Serotonin contributes to high pulmonary vascular tone in a sheep model of persistent pulmonary hypertension of the newborn

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Serotonin contributes to high pulmonary vascular tone in a sheep model of persistent pulmonary hypertension of the newborn. Am J Physiol Lung Cell Mol Physiol 304: L894–L901, 2013. First published April 19, 2013; doi:10.1152/ajplung.00043.2013.—Although past studies demonstrate that altered serotonin (5-HT) signaling is present in adults with idiopathic pulmonary arterial hypertension, whether serotonin contributes to the pathogenesis of persistent pulmonary hypertension of the newborn (PPHN) is unknown. We hypothesized that 5-HT contributes to increased pulmonary vascular resistance (PVR) in a sheep model of PPHN and that selective 5-HT reuptake inhibitor (SSRI) treatment increases PVR in this model. We studied the hemodynamic effects of 5-HT, ketanserin (5-HT2A receptor antagonist), and sertraline, an SSRI, on pulmonary hemodynamics of the late gestation fetal sheep with PPHN caused by prolonged constriction of the ductus arteriosis. Brief intrapulmonary infusions of 5-HT increased PVR from 1.0 ± 0.07 (baseline) to 1.4 ± 0.22 mmHg/ml per minute of treatment (P < 0.05). Ketanserin decreased PVR from 1.1 ± 0.15 (baseline) to 0.82 ± 0.09 mmHg/ml per minute of treatment (P < 0.05). Sertraline increased PVR from 1.1 ± 0.17 (baseline) to 1.4 ± 0.17 mmHg/ml per minute of treatment (P = 0.01). In addition, we studied 5-HT production and activity in vitro in experimental PPHN. Compared with controls, pulmonary artery endothelial cells from fetal sheep with PPHN exhibited increased expression of tryptophan hydroxylase 1 and 5-HT production by twofold and 56%, respectively. Compared with controls, 5-HT2A R expression was increased in lung homogenates and pulmonary artery smooth muscle cell lysates by 35% and 32%, respectively. We concluded that increased 5-HT contributes to high PVR in experimental PPHN through activation of the 5-HT2A receptor and that SSRI infusion further increases PVR in this model.

Serotonin; selective serotonin reuptake inhibitors; pulmonary hypertension; newborn; ketanserin

IN UTERO, PULMONARY VASCULAR RESISTANCE (PVR) is greater than systemic vascular resistance (1, 62). At birth, PVR rapidly falls, allowing for an 8–10-fold increase in pulmonary blood flow (8, 11). Failure of PVR to decrease at birth causes hypoxemia due to extrapulmonary shunting of blood at the ductus arteriosis and foramen ovale, resulting in the clinical syndrome known as pulmonary hypertension of the newborn (PPHN) (41). Although the pathogenesis of PPHN is poorly understood, it is believed to be multifactorial in origin and includes altered NO-cGMP, endothelin (ET), and Rho kinase (ROCK) signaling, resulting in abnormal vascular reactivity, remodeling, and growth (2, 20, 21, 24, 58).

PPHN is a clinical syndrome, associated with a number of neonatal conditions, including meconium aspiration, sepsis, and abnormalities in lung structure (10, 54). In addition to these neonatal conditions, the use of maternal medications has been linked to the development of PPHN (6, 64, 65). Recently, maternal use of selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitors (SSRIs) after the 20th wk of gestation has been associated with the development of PPHN (9, 33, 37). Despite this association, little is known about serotonin signaling in the fetal and newborn pulmonary circulation.

Although it is not completely clear how SSRIs may cause PPHN, SSRIs are known to readily cross the placenta, which leads to the possibility that SSRIs can directly affect the fetus independent of maternal effects (28, 59). We have previously shown that acute intrapulmonary infusions of SSRIs in chronically instrumented fetal sheep cause potent and sustained pulmonary vasoinconstriction (12). However, several studies demonstrate that SSRI treatment attenuates pulmonary vascular remodeling and improves survival in adult rodent models of pulmonary hypertension (25, 42, 48, 63). Whether or not SSRI treatment causes pulmonary vasodilation or further elevates PVR in the setting of neonatal pulmonary hypertension is unknown.

