ORIGINAL ARTICLE

Serotonin-deficient neonatal mice are not protected against the development of experimental bronchopulmonary dysplasia or pulmonary hypertension

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Abstract

Serotonin (5-hydroxytryptamine, 5-HT) is a potent pulmonary vasoconstrictor and contributes to high pulmonary vascular resistance in the developing ovine lung. In experimental pulmonary hypertension (PH), pulmonary expression of tryptophan hydroxylase-1 (TPH1), the rate limiting enzyme in 5-HT synthesis, and plasma 5-HT are increased. 5-HT blockade increases pulmonary blood flow and prevents pulmonary vascular remodeling and PH in neonatal models of PH with bronchopulmonary dysplasia (BPD). We hypothesized that neonatal tph1 knock-out (KO) mice would be protected from hypoxia-induced alveolar simplification, decreased vessel density, and PH. Newborn wild-type (WT) and tph1 KO mice were exposed to normoxia or hypoxia for 2 weeks. Normoxic WT and KO mice exhibited similar alveolar development, pulmonary vascular density, right ventricular systolic pressures (RVSPs), and right heart size. Circulating (plasma and platelet) 5-HT decreased in both hypoxia-exposed WT and KO mice. Tph1 KO mice were not protected from hypoxia-induced alveolar simplification, decreased pulmonary vessel density, or right ventricular hypertrophy, but displayed attenuation to hypoxia-induced RVSP elevation compared with WT mice. Tph1 KO neonatal mice are not protected against hypoxia-induced alveolar simplification, decreased pulmonary vessel density, or RVH. While genetic and pharmacologic inhibition of tph1 has protective effects in adult models of PH, our results suggest that tph1 inhibition would not be beneficial in neonates with PH associated with BPD.

KEYWORDS

BPD, neonate, pulmonary hypertension, serotonin

1 INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a disorder of prematurity that results in potential lifelong respiratory morbidity (Abman et al., 1985; Northway et al., 1967). The incidence of BPD is rising, despite both technological and clinical advances in neonatal care (Stoll et al., 2015). Fourteen to 25% of infants with BPD develop pulmonary

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hypertension (PH; An et al., 2010; Bhat et al., 2012; Mourani et al., 2015). While most therapies for PH associated with BPD are targeted at NO/cGMP signaling, alterations in numerous pathways, including serotonin (5-hydroxytryptamine [5-HT]), have been implicated in the development of PH associated with BPD (Bhatt et al., 2001; Delaney et al., 2018; Le Cras et al., 2002; Mourani et al., 2004, 2009). 5-HT may contribute to PH, by several mechanisms including pulmonary vasoconstriction, pulmonary artery smooth muscle cell proliferation, and activation of pulmonary fibroblasts leading to fibrosis (Chen et al., 2014; Delaney et al., 2013; Lawrie et al., 2005; MacLean et al., 1996; Morecroft et al., 1999). Furthering our understanding of the underlying mechanisms surrounding BPD associated with PH is essential in the prevention and treatment of this prevalent disease.

5-Hydroxytryptamine has largely been studied for its role in the central nervous system, where it regulates mood, memory, and pain. However, the majority of 5-HT is synthesized in the periphery by enterochromaffin cells of the small intestine where it has diverse biologic roles acting as a mitogen, growth factor, regulator of vasomotor tone, and mediator of inflammation (Berger et al., 2009; Fanburg & Lee, 1997; MacLean et al., 1996; Watts, 1996; Watts et al., 1994, 2012). The ability of 5-HT to influence such a wide variety of functions is attributed to its varied receptor system and intricate signaling pathways (Adnot et al., 2013). 5-HT is synthesized from L-tryptophan through the activity of tryptophan hydroxylase (TPH). TPH is the rate limiting step in 5-HT synthesis and exists as two isoforms, tph1 and tph2, encoded by separate genes (Walther et al., 2003). While tph1 expression is highest in enterochromaffin cells of the small intestine, other cell types, including pulmonary artery endothelial cells, express tph1 (Barter & Pearse, 1953; Delaney et al., 2018; Gershon et al., 1977; Walther et al., 2003). Tph2 expression is limited to the brain and enteric nervous system. As tph2 is expressed in the enteric nervous system, tph1 knock-out (KO) mice synthesize peripheral 5-HT via tph2 (Gershon et al., 1977; Walther et al., 2003). Plasma levels of 5-HT are in the low nanomolar range as circulating 5-HT is rapidly taken up by platelets via the 5-HT transporter (SERT) and stored in platelet dense granules (Da Prada & Picotti, 1979; Holmsen & Weiss, 1979).

