76 ± 12% over basal, n = 6) in +/- mice (Fig. 4A). In contrast, in 5-HT<sub>2B</sub>R<sup>-/-</sup> mice (Fig. 4B) or in 5-HT<sub>2B</sub>R antagonist, RS-127445-treated (1 mg/kg, n = 4) +/- mice (Fig. 4C), this increase could not be detected. Interestingly, acute i.p. injection of nordexfenfluramine (10 mg/kg, n = 6) produced a significant increase (P < 0.0001, star, n = 6) in plasma serotonin levels over control values was observed at 10 min after BW-723C86 (10 mg/kg) (+/-) and nordexfenfluramine (10 mg/kg) (+/-) i.p. injection in +/- mice (A), which is not detected in 5-HT<sub>2B</sub>R<sup>-/-</sup> mice (B) or in RS-127445-treated +/- mice (C). However, acute dexfenfluramine i.p. injection (10 mg/kg) produced a significant increase (P < 0.0001, star, n = 6) in plasma serotonin in +/- (DF) mice at 5 min.

Fig. 3. Correlations between individual values of plasma serotonin levels with other parameters involved in pulmonary hypertension; highly significant correlations (P < 0.0001, star) are observed among all wild-type individual values at 5 weeks for plasma serotonin levels and RVSP (A) (n = 58), the number of 5-HT<sub>2B</sub>R-specific binding sites (B) (n = 54), and plasma 5-HIAA levels (C) (n = 58). However, the correlation was not significant between individual plasma serotonin levels and whole-blood serotonin contents (D) (n = 58). Furthermore, the serotonin/5-HIAA plasma ratios were also significantly correlated with plasma DHPG values, confirming they reflect MAOA activity (E) (n = 22). r, rank of correlation; open circles, normoxia; gray circles, hypoxia; open squares, hypoxia with dexfenfluramine; black squares, hypoxia with BW-723C86.

TABLE 1
Parameters of lung serotonin uptake

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<th>Parameters of serotonin uptake were evaluated in lung explants from normoxic +/- mice (VE), hypoxic +/- mice (H5W), hypoxic +/- mice in the presence of dexfenfluramine (DFH5W), hypoxic +/- mice in the presence of 5-HT&lt;sub&gt;2B&lt;/sub&gt;R-specific antagonist RS-127445 (RS4H5W), normoxic +/- mice (VEKO), hypoxic +/- mice (H5WK0), hypoxic +/- mice in the presence of dexfenfluramine (DFH5WK0), or hypoxic +/- mice in the presence of RS-127445 (RS4H5WK0).</th>
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* Statistical difference from normoxic untreated control values (P < 0.0001).
† Statistical difference from chronic hypoxia values (P < 0.0001).
mice. The dexfenfluramine effect was clearly distinct from that of nordexfenfluramine since the nordexfenfluramine as the BW-723C86 effects on peripheral serotonin release required 5-HT\textsubscript{2B}Rs. No variation in whole-blood serotonin could be detected in this time window in either genotype after similar injections (not illustrated). Activation of 5-HT\textsubscript{2B}Rs can thus increase plasma serotonin levels.

The in Vivo 5-HT\textsubscript{2B}R-Dependent Increase in Plasma Serotonin Levels Is SERT-Dependent. We tested the effect of SERT inhibition using a pretreatment by the highly selective SERT blocker paroxetine or SERT-/-- on dexfenfluramine, BW-723C86, or nordexfenfluramine in vivo acute i.p. injection. Strikingly, no change in plasma serotonin levels could be detected after acute injection of nordexfenfluramine or BW-723C86 when the mice were pretreated by paroxetine (1 mg/kg, n = 6) twice a day for 2 days in \textsuperscript{+/+}, 5-HT\textsubscript{2B}R-/--, and SERT-/-- mice (not illustrated). In contrast, a lower but significant increase in plasma serotonin levels over control values (VEP) is observed at 5 min after acute injection of nordexfenfluramine or BW-723C86 (Fig. 5A) and in 5-HT\textsubscript{2B}R-/-- mice after paroxetine treatment (64 ± 8% of plasma level without paroxetine) (Fig. 5B). No variation in total blood serotonin could be detected in this time window in either genotype after similar injections (not illustrated). The BW-723C86- and nordexfenfluramine-dependent increases in plasma serotonin levels appear entirely 5-HT\textsubscript{2B}R- and SERT-dependent, whereas that of dexfenfluramine appears 5-HT\textsubscript{2B}R-independent and only partially SERT-dependent.