5-HT is primarily produced in the enterochromaffin cells of the intestine. Circulating levels of 5-HT are extremely low, as 5-HT is primarily stored in platelets, which pool and release 5-HT via the serotonin transporter (SERT). Recently, it has been recognized that the lung synthesizes 5-HT in pulmonary neuroendocrine and pulmonary artery endothelial cells (PAECs) from tryptophan via the enzyme tryptophan hydroxylase 1 (Tph1) (17). Serotonin levels are increased in patients with idiopathic pulmonary arterial hypertension (iPAH) as well as in rodent models of PAH (7, 17, 29, 38). To date, 14 receptors for 5-HT have been identified. The 5-HT1B, 2A, and 2B receptors, as well as the SERT are all expressed in the adult lung, and alterations in their signaling have been linked to the development of PAH (26, 36, 40, 44, 45, 49). Serotonin intracellular signaling is complex and incompletely understood but is known to include activation of the mitogen activated protein kinase pathway, generation of reactive oxygen species, and ROCK activation (23, 43, 66).

We have previously shown that the 5-HT2A receptor is expressed in fetal ovine lung and that intrapulmonary infusions of ketanserin, the 5-HT2A receptor antagonist, increases pulmonary blood flow in chronically instrumented fetal sheep. These findings indicate that 5-HT contributes to high PVR in the normal fetus. Based on our prior work and the contribution of serotonin to the development of PAH in adults, we hypothesized that 5-HT and SSRIs would increase PVR and that blockade of the 5-HT2A receptor may increase pulmonary blood flow in fetal sheep with experimental PPHN. We further hypothesized that increased pulmonary serotonin production
and activity contribute to the pathogenesis of PPHN in this model.

MATERIALS AND METHODS

Pregnant, mixed-breed (Colombia-Rambouillet) ewes were used in this study. All procedures and protocols were reviewed and approved by the Animal Care and Use Committee of the University of Colorado Denver and followed the Guide for the Care and Use of Laboratory Animals established by the National Research Council.

Fetal Surgical Preparation

Surgery was performed between 124 and 129 days gestation (full term = 147 days) according to previously published methods (3). Under isoflurane inhalational anesthesia, the left fetal forelimb was exposed through a hysterotomy and a left thoracotomy was performed. Polyvinyl catheters (20 gauge) were placed in the left axillary artery and vein and advanced in the ascending aorta and superior vena cava, respectively. Using a 16-gauge intravenous placement unit (Angiocath; Travenol, Deerfield, IL), a 22-gauge catheter was placed through purse-string sutures in the left pulmonary artery (LPA) to allow for selective drug infusions. A 14-gauge intravenous placement unit (Angiocath) was used to place 20-gauge catheters in the main pulmonary artery (MPA) and left atrium. After gentle, blunt dissection of the bifurcation of the MPA, a flow transducer (Transonic Systems, Ithaca, NY) was placed around the LPA to measure blood flow to the left lung (QLPA). A cotton umbilical tie was placed around the ductus arteriosus and tied to cause constriction.

Western Blot Analysis

Western blot analysis for pulmonary artery smooth muscle cell (PASMC) and PAEC expression of Tph1 and 5-HT 2A R was performed by standard methods. Membranes were incubated overnight at 4°C with antibodies raised against the 5-HT 2A receptor (catalog no. sc-32538; Santa Cruz Biotechnology, Santa Cruz, CA; dilution 1:200), or Tph1 (catalog no. ab-78969; Abcam, Cambridge, MA; dilution 1:1000). The membranes for 5-HT 2A R were washed and incubated for 1 h at room temperature with donkey anti-goat IgG-horseradish peroxidase (HRP) (catalog no. sc-2033; Santa Cruz Biotechnology; 1:4,000 dilution). Immunocomplexes were visualized using the Enzyme-linked Immunosorbent Assay (ELISA) kit (catalog no. Biorad 1706515; Bio-Rad, Hercules, CA; Biotechnology; 1:4,000 dilution). The membranes for Tph1 were washed and incubated for 1 h at room temperature with goat anti-rabbit HRP (catalog no. Biorad 1706515; Bio-Rad, Hercules, CA; 1:2000 dilution). Immunocomplexes were visualized using the Enhanced Chemiluminescence Plus kit and identified by molecular weight as designated by the manufacturer. Membranes were stripped and reprobed with an antibody to β-actin (catalog no. A5316; Sigma, St. Louis, MO). Densitometry was performed using NIH Image J software. Changes in protein expression were analyzed after normalization for β-actin expression.