The "serotonin hypothesis of PH" was developed in the 1960s after the development of PH was observed in patients consuming diet pills that increase 5-HT bioavailability (Eddahibi & Adnot, 2002; MacLean, 2018). Increasing clinical and experimental evidence support a role for aberrant 5-HT signaling in the pathogenesis of neonatal PH and BPD. 5-HT immunoreactive cells are present from 8 weeks gestation onward in human fetal lungs and infants who died from severe BPD exhibit a 34-fold increase in pulmonary 5-HT when compared with age-matched controls (Cutz et al., 1985; Johnson et al., 1985). Maternal selective 5-HT reuptake inhibitor (SSRI) use in the third trimester of gestation is associated with a sixfold increased risk of persistent pulmonary hypertension of the newborn (PPHN; Chambers et al., 2006). In addition, absent SERT expression in the neonatal lung is associated with alveolar capillary dysplasia, a fatal disease characterized by PH (Castro et al., 2017). Newborn rats exposed to an SSRI, fluoxetine, in utero develop pulmonary vascular remodeling, abnormal oxygenation, and higher mortality when compared with controls (Belik, 2008).

Our lab has demonstrated that 5-HT is a potent pulmonary vasoconstrictor in the ovine fetus and that 5-HT contributes to fetal pulmonary vascular resistance (Delaney et al., 2011). Pulmonary TPH1 expression and pulmonary artery endothelial cell (PA-EC) synthesis of 5-HT is increased in fetal sheep with PH (Delaney et al., 2013). Our lab has also demonstrated that both lung TPH1 expression and plasma 5-HT are increased in neonatal mice with bleomycin-induced PH and BPD (Delaney et al., 2018). Additionally, we have shown increased activation and accumulation of platelets, the primary source of circulating 5-HT, in the lungs of mice with bleomycin-induced neonatal PH (Davison-Castillo et al., 2020).

Due to increasing support regarding the role of 5-HT in promoting PH, genetic or pharmacologic depletion of 5-HT has been the focus of multiple studies. Overall, the results demonstrate that 5-HT depletion affords varying degrees of protection in several experimental adult rodent models of PH (Abid et al., 2012; Aiello et al., 2017; Izikki et al., 2007; Morecroft et al., 2007). From a neonatal perspective, pharmacologic blockade of 5-HT via inhibition of the 5A receptor not only decreases fetal pulmonary vascular resistance in the ovine fetus with PH, but also protects against the development of PH and pulmonary vascular remodeling in a neonatal murine model of bleomycin-induced BPD and PH (Delaney et al., 2013, 2018). In the present study, we hypothesized that circulating (platelet and plasma) and pulmonary 5-HT is increased in neonatal WT hypoxic mice and tph1 KO neonatal mice would be protected against hypoxia-induced alveolar simplification, decreased pulmonary vessel density, and PH. To study our hypothesis, we utilized a hypobaric hypoxia neonatal murine model of PH and BPD.

2 | METHODS

2.1 | Mouse model

The University of Colorado Denver Institutional Animal Care and Use Committee (IACUC) approved all animal studies. C57BL/6 wild-type (WT) mice were purchased from Jackson Laboratory and bred in Denver. Tph1 KO
mice were engineered by Dr. M. Bader (Max-Delbrück-Center for Molecular Medicine) on a C57BL/6 background (Deruelle et al., 2006). These mice were kindly donated to our laboratory and bred in Denver. Offspring were obtained from crosses of WT mice, tph1 heterozygous mice, and tph1 KO mice. Offspring were either raised at Denver altitude (633 mmHg) or placed in hypobaric chambers 24–36 h after birth at 446 mmHg to simulate 12% FiO2 at sea level. This murine injury model of PH and BPD produces similar major pathologic findings to infants with PH and BPD including impaired alveolar development (decreased surface area [SA], increased mean linear intercept), vascular injury (decreased vessel density), and pulmonary hypertension (elevated right ventricular systolic pressure and right ventricular hypertrophy; Balasubramaniam et al., 2003; Deruelle et al., 2006; Tang et al., 2000). Hemodynamic assessment and euthanization for blood and tissue collection took place at 2 weeks of age.

2.2 Preparation of mouse blood and measurement of platelet and plasma 5-HT by ELISA

Mice were anesthetized with 1%–2% isoflurane and blood was obtained via cardiac puncture of the right ventricle (RV) after performing a bilateral thoracotomy using a 21-gauge needle containing heparin. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 100 g for 10 min. PRP was supplemented with prostacyclin (PGI2; 1 μg/mL) and incubated at room temperature for 3 min prior to centrifugation at 2000 g for 2 min to obtain platelet poor plasma (PPP) or platelet pellets. 5-HT levels were measured using the mouse 5-HT ELISA kit (GenWay Biotech) following the manufacturer’s instructions.

2.3 Preparation of mouse lung homogenates and measurement of lung 5-HT by ELISA

Flushed lungs were obtained from storage at −80°C and placed on ice. The reagents, Pierce’s Tissue Protein Extraction Reagent, Sigma’s Phosphatase Inhibitor Cocktail 2, Sigma’s Phosphatase Inhibitor Cocktail 3, and Sigma’s Protease Inhibitor Cocktail were prepared per manufacturer’s instructions. Tissue samples (25–30 mg) were separated and placed in the Bead Ruptor12 (Omni International), run at High Speed for 45 s, and then transferred to ice ×3 cycles. Homogenized samples were incubated for 30 min on ice, then centrifuged at 10,000 g for 5 min. 5-HT levels were measured using the 5-HT ELISA kit (ALPCO) following the manufacturer’s instructions.

