Extracellular superoxide enhances 5-HT-induced murine pulmonary artery vasoconstriction

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Liu, John Q., and Rodney J. Folz. Extracellular superoxide enhances 5-HT-induced murine pulmonary artery vasoconstriction. Am J Physiol Lung Cell Mol Physiol 287: L111–L118, 2004.—Accumulating evidence suggests that changes in both 5-hydroxytryptamine (5-HT) receptor activity and in the levels of reactive oxygen species (ROS) play an important role in regulating pulmonary artery (PA) vascular responsiveness, particularly in the setting of pulmonary hypertension. Therefore, we hypothesized that increased levels of superoxide enhance 5-HT-induced PA constriction. With the use of a small-vessel bioassay, 5-HT (0.01–10 μM) induced a concentration-dependent vasoconstriction in isolated wild-type murine intrapulmonary arteries (100–150 μm diameter) that was enhanced by both removal of the endothelium and by treatment with either α-nitro-l-arginine methyl ester (30 μM) or xanthine (10 μM) + xanthine oxidase (0.005 U/ml). PA isolated from extracellular superoxide dismutase (EC-SOD) knockout mice also showed enhanced constriction. On the other hand, PA constriction to 5-HT was attenuated by either the addition of GR-127935 (0.1 μM, a selective inhibitor of 5-HT1B/1D receptor) or copper/zinc-containing superoxide dismutase (Cu/Zn SOD, 150 U/ml) and in PA isolated from transgenic mice overexpressing human EC-SOD. With the use of both oxidative fluorescent confocal microscopy and lucigenin-enhanced chemiluminescence, superoxide levels were increased significantly after 5-HT-induced PA vasoconstriction. This increase in superoxide levels could be blocked by the exogenous addition of Cu/Zn SOD (150 U/ml) or by apocynin (30 μM, an inhibitor of NADPH oxidase) but was not affected by gp91phox knockout mice. Overall, our results are consistent with 5-HT increasing vascular smooth muscle superoxide production via an NADPH oxidase pathway that is independent of gp91phox, which leads to increases in extracellular superoxide levels, which in turn enhances 5-HT-induced murine pulmonary vasoconstriction.

extracellular superoxide; 5-hydroxytryptamine; murine pulmonary artery; reduced nicotinamide adenine dinucleotide

5-HYDROXYTRYPTAMINE (5-HT), also known as serotonin, is a potent pulmonary artery (PA) vasoconstrictor (33). It binds to G protein-coupled receptors (primarily 5-HT1B/1D receptors) to induce vasoconstriction. Seven different 5-HT receptor families (5-HT1 to 5-HT7) have been identified, each with numerous subclasses. However, the 5-HT1B/1D and 5-HT2A receptors have been implicated in 5-HT-induced pulmonary vasoconstriction under normoxic conditions (4, 24, 32, 33, 38). In a murine model of chronic hypoxia-induced pulmonary hypertension, enhanced PA pressures require the presence of functional 5-HT2B receptors, functional 5-HT1B/1D receptors, and a functional 5-HT transporter, since knockouts (KOs) of each of these genes show attenuated pulmonary hypertensive responses (10, 20, 24).

In bovine PA smooth muscle cells, 5-HT stimulates the generation of reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide (25–27). Superoxide anions are a short-lived ROS formed by the univalent reduction of molecular O2. Superoxide is generated under basal conditions (3), but its production can be greatly augmented under pathophysiological conditions (28–30, 45, 46). Both endothelium- and vascular smooth muscle (VSM) can generate superoxide under hypoxia-reoxygenation conditions (49). Xanthine oxidase (XO) is the major source of superoxide in endothelial cells (49). However, in VSM, a NADPH oxidase pathway accounts for the majority of superoxide produced (12). Following ANG II, Landmesser et al. (23) showed that cultured endothelial cells obtained from wild-type (wt) mice, but not p47phox (a cytosolic subunit of NADPH oxidase)-deficient animals, had a marked increase in superoxide levels, as revealed by electron spin resonance spectroscopy, suggesting that endothelial NADPH oxidase may be a major source of superoxide.