Discussion

We are presenting here the first evidence that 5-HT\textsubscript{2B}Rs are required to control the plasma serotonin levels in mice. In chronic \textsuperscript{+/+} hypoxic mice, the plasma serotonin levels parallel lung expression of 5-HT\textsubscript{2B}R and are significantly increased before any significant vascular remodeling could be evidenced and potentiated by dexfenfluramine. In mice with either permanent (5-HT\textsubscript{2B}R homozygous mutation) or transient (5-HT\textsubscript{2B}R selective antagonist) inactivation of 5-HT\textsubscript{2B}Rs, plasma serotonin levels are not modified by chronic hypoxia. We also observed that the values for plasma serotonin levels and 5-HT\textsubscript{2B}Rs expression are significantly correlated. In addition, we show that acute injection of BW-723C86, a preferential 5-HT\textsubscript{2B}R agonist, triggers a rapid increase in plasma serotonin levels, which is entirely SERT- and 5-HT\textsubscript{2B}R-dependent as shown by genetic and pharmacologic ablation of either protein and mimicked by nordexfenfluramine but not dexfenfluramine. These data demonstrate the participation of both 5-HT\textsubscript{2B}R and SERT in the control of plasma serotonin levels in vivo.

An interference of the 5-HT\textsubscript{2B}R-/-- mice congenital defects has been ruled out since treatment of \textsuperscript{+/+} mice by RS-127445, the most selective 5-HT\textsubscript{2B}R antagonist, reproduces the results obtained with 5-HT\textsubscript{2B}R-/-- mice. The serotonergic anorectic agent and amphetamine derivative, dexfenfluramine, increases the risk of developing pulmonary hypertension (Rich et al., 2000). In vitro, dexfenfluramine and its main metabolite nordexfenfluramine have been shown to inhibit serotonin reuptake and to stimulate its release from brain synaptosomes (Mennini et al., 1981). Moreover, both compounds bind to 5-HT\textsubscript{Rs with appreciable affinity. In fact, dexfenfluramine binds weakly to rodent and human 5-HT\textsubscript{2A/B/C}Rs, but nordexfenfluramine behaves as a high-affinity agonist for 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C}Rs and more moderately for 5-HT\textsubscript{2A}Rs (Porter et al., 1999; Fitzgerald et al., 2000; Rothman and Baumann, 2002; Setola et al., 2003; Supplementary table). In mice, chronic exposure to dexfenfluramine 2.5 mg/kg/day leads to complete conversion into nordexfenfluramine with a final plasma concentration of about 500 nM (Caccia et al., 1985; Launay et al., 2002), fully stimulating 5-HT\textsubscript{2B}Rs but not 5-HT\textsubscript{2A}Rs (Supplementary table), 5-HT\textsubscript{2C}Rs being below detection level in the periphery. Chronic effects of dexfenfluramine therefore appeared nordexfenfluramine-mediated.

The mechanisms leading to hypoxia-induced plasma serotonin elevation might be a consequence of platelet defects or of a slower serotonin uptake by platelets due to a kinetic change in SERT activity (Awabdy et al., 2003). We did not observe any significant variation in whole-blood serotonin content (>99% of which is represented by platelets) in hypoxic mice (Fig. 2B), thus eliminating major changes in serotonin synthesis, platelet number, or their serotonin uptake capacity. Furthermore, the absence of correlation between individual values of plasma and blood serotonin (Fig. 3D) indicates that mechanisms controlling serotonin platelet up-
take and plasma levels are not coordinately regulated by hypoxia. Moreover, mature platelets express 5-HT_{2AR}S but not 5-HT_{2BR}S, and ketanserin, a selective 5-HT_{2AR} antagonist, does not significantly change pulmonary artery pressure in hypoxic mice (Marcos et al., 2003). Recently, platelets serotonin uptake inhibition by both dexfenfluramine and nordexfenfluramine (IC_{50}, dexfenfluramine approximately 3 μM; nordexfenfluramine approximately 10 μM) has been reported, but no serotonin efflux occurs at these concentrations (Johnson et al., 2003). Together with our chronic in vivo results, these findings indicate that regulation of platelets serotonin content and that of plasma serotonin are under different molecular controls in hypoxic mice.

A change in metabolism might also explain the increase in plasma serotonin levels in hypoxic pulmonary hypertension. Lung endothelial cells control serotonin clearance by catabolism into 5-HIAA by MAOA, and lung is known as the main site of serotonin removal from plasma (Gillis and Pitt, 1982). Low oxygen levels are expected to directly decrease MAO activity and thus serotonin metabolism. The nonsignificant reduction in serotonin degradation observed in hypoxic 5-HT_{2BR}−/− or +/+ mice (Fig. 3, C–E) indicates that 5-HT_{2BR}Rs have no major contribution to the MAOA activity under hypoxia, and the increase in plasma serotonin cannot be explained solely by changes in MAOA activity. In conclusion, a generalized deficit of the serotonin metabolism could not explain the increase in plasma serotonin encountered during hypoxic pulmonary hypertension with no changes in whole-blood serotonin.

Since the lung endothelium is the major site of carrier-mediated peripheral serotonin fast removal (Gillis and Pitt, 1982), reduced SERT uptake activity in lungs might also raise plasma serotonin levels. Recent evidence obtained by positron emission tomography scan of SERT ligands in healthy volunteers confirmed that lung is the main fast high-affinity organs in the body (Suhara et al., 1998; Takano et al., 2002). Our result showing a reduced rate of lung serotonin uptake upon chronic hypoxia, which is not observed when the 5-HT_{2BR}Rs are inactivated (Table 1), agrees with previous reports in lung of hypoxic rats and mice (Jeffery et al., 2000). In perfused rat lung, SERT can mediate an efflux of serotonin (James and Bryan-Lluaka, 1987), and the serotonin efflux caused by SERT substrates (such as amphetamine derivatives) is carrier-mediated (Hilber et al., 2005). Together, the reduced lung serotonin uptake and the increased plasma serotonin levels in hypoxic mice suggest that hypoxia-dependent 5-HT_{2BR} activation can trigger the increase in plasma serotonin, probably via a negative regulation of SERT activity. Whether this regulation is restricted to lungs remains to be determined.