2.4 Immunohistochemistry

Lungs were inflation-fixed at 25 cm H2O for 30 min with 4% paraformaldehyde for paraffin embedding. Lung sections were stained with hematoxylin and eosin to assess alveolar structure, and rabbit anti-vWF (1:1500; Sigma-Aldrich), and ready-to-use horse radish peroxidase (HRP) conjugated anti-rabbit IgG (Dako EnVision+ Dual Link System- HRP [DAB+]) to assess vessel density. von Willebrand Factor (vWF)-stained slides were developed with Vector very intense purple peroxidase (HRP) substrate kit (Vector Laboratories) and counterstained with light green counterstain.

2.5 Evaluation of alveolar development and pulmonary vessel density

Surface area (mm²/HPF) and mean linear intercept (MLI) were obtained with Metamorphic Basic (Molecular Devices Sunnyvale). Ten randomly selected non-overlapping sections per mouse at 20× magnification were assessed. Fields with large airways or vessels were excluded. Vessel density was assessed by counting the number of vessels <30 μm staining positive for vWF per high-power field (20×). Again, lung fields containing large vessels or airways were excluded, and greater than six fields were included per mouse. An investigator blinded to the experimental group performed the analysis.

2.6 Hemodynamic measurements and evaluation of right ventricular hypertrophy

At 2 weeks of life, mice underwent direct RV puncture via closed chest to obtain right ventricular systolic pressures (RVSPs). Hearts were removed and dissected to isolate the free wall of the RV from the left ventricle (LV) and septum (S). Fulton’s index, the ratio of RV weight over LV+S weight (RV/LV+S), was used as an index of RV hypertrophy resulting from PH, as previously described (Delaney et al., 2018). An investigator blinded to the experimental group performed the analysis.

2.7 RNA isolation and qPCR

RNA was isolated as previously described using a RNeasy kit (Qiagen); then cDNA was prepared using the iScript cDNA synthesis kit (Bio-Rad; Good et al., 2018). qPCR was performed on a QuantStudio 6 Flex qPCR machine (Thermo Fisher Scientific) using TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific). The following
Taqman probe was used: tph1 (Mm01202614_m1), tph2 (Mm00557722_m1). All samples were run in triplicate fashion, analyzed, and presented by $2^{-\Delta\Delta Ct}$ method.

2.8 Statistical analysis

Data were analyzed using Prism (GraphPad Software) by one-way ANOVA, two-way ANOVA, and unpaired t-test when appropriate. Post hoc analysis was performed using Tukey’s posttest when significant differences existed between groups. Data were expressed as mean ± SD and significance defined as $p < 0.05$.

3 RESULTS

3.1 Circulating and lung 5-HT is decreased in tph1 KO mice

We evaluated our model by first quantifying pulmonary tph1 gene expression and circulating 5-HT in WT and tph1 KO mice. Pulmonary expression of the tph1 gene was not detected in tph1 KO mice (Figure 1a). As expected, we found that 5-HT is primarily located within platelets and 5-HT levels in tph1 KO mice are decreased in both platelet poor plasma (PPP) and platelets compared with WT mice (Figure 1b, *$p < 0.0001$, **$p < 0.01$, ***$p < 0.0001$, ****$p < 0.005$). Although greatly decreased, tph1 KO mice synthesize 5-HT in the periphery. The average plasma 5-HT level in tph1 KO mice is ~6 ng/ml, while the average platelet 5-HT level in tph1 KO mice is ~30 ng/ml, compared with average 5-HT levels of ~30 and ~285 ng/ml, respectively, in WT mice (Figure 1b).

3.2 Two-week-old tph1 KO normoxic mice exhibit similar alveolar development, pulmonary vessel density, pulmonary pressures, and right heart size to WT normoxic mice

We evaluated alveolar development by examining alveolar SA and MLI. We quantified the density of vWF stained vessels <30 μm. Alveolar development (Figure 2a–d) and pulmonary vessel density (Figure 3a–c) are not altered at 2 weeks of age in tph1 KO mice compared with WT mice at baseline. Additionally, there is no difference in RVSP or RVH in tph1 KO mice compared with WT mice at baseline (Figure 3d–e).