Although 5-HT receptors and ROS have each been studied intensively, the mechanism by which ROS interact with and modulate 5-HT receptor function remains unclear. Understanding these responses is important, since it will provide useful information to clarify the role 5-HT receptor(s) play in chronic hypoxia-induced pulmonary hypertension. Here, we propose that 5-HT enhances superoxide production in PA, and this resultant increase in superoxide levels facilitates 5-HT-induced pulmonary vasoconstriction. In the present studies, we characterized the nature and source of these interactions.

MATERIALS AND METHODS

General preparations. Male wt mice with C57BL/6 and C57BL/6×129 genotype were obtained from Jackson Laboratory (Bar Harbor, ME). Transgenic mice overexpressing human extracellular (EC) superoxide dismutase (SOD) using the B-actin promoter (41) were maintained in a C57BL/6 genotype. Homozygous EC-SOD null mice (EC-SOD−/−) are maintained in a C57BL/6×129 genotype (5). Hemizygous male NADPH gp91phox KO mice (−/Y) (C57BL/6) were obtained from Jackson Laboratories (stock no. 002365). All mice were 10–20 wk of age and weighed between 22 and 30 g. On the day of the experiment, the mouse was anesthetized by an intraperitoneal injection of pentobarbital sodium (80 mg/kg body wt). The chest cavity was opened, and the lungs were removed rapidly and placed in Krebs-Ringer bicarbonate solution (KRBS) bubbled with 21% O2. The KRBS contains (in mM) 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 25.0 NaHCO3, and 10 glucose.

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PA ring contractility studies. PA rings, ~100–50 μm internal diameter and 2–3 mm long, were isolated from the intrapulmonary artery (3rd to 4th generation) using a dissection microscope. The PA was placed in a small vessel wire myograph chamber (DMT, Aarhus, Denmark), after being mounted as ring preparations by threading two steel wires into the lumen and securing the wires to two supports. One support was then attached to a micrometer, allowing for the control of ring circumference; the other support was attached to a force transducer for measurement of isometric tension. In some vessels, endothelial cells were removed by gently rubbing the intraluminal surface with a steel wire. These vessels were then perfused with 2 ml air bubbles, followed by 2 ml KRBS (perfusion pressure <5 mmHg), before being mounted in the chamber. The viability of endothelium-denuded vessels was determined by demonstrating these vessels to have both a vigorous constrictor response to U-46619 and no dilator response to Ach (Fig. 1). The whole preparation was kept within a chamber filled with KRBS (pH 7.35–7.45), bubbled with 21% O2-5% CO2-5% N2, and maintained at 37°C. A Plexiglas cover was placed over the chamber to control oxygen tension over the superfusate. It should be noted that 21% O2 likely represents a relative hypoxic environment for pulmonary arteries. The temperature and PA tension were recorded using a data acquisition and analysis program (DMT).

Initially, isolated murine PA rings were allowed to equilibrate in the chamber for 10–5 min at initial tension of 0 mN (1 g = 4.905 mN). Tension was then increased to 5 mN in 2.5-mN steps at 4- to 5-min intervals and held constant thereafter. Preliminary experiments were performed to optimize the resting tension for maximal contractile response. In these studies, resting tension levels of 2.5, 5, and 7.5 mN were compared, and we found that the use of 5 mN as resting tension showed maximal contractile responses (data not shown). To assess vascular viability, PA were treated with 60 mM KCl, and tension showed maximal contractile responses (data not shown). To test whether the use of 5 mN as resting tension was recorded. Data are expressed as means ± SE, n, no. of animals. After being washed extensively with KRBS, the PA was exposed to U-46619 (0.01 μM, thromboxane A2 agonist), followed by Ach (1 μM), and the resulting tension was recorded. After these responses stabilized for 5–10 min, the agonists were washed out of the myograph chamber with KRBS.