Another site known to express 5-HT_{2BR}S is the gut, which is the main site of peripheral serotonin synthesis by enterochromaffin cells. The recent observation that, in perfused rat ileum, dexfenfluramine (EC_{50} around 100 μM) or nordexfenfluramine (EC_{50} around 10 μM) can increase serotonin levels in the venous effluent strongly supports their ability to increase its release from the small intestine (Rezaie-Majd et al., 2004). Given the plasma concentrations of dexfenfluramine and nordexfenfluramine in submicromolar range (Caccia et al., 1985; Launay et al., 2002) and the nanomolar affinity of nordexfenfluramine for 5-HT_{2AR}S (Supplementary table), a direct action at 5-HT_{2AR}S to the regulation of serotonin release from intestine seems unlikely but cannot be totally excluded.

In a different experimental in vivo setup, our data show that acute injection of the 5-HT_{2BR} selective agonist BW-723C86, as nordexfenfluramine (another preferential 5-HT_{2AR}S agonist), significantly increases plasma serotonin levels with identical kinetic and 5-HT_{2BR}R dependence, whereas dexfenfluramine acts with a faster kinetic and generates a serotonin increase that is independent of 5-HT_{2BR}Rs (Fig. 4). Evidence that the mechanism by which nordexfenfluramine induces serotonin efflux is different from that underling dexfenfluramine-induced release had been documented previously: work in synaptosomes showed that nordexfenfluramine acts, at least in part, on the release of a serotonin cytoplasmic pool (Mennini et al., 1981), whereas dexfenfluramine can induce a Ca^{2+}-dependent serotonin release from a vesicular pool (Gobbi et al., 1998); independent work using microdialysis showed also that dexfenfluramine, but not nordexfenfluramine, uses calcium to increase extracellular serotonin (Puig de Parada et al., 1995).

Although evaluated in the periphery, our work substantiates a dual mode of action for these compounds in modulating plasma serotonin levels. Our observations that acute injection of dexfenfluramine triggers serotonin releasing effects that are rapid, mainly paroxetine-insensitive and independent of 5-HT_{2BR}Rs, support a rapid mechanism of serotonin release by dexfenfluramine. The modulation by BW-723C86 or nordexfenfluramine of serotonin plasma levels is blocked by pharmacological (paroxetine at 1 mg/kg, Fig. 5) or genetic (mutant mice, not illustrated) SERT inhibition. In humans, similar paroxetine treatment leads to plasma concentration less than 500 nM (Lindsay De Vane, 1999). Thus, assuming a similar bioavailability in mice, it is unlikely that this concentration could affect 5-HT_{2AR}, 5-HT_{2CR}, or 5-HT_{2BR}S (Supplementary table). The elimination of both BW-723C86- and nordexfenfluramine-induced plasma serotonin increases in 5-HT_{2BR}−/− mice, in 5-HT_{2BR} antagonists-treated +/+ mice, by selective SERT blocker and in SERT−/− mice, strongly supports the hypothesis that serotonin release is controlled by 5-HT_{2BR}S via a regulation of SERT uptake activity. Reports showing that both dexfenfluramine and nordexfenfluramine at micromolar per liter concentrations generated no efflux of serotonin from platelets (Johnson et al., 2003) and that in ileum, both compounds increased serotonin levels but at concentrations over 10 μM (Rezaie-Majd et al., 2004) support the implication of other organs such as the lungs in this serotonin release.

A 5-HT_{2BR}R-dependent SERT phosphorylation (Launay et al., 1998) in vascular endothelium could be an essential component of the 5-HT_{2BR}R-dependent regulation of plasma serotonin independently of platelets. However, the molecular pathway leading to the control of SERT activity by 5-HT_{2BR}S remains to be molecularly detailed. Our data support the hypothesis that the 5-HT_{2BR}R is an essential partner in signaling pathways regulating the plasma serotonin levels via a control of SERT activity. The previous observations that, in response to hypoxia, increases in RVSP and in lung vascular remodeling are reduced in mice deficient for SERT, together with our finding that paroxetine blocks 5-HT_{2BR}R efflux effects, may explain the paradoxical reports showing that some SERT inhibitors have putative beneficial effects in pulmonary hypertension (Marcos et al., 2003), whereas injection of
serotonin potentiates the development of pulmonary hypertension in rats exposed to chronic hypoxia (Eddahibi et al., 1997). Nevertheless, our work demonstrates that 5-HT\(_{2BR}\) can control plasma serotonin levels in mice and further suggests that such a control may participate in other types of pulmonary hypertension in other organisms.

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