3.3 Tph1 KO mice display comparable hypoxia-induced alveolar simplification and reduction in pulmonary vessel density to WT mice

Tph1 KO mice display comparable hypoxia-induced alveolar simplification to WT mice, with similar decreased alveolar SA and increased MLI (Figure 4a–f). SA decreased

FIGURE 1 PPP and platelet 5-HT (ng/ml) are decreased in 2-week-old tph1 KO mice compared with WT mice. (a) Lung tph1 gene expression in WT and tph1 KO mice, *$p < 0.0001$ by unpaired test, $n = 5$ WT (3M, 2F), $n = 9$ KO (5M, 4F), ns for sex. (b) Baseline 5-HT (ng/ml) in PPP and platelet pellets of WT and tph1 KO mice, *$p < 0.0001$, **$p < 0.01$, ***$p < 0.0001$, ****$p < 0.005$ by one-way ANOVA, $n = 20$ WT (12M, 8F), $n = 16$ KO (9M, 7F), ns for sex. 5-HT, 5-hydroxytryptamine; PPP, platelet poor plasma; WT, wild-type.
18% in hypoxia-exposed KO mice compared with a 14% decrease in WT mice (Figure 4e, *p < 0.0001, "p < 0.0001). Hypoxia exposed KO mice display a 15% increase in MLI compared with a 14% increase in WT mice (Figure 4f, *p < 0.0001, "p < 0.0001). Neonatal tph1 KO mice and WT mice have similar decreased density of small pulmonary arteries following hypoxia (Figure 5a–e). The density of vWF stained vessels <30μm decreased 28% in tph1 KO mice and 26% in WT mice with hypoxia exposure (Figure 5e, *p < 0.01, "p < 0.01).
3.4 | Hypoxia-induced increase in RVSP is attenuated in tph1 KO mice compared with WT mice; however, hypoxia-induced RVH is comparable between KO and WT mice

Prior studies in our lab have shown that pharmacologic inhibition of 5-HT signaling via the 2A receptor prevents PH in neonatal mice (decreased RVSP and RVH) treated with bleomycin (Delaney et al., 2018). In the present study, we assessed whether tph1 deletion, resulting in decreased peripheral 5-HT, protects against hypoxia-induced PH. We found that tph1 KO mice display attenuation of hypoxia-induced increase in RVSP compared with WT mice; however, RVH was not different between KO and WT mice. RVSP increased 26% in tph1 KO mice compared with a 36% increase in WT mice (Figure 5f, *p < 0.0001 and **p < 0.0001 by two-way ANOVA, n = 22 WT NMX (13M, 9F), n = 24 KO NMX (12M, 12F), n = 17 WT and KO HPX (8M, 9F), ns for sex. (f) MLI of WT and tph1 KO mice in NMX and HPX, *p < 0.0001 and **p < 0.0001 by two-way ANOVA, n = 22 WT NMX (13M, 9F), n = 24 KO NMX (12M, 12F), n = 17 WT HPX (8M, 9F), n = 18 KO HPX (10M, 8F), ns for sex. HPX, hypoxia; KO, knock-out; MLI, mean linear intercept; NMX, normoxia; SA, surface area; WT, wild-type).

3.5 | Platelet and plasma 5-HT is decreased in hypoxia-exposed WT mice compared with WT normoxic mice

Based on our prior work demonstrating both increased plasma 5-HT and pulmonary tph1 expression, as well as accumulation of platelets, the primary source of 5-HT within the lungs of neonatal mice with bleomycin-induced PH, we hypothesized that circulating and pulmonary 5-HT would be increased in neonatal mice following hypoxia exposure (Davison-Castillo et al., 2020). As the majority of circulating 5-HT is stored within platelet dense granules, we measured both platelet and platelet poor plasma 5-HT levels. Surprisingly, 5-HT was decreased in both platelet and platelet poor plasma following 2 weeks of hypoxia in neonatal mice (Figure 6a, *p < 0.0001; Figure 6b, *p < 0.0001). Lung 5-HT in WT normoxic and hypoxic mice was not different. Lung 5-HT decreased 23% in hypoxia-exposed KO mice when compared with lung 5-HT of normoxic KO mice; of note, these values were low, close to the limit of detection of the assay (Figure 6c, *p < 0.001).
DISCUSSION