Confocal microscopy and oxidative fluorescent measurement. The isolated murine PA was placed in a confocal pressure myograph chamber (DMT), cannulated at both ends with glass micropipettes, and secured with 12–0 nylon monofilament suture. Both cannulas were connected to a reservoir that can be raised or lowered to control transmural pressure. In the vascular chamber, vessels were superfused constantly with KRBS, gassed with 21% O2-5% CO2-5% N2, and maintained at 37°C. A custom-built Plexiglas cover was placed over the chamber to control oxygen tension over the superfusate. Dihydroethidium (DHE, 0.1 mM) was added to this chamber, incubated with murine PA at 37°C for 45 min, and then washed out for 30 min. Intracellular DHE reacts with ROS to produce a fluorescent oxidized product (1, 2, 21). PA images were scanned by a Zeiss LSM-510 inverted laser scanning confocal microscope (Carl Zeiss) with a ×20 lens (numerical aperture = 1.2). DHE was excited by the 480-nm-line argon laser, and fluorescence was measured at 620 nm. LSM 5 software was used for image processing and analysis.

Measurement of superoxide. The measurement of superoxide anion levels in isolated murine PA was accomplished using a lucigenin-enhanced chemiluminescence technique (3, 14) and detected using a scintillation counter (Luminometer) with two photomultiplier tubes (Biolumat LB 9506; Berthold, Wildbad, Germany). Lucigenin (5 μM) was dissolved in a Krebs-HEPES buffer with the following composition (in mM): 10.0 HEPES acid, 135.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.8 CaCl2, 0.026 Na-EDTA, and 11.1 glucose. The total volume of lucigenin-buffer solution was 1 ml. After background chemiluminescence activity had stabilized for 5 min, photon emissions from the isolated murine PA were recorded continuously. The specific chemiluminescence signal (emitted light) was recorded as relative light units per second (RLUs).

Reagents. 5-HT, Ach, N5-nitro-l-arginine methyl ester (l-NAME), bovine copper/zinc-containing superoxide dismutase (Cu/Zn SOD), catalase, xanthine (X), XO, apocynin, and lucigenin were purchased from Sigma Chemical (St. Louis, MO). U-46619 was purchased from Cayman Chemical (Ann Arbor, MI). DHE was obtained from Molecular Probes (Eugene, OR). GR-127935 was purchased from Tocris Cookson (Ellisville, MO). Concentrations expressed are the final molar concentration in the perfusate.

Data analysis. 5-HT- or U-46619-induced murine pulmonary vasconstriction are expressed as a percentage change of baseline tension or increases in tension (mN). t-Tests and ANOVA were used for statistical comparisons, taking repeated measures into account when appropriate. When ANOVA yielded a significant F-ratio, least-significant differences were calculated for pairwise comparison between means. P values ≤0.05 indicated statistical significance.

RESULTS

Effects of U-46619 and Ach in isolated murine PA rings. To characterize the vascular contractility of isolated murine intrapulmonary arteries, we first used U-46619 (0.01 μM) to induce vasoconstriction. As shown in Fig. 1, U-46619 caused constriction (2.83 ± 0.19 mN, n = 22). Removal of endothelium, treatment with Cu/Zn SOD (150 U/ml) or l-NAME (30 μM), PA isolated from EC-SOD KO mice, and PA isolated from gp91 KO mice did not affect the U-46619-induced PA vasoconstrictor response. In contrast, the addition of Ach (1 μM) to U-46619-preconstricted PA showed some significant differences. As expected, Ach caused relaxation in preconstricted wt PA rings (2.14 ± 0.20 mN, n = 22). In the absence of endothelium (wt + denuded), Ach showed a slight further contractile response (3.28 ± 0.27 mN, n = 7, P < 0.01). The
addition of Cu/Zn SOD (150 U/ml) to wt PA rings did not significantly affect ACh-induced, endothelium-dependent, vasodilatation (1.65 ± 0.23, n = 8), whereas the addition of L-NAME (30 μM) completely blocked ACh-induced relaxation responses (3.43 ± 0.19, n = 8, P < 0.01). PA isolated from EC-SOD KO mice (−/−) or from hemizygous gp91phox KO mice did not show a significant decrease in the ACh-induced relaxation response (2.39 ± 0.26, n = 13, and 1.85 ± 0.30, n = 7, respectively) compared with wt PA (2.14 ± 0.20 mN, n = 22). These results are consistent with superoxide playing no role (or a limited role) in U-46619-mediated vasoconstriction or the ACh-mediated relaxation response under normoxic conditions.