We previously reported that both pulmonary expression of TPH1, the rate limiting step in circulating 5-HT synthesis, and plasma 5-HT are increased in neonatal murine bleomycin-induced PH and BPD (Delaney et al., 2018). Additionally, we reported that pharmacologic blockade of the 5-HT 2A receptor prevents bleomycin-induced pulmonary vascular remodeling and PH (Delaney et al., 2018). Based upon our prior work and studies in adult mice demonstrating that genetic deletion of tph1 protects against the development of PH, we hypothesized that hypoxia would result in increased lung and circulating 5-HT in WT mice and genetic deletion of tph1 would ameliorate hypoxia-induced alveolar simplification, reduction in vessel density, and PH in neonatal mice. We tested this hypothesis in WT and tph1 KO neonatal mice that either remained at Denver altitude or were exposed to hypobaric hypoxia for 2 weeks. We report that tph1 KO neonatal mice display mild attenuation of hypoxia-induced increase in RVSPs, tph1 KO neonatal mice were not protected against hypoxia-induced alveolar...
However, reports are conflicting regarding whether circulating 5-HT is increased in adults with PH or rodents with experimental PH (Herve et al., 1990, 1995; Kereveur et al., 2000; Lederer et al., 2008; Zeinali et al., 2014). Similar to prior reports in adult hypoxic mice, we found that both platelet and plasma 5-HT are decreased in neonatal mice with hypoxia-induced PH (Abid et al., 2012). As lung 5-HT levels do not change and both plasma and platelet 5-HT decrease following hypoxia, we speculate that hypoxia results in decreased peripheral synthesis of 5-HT. Our findings in hypoxic 2-week-old mice differ from our prior findings in bleomycin-treated neonatal mice where we found an increase in plasma 5-HT (Delaney et al., 2018). While the mechanisms for these differences are unclear, bleomycin causes transient thrombocytopenia and induces endothelial injury, potentially increasing plasma 5-HT via platelet activation and subsequent release of 5-HT (Hilgard & Hossfeld, 1978). The results of our studies, in which we utilize two different models of neonatal PH, highlight the importance of evaluating disease processes at various time points and in multiple models to broaden our understanding of complex interactions that underlie disease etiology.

TPH1 pharmacologic inhibition attenuates PH in several adult rodent models (Abid et al., 2012; Aiello et al., 2017; Ciuclan et al., 2013). Blockade of 5-HT via inhibition of the 2A receptor decreases fetal pulmonary vascular resistance in the ovine fetus with PH and prevents the development of PH and pulmonary vascular remodeling in a neonatal murine model of bleomycin-induced BPD and PH (Delaney et al., 2013, 2018). Based on these findings, we hypothesized that hypoxia-induced PH would be prevented in tph1 KO neonatal mice. While we found that hypoxia-induced increase in RVSP was attenuated in tph1 KO mice compared with WT mice, tph1 KO mice were not protected against hypoxia-induced

Our first major finding is that alveolar development, pulmonary vascular density, RVSPs and right heart size are similar between tph1 KO and WT mice at baseline. 5-HT is detected in fetal human lungs as early as 8 weeks gestation and increases lung fluid absorption in fetal guinea pigs close to term (Chua & Perks, 1999; Cutz et al., 1985; Pan et al., 2006). However, to our knowledge there are no studies evaluating 5-HT’s impact on fetal lung alveologenesis or angiogenesis. We report that peripheral 5-HT depletion does not disrupt the parameters of alveolar development and vascular development measured at the time point of our study. It is possible that 5-HT is not a critical mitogen or growth factor required for normal murine lung development or that tph1 KO mice have sufficient levels of 5-HT required for normal lung development as tph1 KO mice synthesize peripheral 5-HT via tph2 within enteric neurons (Walther et al., 2003). We found that RVSPs in 2-week-old 5-HT deficient mice (tph1 KO) are similar to WT mice. Our findings in 2-week-old mice agree with findings in adult tph1 KO mice (Le Cras et al., 2002). Deletion of the 5-HT 2B receptor gene leads to embryonic and neonatal death attributed to cardiac defects (Nebigil et al., 2000). While we found that heart size did not differ between KO and WT mice, our cardiac evaluation of tph1 KO mice was limited to RV and LV mass. As such, we cannot draw additional conclusions regarding whether 5-HT deficiency affects cardiac development or function.

Exogenously administered 5-HT potentiates the development of PH in rats (Eddahibi et al., 1997). Decreased platelet storage of 5-HT, resulting in elevated plasma 5-HT, leads to the development of PH in patients with platelet storage disease and fawn hooded rats (Herve et al., 1990; Le Cras et al., 1999). However, reports are
RVH. The reasons for lack of protection against PH is unclear, but not surprising, as 5-HT did not increase in this model. Additionally, we did not measure pulmonary 5-HT transporter or receptor expression, it is feasible that changes in receptor expression may also drive the lack of protection.

There are a few potential limitations to our study. Alveolar development, pulmonary vascular development, and pulmonary pressures are dynamic measures, and our study evaluated a single time point during the alveolar stage of lung development. This raises the question of whether assessment at an earlier timepoint would reveal baseline differences in alveolar development or vascular density in tph1 KO mice compared with WT mice. We did not perform pulmonary function studies to assess whether similar alveolar development between WT and tph1 KO mice correlated with lung function as determined by lung compliance and airway reactivity. Furthermore, we measured 5-HT levels in the circulation and lung at the end of our study. Whether these findings reflect levels across the 2-week study period is unknown. Additionally, 5-HT modulates systemic blood pressure, a value we did not collect in the present study (Watts et al., 2012). Systemic hypotension may falsely lower RVSP values and impact data analysis. A consideration for future studies is to obtain neonatal echocardiograms to assess cardiac output.

We conclude that tph1 KO neonatal mice are not protected against hypoxia-induced alveolar simplification, reduction in pulmonary vessel density, or RVH. While genetic and pharmacologic inhibition of tph1 has protective effects in adult models of PH, our results suggest that tph1 inhibition may have some utility in decreasing pulmonary vasoconstriction but would not be beneficial in treating PH in neonates with PH associated with BPD.

INFORMED CONSENT
No patient consent was required for this manuscript.

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REFERENCES
Abid, S., Houssaini, A., Chevarin, C., Marcos, E., Tissot, C. M., Gary-Bobo, G., Wan, F., Mouraret, N., Amsellem, V., Dubois-Rande, J. L., Hamon, M., & Adnot, S. (2012). Inhibition of gut- and lung-derived serotonin attenuates pulmonary hypertension in mice. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 303, L500–L508.

Abman, S. H., Wolfe, R. R., Accurso, F. J., Koops, B. L., Bowman, C. M., & Wiggins, J. W., Jr. (1985). Pulmonary vascular response to oxygen in infants with severe bronchopulmonary dysplasia. *Pediatrics*, 75, 80–84.

Adnot, S., Houssaini, A., Abid, S., Marcos, E., & Amsellem, V. (2013). Serotonin transporter and serotonin receptors. *Handbook of Experimental Pharmacology*, 218, 365–380.

Aiello, R. J., Bourassa, P. A., Zhang, Q., Dubins, J., Goldberg, D. R., De Lombaert, S., Humbert, M., Guignabert, C., Cavasin, M. A., McKinsey, T. A., & Parikal, V. (2017). Tryptophan hydroxylase 1 inhibition impacts pulmonary vascular remodeling in two rat models of pulmonary hypertension. *The Journal of Pharmacology and Experimental Therapeutics*, 360, 267–279.

An, H. S., Bae, E. J., Kim, G. B., Kwon, B. S., Beak, J. S., Kim, E. K., Kim, H. S., Choi, J. H., Noh, C. I., & Yun, Y. S. (2010). Pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. *Korean Circulation Journal*, 40, 131–136.

Balasubramaniam, V., Tang, J. R., Maxey, A., Plopper, C. G., & Abman, S. H. (2003). Mild hypoxia impairs alveolarization in the endothelial nitric oxide synthase-deficient mouse. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 284, L964–L971.

Barter, R., & Pearse, A. G. (1953). Detection of 5-hydroxytryptamine in mammalian enterochromaffin cells. *Nature*, 172, 810.

Belik, J. (2008). Fetal and neonatal effects of maternal drug treatment for depression. *Seminars in Perinatology*, 32, 350–354.

Berger, M., Gray, J. A., & Roth, B. L. (2009). The expanded biology of serotonin. *Annual Review of Medicine*, 60, 355–366.

Bhat, R., Salas, A. A., Foster, C., Carlo, W. A., & Ambalavanan, N. (2012). Prospective analysis of pulmonary hypertension in extremely low birth weight infants. *Pediatrics*, 129, e682–e689.

Bhatt, A. J., Pryhuber, G. S., Huyck, H., Watkins, R. H., Metlay, L. A., & Maniscalco, W. M. (2001). Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Fli-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. *American Journal of Respiratory and Critical Care Medicine*, 164, 1971–1980.

Castro, E. C., Sen, P., Parks, W. T., Langston, C., & Galambos, C. (2017). The role of serotonin transporter in human lung development and in neonatal lung disorders. *Canadian Respiratory Journal*, 2017, 9064046.

Chambers, C. D., Hernandez-Diaz, S., Van Marter, L. J., Werler, M. M., Louik, C., Jones, K. L., & Mitchell, A. A. (2006). Selective
serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. The New England Journal of Medicine, 354, 579–587.

Chen, C., Han, X., Fan, F., Liu, Y., Wang, T., Wang, J., Hu, P., Ma, A., & Tian, H. (2014). Serotonin drives the activation of pulmonary artery adventitial fibroblasts and TGF-beta1/Smad3-mediated fibrotic responses through 5-HT2A receptors. Molecular and Cellular Biochemistry, 397, 267–276.

Chua, B. A., & Perks, A. M. (1999). The pulmonary neuroendocrine system and drainage of the fetal lung: Effects of serotonin. General and Comparative Endocrinology, 113, 374–387.

Ciucian, L., Hussey, M. J., Burton, V., Good, R., Duggan, N., Beach, S., Jones, P., Fox, R., Clay, I., Bonnaue, O., Konstantinova, I., Pearce, A., Rowlands, D. J., Jarai, G., Westwick, J., MacLean, M. R., & Thomas, M. (2013). Imatinib attenuates hypoxia-induced pulmonary arterial hypertension pathology via reduction in 5-hydroxytryptamine through inhibition of tryptophan hydroxylase 1 expression. American Journal of Respiratory and Critical Care Medicine, 187, 78–89.

Cutz, E., Gillan, J. E., & Bryan, A. C. (1985). Neuroendocrine cells in the developing human lung: Morphologic and functional considerations. Pediatric Pulmonology, 1, S21–S29.