**Role of endothelium and endothelium-derived nitric oxide in 5-HT-induced PA vasoconstriction.** Isolated murine PA showed a dose-dependent contractile response to 5-HT (Fig. 2A; EC50 = 0.27 ± 0.05 μM, maximum constriction (Emax) = 2.97 ± 0.20 mN, n = 22). Denuding the endothelium significantly shifted the 5-HT dose-response curve to the left (EC50 = 0.08 ± 0.01 μM, n = 7, P < 0.01) but did not significantly change the 5-HT-induced maximum constriction (Emax = 3.52 ± 0.30 mN, n = 7, P = 0.150).

Similarly, we show that inhibition of nitric oxide (NO) synthesis with L-NAME (30 μM, Fig. 3) caused a significant shift to the left of the 5-HT dose-response curve (EC50 = 0.12 ± 0.01 μM, n = 8, P < 0.05) and significantly increased the maximum level of constriction (Emax = 3.56 ± 0.20 mN, n = 8, P = 0.03). These findings are consistent with NO being the endothelium-derived relaxing factor capable of reducing 5-HT-induced PA vasoconstriction.

**Effects of GR-129375 on 5-HT-induced PA vasoconstriction.** Isolated murine PA were treated with GR-129375 (0.1 μM, a selective inhibitor of 5-HT1B/1D receptors) to identify the major 5-HT receptor responsible for vasoconstriction. As shown in Fig. 4, the addition of GR-129375 markedly reduced the 5-HT-induced vasoconstrictor responses (n = 8, P < 0.001). These results suggest that 5-HT1B/1D receptors are the dominant 5-HT receptor target in murine PA.

**Effects of antioxidants and oxidants on 5-HT-induced PA vasoconstriction.** We added Cu/Zn SOD to the vascular ring bioassay to reduce extracellular vascular superoxide levels. In the presence of Cu/Zn SOD (150 U/ml), 5-HT-induced PA vasoconstriction was reduced significantly from a maximum constriction of 2.97 ± 0.20 mN in wt to 2.01 ± 0.27 mN when Cu/Zn SOD was added (n = 12, P < 0.01; Fig. 5). The addition of Cu/Zn SOD did not significantly change the EC50 of the PA ring for 5-HT (EC50 = 0.20 ± 0.04 μM, n = 12, P = 0.482).

To determine the effects of superoxide on 5-HT-induced contraction, we added X (10 μM) and XO (0.005 U/ml) to the myograph chamber for 20 min before determining the 5-HT dose-response curve. We found that, in the presence of X/XO, 5-HT-induced vasoconstriction was enhanced significantly.
over wt controls to a maximum contraction of 4.09 ± 0.53 mN (n = 4, P < 0.01; Fig. 5). However, the EC50 for 5-HT was not significantly shifted in the presence of X/XO (EC50 = 0.11 ± 0.02 μM, P = 0.076).