Da Prada, M., & Picotti, G. B. (1979). Content and subcellular localization of catecholamines and 5-hydroxytryptamine in human and animal blood platelets: Monoamine distribution between platelets and plasma. British Journal of Pharmacology, 65, 653–662.

Davizon-Castillo, P., Allawzi, A., Sorrells, M., Fisher, S., Baltrunaite, K., Neeves, K., Nozik-Grayck, E., DiPaola, J., & Delaney, C. (2020). Platelet activation in experimental murine neonatal pulmonary hypertension. Physiological Reports, 8, e14386.

Delaney, C., Gien, J., Grover, T. R., Roe, G., & Abman, S. H. (2011). Pulmonary vascular effects of serotonin and selective serotonin reuptake inhibitors in the late-gestation ovine fetus. American Journal of Physiology. Lung Cellular and Molecular Physiology, 301, L937–L944.

Delaney, C., Gien, J., Roe, G., Isenberg, N., Kailey, J., & Abman, S. H. (2013). Serotonin contributes to high pulmonary vascular tone in a sheep model of persistent pulmonary hypertension of the newborn. American Journal of Physiology. Lung Cellular and Molecular Physiology, 304, L894–L901.

Delaney, C., Sherlock, L., Fisher, S., Maltzahn, J., Wright, C., & Nozik-Grayck, E. (2018). Serotonin 2A receptor inhibition protects against the development of pulmonary hypertension and pulmonary vascular remodeling in neonatal mice. American Journal of Physiology. Lung Cellular and Molecular Physiology, 314, L871–L881.

Dervelle, P., Balasubramaniam, V., Kunig, A. M., Seedorf, G. J., Markham, N. E., & Abman, S. H. (2006). BAY 41-2272, a direct activator of soluble guanylate cyclase, reduces right ventricular hypertrophy and prevents pulmonary vascular remodeling during chronic hypoxia in neonatal rats. Biology of the Neonate, 90, 135–144.

Eddahibi, S., & Adnot, S. (2002). Anorexigen-induced pulmonary hypertension and the serotonin (5-HT) hypothesis: Lessons for the future in pathogenesis. Respiratory Research, 3, 9.

Eddahibi, S., Raffestin, B., Pham, I., Launay, J. M., Aegerter, P., Sitbon, M., & Adnot, S. (1997). Treatment with 5-HT potentiates development of pulmonary hypertension in chronically hypoxic rats. The American Journal of Physiology, 272, H1173–H1181.

Fanburg, B. L., & Lee, S. L. (1997). A new role for an old molecule: Serotonin as a mitogen. The American Journal of Physiology, 272, L795–L806.

Gershon, M. D., Dreyfus, C. F., Pickel, V. M., Joh, T. H., & Reis, D. J. (1977). Serotonergic neurons in the peripheral nervous system: Identification in gut by immunohistochemical localization of tryptophan hydroxylase. Proceedings of the National Academy of Sciences of the United States of America, 74, 3086–3089.

Good, R. J., Hernandez-Lagunas, L., Allawzi, A., Maltzahn, J. K., Vohwinkel, C. U., Upadhay, A. K., Kompella, U. B., Birukov, K. G., Carpenter, T. C., Sucharov, C. C., & Nozik-Grayck, E. (2018). MicroRNA dysregulation in lung injury: The role of the miR-26a/EphA2 axis in regulation of endothelial permeability. American Journal of Physiology. Lung Cellular and Molecular Physiology, 315, L584–L594.

Herve, P., Drouet, L., Dosquet, C., Launay, J. M., Rain, B., Simonneau, G., Caen, J., & Duroux, P. (1990). Primary pulmonary hypertension in a patient with a familial platelet storage pool disease: Role of serotonin. The American Journal of Medicine, 89, 117–120.

Herve, P., Launay, J. M., Scrobhaci, M. L., Brenot, F., Simonneau, G., Petitpretz, P., Pouboue, P., Cerrina, J., Duroux, P., & Drouet, L. (1995). Increased plasma serotonin in primary pulmonary hypertension. The American Journal of Medicine, 99, 249–254.

Hilgard, P., & Hossfeld, D. K. (1978). Transient bleomycin-induced thrombocytopenia. A clinical study. European Journal of Cancer, 14(11), 1261–1264.

Holmsen, H., & Weiss, H. J. (1979). Secretable storage pools in platelets. Annual Review of Medicine, 30, 119–134.

Izikki, M., Hanoun, N., Marcos, E., Savale, L., Barlier-Mur, A. M., Saurini, F., Eddahibi, S., Hamon, M., & Adnot, S. (2007). Tryptophan hydroxylase 1 knockout and tryptophan hydroxylase 2 polymorphism: Effects on hypoxic pulmonary hypertension in mice. American Journal of Physiology. Lung Cellular and Molecular Physiology, 293, L1045–L1052.

Johnson, D. E., Kulik, T. J., Lock, J. E., Elde, R. P., & Thompson, T. R. (1985). Bombesin-, calcitonin-, and serotonin-immunoreactive pulmonary neuroendocrine cells in acute and chronic neonatal lung disease. Pediatric Pulmonology, 1, S13–S20.