We also examined whether changes in endogenous EC-SOD levels would affect 5-HT-induced PA vasoconstriction. Using PA isolated from EC-SOD KO mice (Fig. 6), we found that 5-HT-induced vasoconstriction was increased significantly at 0.1 and 0.3 μM compared with wt controls (B6 × 129). However, just the opposite effect was seen in PA isolated from transgenic mice overexpressing human EC-SOD (Fig. 6). Here, the vasoconstrictor response to 5-HT was reduced significantly (2.25 ± 0.28 mN, n = 8, P < 0.05) when compared with wt PA controls (2.97 ± 0.20 mN, n = 22).

Confocal microscopy and oxidative fluorescent measurement. We used an inverted laser-scanning confocal microscopy on PA rings loaded with the oxidative fluorescent dye DHE (0.1 mM; Molecular Probes) to study 5-HT-induced ROS generation. We found that treatment of PA with 5-HT results in significantly increased ROS generation, as demonstrated by an increase in DHE fluorescence intensity of the contracting PA (Fig. 7).

Source of superoxide production. We used lucigenin-enhanced chemiluminescence (RLU/s) as an indirect measure of superoxide generation in isolated PA. When isolated PA were treated with 5-HT (1 μM), the chemiluminescence signal increased 66 ± 0.1% relative to wt controls (19.8 ± 0.8 vs. 12.0 ± 0.4 RLU/s, P < 0.001; Fig. 8). Removal of endothelium had no affect on baseline chemiluminescence (wt + denuded) nor did it affect 5-HT-induced superoxide production (wt + denuded + 5-HT). X (10 μM) and XO (0.005 U/ml) significantly increased PA baseline levels of the chemiluminescence signal (14.8 ± 0.3 RLU/s, n = 3, P < 0.01; Fig. 8). Chemiluminescence was further increased in X/XO-treated vessels by the addition of 5-HT (23.9 ± 0.7 RLU/s, n = 3, P < 0.01; Fig. 8). The wt PA treated with either Cu/Zn SOD (150 U/ml) or apocynin (30 μM), or PA isolated from transgenic mice overexpressing human EC-SOD, completely abolished 5-HT-induced superoxide production (Fig. 8). However, PA isolated from hemizygous gp91phox KO mice or EC-SOD KO mice showed similar increased levels of 5-HT-induced superoxide production compared with wt mice (Fig. 8).

Because apocynin completely blocked 5-HT-induced chemiluminescence, we evaluated the effects of apocynin on 5-HT-mediated PA contraction. We found that apocynin (30 μM) significantly reduced 5-HT maximal contraction from 3.14 ± 0.24 to 2.19 ± 0.24 mN (n = 8, P < 0.01; Fig. 9) and significantly shifted to the right the dose-response curve for 5-HT (EC50 = 0.97 ± 0.10 μM, n = 8, P < 0.001). This effect seemed to be specific for 5-HT, since apocynin did not affect KCl-induced contraction of the PA rings (see Fig. 9, inset). PA isolated from hemizygous gp91phox KO animals showed an identical dose-response curve compared with wt PA (Fig. 9).

**DISCUSSION**

5-HT can cause contraction of blood vessels by directly acting on VSM (17). In the pulmonary vasculature, 5-HT binds to G protein-coupled receptors such as 5-HT1B,2A to produce vasoconstriction (22, 37). Recently, several studies have indicated that changes in the activity of 5-HT receptors may contribute to the development of chronic hypoxia-induced
pulmonary hypertension (20, 24, 34). Likewise, an increase in superoxide production has been observed in 5-HT-stimulated bovine PA smooth muscle cells under normoxic conditions (25–27) and in pulmonary arteries under hypoxic conditions (28, 35). Taken together, it is reasonable to propose that an interaction between the activity of 5-HT receptor(s) and the generation of superoxide anions play an important role in the regulation of pulmonary arterial responsiveness.

Fig. 7. Representative videomicroscopic image (A) and confocal microscopic image (B) of 5-HT-induced vasoconstriction of wt PA. A: 5-HT decreases intraluminal diameter. B: 5-HT increased dihydroethidium (DHE) fluorescence intensity. C: the intensity of DHE fluorescence from B is measured and plotted.

Fig. 8. Measurement of baseline and 5-HT (1 μM)-induced chemiluminescence signal in murine PA. PA were isolated from wt mice, EC-SOD KO mice, transgenic mice overexpressing human EC-SOD, or gp91phox KO mice. These rings had their endothelium denuded or were either treated with GR-127935 (0.1 μM), Cu/Zn SOD (150 U/ml), X (10 μM) and XO (0.005 U/ml), or apocynin (30 μM), as indicated. Changes in chemiluminescence signal are expressed as means ± SE; n, no. of animals.

Fig. 9. Vasoconstrictor dose-response curves to 5-HT in isolated wt PA and wt PA treated with apocynin (30 μM) or gp91phox KO PA. Inset: effect of KCl (60 mM) on isolated wt murine PA with and without apocynin (30 μM). PA contractions (changes in tension from baseline) are expressed as means ± SE; n, no. of animals.

pulmonary hypertension (20, 24, 34). Likewise, an increase in superoxide production has been observed in 5-HT-stimulated bovine PA smooth muscle cells under normoxic conditions (25–27) and in pulmonary arteries under hypoxic conditions (28, 35). Taken together, it is reasonable to propose that an interaction between the activity of 5-HT receptor(s) and the generation of superoxide anions play an important role in the regulation of pulmonary arterial responsiveness.
5-HT induces PA superoxide overproduction. Using both oxidative fluorescent confocal microscopy and lucigenin-enhanced chemiluminescence, we demonstrate that, under normoxic conditions, 5-HT significantly increases superoxide levels in wt PA, both with and without intact endothelium (Figs. 7 and 8). These results indicated that VSM cells are the likely source of 5-HT-induced superoxide overproduction. These findings are consistent with those of Lee et al. (25–27), who report that 5-HT can increase superoxide production in bovine PA smooth muscle cells grown in culture under normoxic conditions. Although superoxide is normally produced under basal conditions (3), its production can be augmented greatly under pathophysiological conditions (28–30, 45, 46). In systemic vessels, for example, ANG II has been reported to cause increased superoxide production and hypertension (42, 44), suggesting that changes in superoxide levels can have a significant impact on vascular function. Gr-127935 did not abolish 5-HT-induced PA superoxide production, suggesting that 5-HT-induced superoxide production is independent of activation of 5-HT1B/D receptor(s).

Superoxide augments 5-HT-mediated PA vasoconstriction. 5-HT-induced vasoconstriction was enhanced in wt PA pre-treated with X/XO (Fig. 5) and in PA isolated from EC-SOD KO mice (Fig. 6), suggesting that increasing superoxide levels, particularly in the extracellular space, augment 5-HT-mediated PA vasoconstriction. It has been demonstrated previously that superoxide modulates vasconstriction through various mechanisms. For example, it can directly cause contraction of VSM cells. Additionally, superoxide can rapidly destroy endothelium-derived relaxing factors (otherwise known as NO) or regulate the release of NO, depending on the activity of endothelial SOD (9, 13, 15, 31, 39, 45). Finally, it may affect the release of other endothelium-derived contracting factors, such as endothelins (18, 19).

In our studies, baseline tension of wt PA was completely unaffected by the addition of X/XO (data not shown), which suggests that superoxide, at least at the concentrations used in this study, does not cause direct vasoconstriction. In addition, superoxide does not seem to affect NO bioactivity, since PA rings from both EC-SOD KO and wt + Cu/Zn SOD mice showed similar ACh-induced, endothelium-dependent, vasodilatory responses (see Fig. 1). However, endothelium-derived NO is still an important determinant of vasomotor tone, since both removal of the endothelium and treatment with 1-NAME result in an enhanced vasoconstriction response to 5-HT (Figs. 2 and 3). Since denuding the artery shifted the 5-HT EC50 more leftward than 1-NAME alone, suggests that there may be additional endothelial derived mediators involved in relaxation other than NO. Nevertheless, the precise mechanism by which superoxide enhances 5-HT vasoconstriction in PA remains unclear.