Kereveur, A., Callebert, J., Humbert, M., Herve, P., Simonneau, G., Launay, J. M., & Drouet, L. (2000). High plasma serotonin levels in primary pulmonary hypertension. Effect of long-term epoprostenol (prostacyclin) therapy. Arteriosclerosis, Thrombosis, and Vascular Biology, 20, 2233–2239.

Lawrie, A., Spiekerooet, E., Martinez, E. C., Ambartsouman, N., Sheward, W. J., MacLean, R. M., Harmar, A. J., Schmidt, A. M., Lukandin, E., & Rabinovitch, M. (2005). Interdependent serotonin transporter and receptor pathways regulate S100A4/Mts1, a gene associated with pulmonary vascular disease. Circulation Research, 97, 227–235.

Le Cras, T. D., Markham, N. E., Shannon, J. M., Tudor, R. M., & Abman, S. H. (1999). Abnormal lung growth and the development of pulmonary hypertension in the Fawn-Hooded rat. The American Journal of Physiology, 277, L709–L718.

Le Cras, T. D., Markham, N. E., Tudor, R. M., Voelkel, N. F., & Abman, S. H. (2002). Treatment of newborn rats with a VEGF receptor inhibitor causes pulmonary hypertension and abnormal lung structure. American Journal of Physiology. Lung Cellular and Molecular Physiology, 283, L555–L562.
Lederer, D. J., Horn, E. M., Rosenzweig, E. B., Karmally, W., Jahnes, M., Barst, R. J., & Kawut, S. M. (2008). Plasma serotonin levels are normal in pulmonary arterial hypertension. *Pulmonary Pharmacology & Therapeutics*, 21, 112–114.

MacLean, M. M. R. (2018). The serotonin hypothesis in pulmonary hypertension revisited: Targets for novel therapies (2017 Grover conference series). *Pulmonary Circulation*, 8, 2045894018759125.

MacLean, M. R., Clayton, R. A., Templeton, A. G., & Morecroft, I. (1996). Evidence for 5-HT1-like receptor-mediated vasoconstriction in human pulmonary artery. *British Journal of Pharmacology*, 119, 277–282.

Morecroft, I., Dempstie, Y., Bader, M., Walther, D. J., Kotnik, K., Loughlin, L., Nilsen, M., & MacLean, M. R. (2007). Effect of tryptophan hydroxylase 1 deficiency on the development of hypoxia-induced pulmonary hypertension. *Hypertension*, 49, 232–236.

Morecroft, I., Heeley, R. P., Prentice, H. M., Kirk, A., & MacLean, M. R. (1999). 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: Importance of the 5-HT1B receptor. *British Journal of Pharmacology*, 128, 730–734.

Mourani, P. M., Ivy, D. D., Gao, D., & Abman, S. H. (2004). Pulmonary vascular effects of inhaled nitric oxide and oxygen tension in bronchopulmonary dysplasia. *American Journal of Respiratory and Critical Care Medicine*, 170, 1006–1013.

Mourani, P. M., Sontag, M. K., Ivy, D. D., & Abman, S. H. (2009). Effects of long-term sildenafil treatment for pulmonary hypertension in infants with chronic lung disease. *The Journal of Pediatrics*, 154(3), 379–384.

Mourani, P. M., Sontag, M. K., Younoszai, A., Miller, J. I., Kinsella, J. P., Baker, C. D., Poinzter, B. B., Ingram, D. A., & Abman, S. H. (2015). Early pulmonary vascular disease in preterm infants at risk for bronchopulmonary dysplasia. *American Journal of Respiratory and Critical Care Medicine*, 191, 87–95.

Nebigil, C. G., Choi, D. S., Dierich, A., Hickel, P., Le Meur, M., Messaddeq, N., Launay, J. M., & Maroteaux, L. (2000). Serotonin 2B receptor is required for heart development. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 9508–9513.

Northway, W. H., Jr., Rosan, R. C., & Porter, D. Y. (1967). Pulmonary disease following respirator therapy of hyaline-membrane disease: bronchopulmonary dysplasia. *New England Journal of Medicine*, 276, 357–368.

Pan, J., Copland, I., Post, M., Yeger, H., & Cutz, E. (2006). Mechanical stretch-induced serotonin release from pulmonary neuroendocrine cells: Implications for lung development. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 290, L185–L193.

Stoll, B. J., Hansen, N. I., Bell, E. F., Walsh, M. C., Carlo, W. A., Shankaran, S., Laptook, A. R., Sanchez, P. J., Van Meurs, K. P., Wyckoff, M., & Delaney, C. A. (2022). Serotonin-deficient neonatal mice are not protected against the development of experimental bronchopulmonary dysplasia or pulmonary hypertension. *Physiological Reports*, 10, e15482. https://doi.org/10.14814/phy2.15482

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