Site and/or sources of 5-HT-induced superoxide generation. That 5-HT increases superoxide levels in murine PA even after denuding the endothelium indicates that the cellular source of superoxide generation was independent of the endothelium. Although other studies have demonstrated that the endothelium can be an important source of superoxide, our current study suggests that, at least in isolated wt murine PA under normoxic conditions, the endothelium is not responsible for 5-HT-induced superoxide overproduction.

Because the 5-HT-induced increase in superoxide levels is reduced by both the exogenous addition of Cu/Zn SOD and with PA isolated from transgenic mice overexpressing human EC-SOD (Fig. 8), this suggests that this increased superoxide is localized to the extracellular space. Furthermore, treatment of the PA with apocynin prevented 5-HT-enhanced superoxide levels, suggesting that the relevant biochemical pathway for superoxide production is via an NADPH oxidase source. NADPH oxidases have been described in endothelium and VSM (11, 16, 36) and can be a major source of superoxide in VSM cells (11, 12, 47). Several studies have reported that VSM NADPH oxidase can be activated by ANG II (11, 44), stretch (40), or changes of oxygen sensing (hypoxia or hyperoxia; see Refs. 35 and 43). Consistent with our results, diphenyliodonium, a nonspecific inhibitor of NADPH oxidase, has been shown to block superoxide formation in bovine PA smooth muscle cells grown in culture and treated with 5-HT (27).

Concomitant with preventing 5-HT-enhanced superoxide levels, apocynin also significantly inhibited 5-HT-induced PA vasoconstriction without affecting the vasoconstrictor response to KCl (Fig. 9). However, in PA isolated from gp91phox KO mice, 5-HT caused comparable vasoconstriction and similar levels of superoxide production when compared with PA isolated from wt mice. These results suggest that the functional NADPH oxidase present in PA smooth muscle cells is not dependent on gp91phox, a finding that is consistent with structural models of neutrophil and VSM NADPH oxidase (12). In contrast, the NADPH oxidase subunit proteins gp91phox and p47phox have been found to play an essential role in ANG II-induced superoxide generation in murine aorta (23, 25).

Importance of antioxidant enzymes in regulating superoxide effects on PA tone. PA vasoconstrictor responses to 5-HT were reduced by the exogenous addition of Cu/Zn SOD (Fig. 5) and if the PA were isolated from transgenic mice overexpressing human EC-SOD (Fig. 6). Because the addition of Cu/Zn SOD also prevented 5-HT-induced superoxide overproduction (Fig. 8), we infer that expression levels of these antioxidant enzymes can play a significant role in regulating PA vasomotor tone. Depending on the site and/or sources of superoxide production, different isoforms of SOD, such as Cu/Zn SOD (intracellular), MnSOD (mitochondria), and EC-SOD (extracellular), can be predicted to protect against superoxide-mediated pathophysiological responses in the cardiopulmonary system (6–8, 48).

In summary, our studies demonstrate 5-HT to have two distinct effects. First, 5-HT specifically interacts with the 5-HT1B/D receptor in murine intrapulmonary arteries, resulting in contraction. Second, 5-HT increases the generation of superoxide, independent of 5-HT1B/D receptor activation, likely via a smooth muscle-specific NADPH oxidase in which the oxidase itself is independent of gp91phox. The enhanced levels of superoxide appear to further augment 5-HT-induced PA contraction. We postulate that modulating levels of SOD activity (with either SOD enzyme activity or through small-molecular-weight SOD mimetics), specifically in the extracellular space, can provide a novel pharmacological pathway for treating and/or preventing the development of chronic pulmonary vascular diseases, such as pulmonary hypertension.

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