Serotonin ELISA Assay

ELISA was performed using the GenWay 5-HT ELISA kit (catalog no. 40 –371-25002; GenWay Biotech, San Diego, CA), according to the manufacturer’s instructions. Briefly, PAECs from control (N = 3) and PPHN (N = 4) lambs were grown on 150-mm dishes in DMEM supplemented with 10% fetal bovine serum to 80–90% confluence. The supernatant was collected and stored in −20°C and cell number was recorded. The 5-HT ELISA assay was performed in triplicate, and 5-HT signal was determined by measurement of absorbance at 405 nm using a microplate spectrophotometer. Differences in absorbance between normal and PPHN PAECs were measured and quantified.

Drug Preparation

A solution of 5-HT, serotonin creatinine sulfate monohydrate complex (3 µg/ml, Sigma H7752) was made immediately before each study by dissolving the drug in normal saline. Ketanserin (50 mg/ml DMSO, Sigma S006) solution was made immediately before each experiment. Sertraline hydrochloride (20 mg/ml DMSO, Sigma S6319) was made and stored at −20°C.

Study Design

Physiological studies were performed at least 5 days after surgery. During each study, pulmonary arterial, aortic, and left atrial pressures were measured by connecting externalized catheters to computer-driven pressure transducers (model MP100A; Biopac Systems, Santa Barbara, CA). Pressure measurements were referenced to simultaneously recorded amniotic pressure. The flow transducer was connected to an internally calibrated flowmeter (Transonsics Systems) to measure flow to the left lung (QLPA). Before infusion of study drugs, a 30-min period of stable baseline hemodynamics was established. Hemodynamic variables, including main pulmonary artery pressure (MPAP), left atrial pressure (LAP), aortic pressure (AoP), and left pulmonary artery blood flow (QLPA), were measured continuously for the duration of each study protocol and recorded every 10 min. Left lung PVR was calculated as follows: PVR = (MPAP − LAP)/QLPA. Heart rate (HR) was determined from phasic pressure traces. Arterial blood gas measurements were obtained before and after each drug infusion and included pH, PCO2, and PO2 (modelABL 800; Radiometer, Copenhagen, Denmark).

Experimental Design

Protocol 1: pulmonary hemodynamic effects of 5-HT in experimental PPHN. The purpose of this study was to investigate the pulmonary effects of selective 5-HT infusions. Baseline hemodynamic measurements were recorded every 10 min for QLPA, MPAP, AoP, LAP, and HR. After baseline measurements were stable for a 30-min period, 5-HT (12–20 µg) was infused into the LPA over 20 min. Hemodynamic measurements were recorded every 10 min for 40 min after the infusion concluded. Arterial blood gas tensions were obtained at baseline and at the conclusion of each infusion.

Protocol 2: pulmonary hemodynamic effects of ketanserin, the 5-HT 2A receptor blocker. The purpose of these studies was to investigate whether blockade of the 5-HT 2A receptor decreases PVR and increases pulmonary blood flow. Baseline hemodynamic measurements were recorded every 10 min for QLPA, MPAP, AoP, and HR. After baseline measurements were stable for a 30-min period, ketanserin (20 mg) was infused into the LPA over 20 min. Hemodynamic measurements were recorded every 10 min for 40 min after the infusion concluded. Arterial blood gas tensions were obtained at baseline and at the conclusion of each infusion.

Protocol 3: pulmonary hemodynamic effects of the SSRI sertraline in experimental PPHN. The purpose of these studies was to determine the pulmonary hemodynamic effects of SSRIs in a model of PPHN. Baseline hemodynamic measurements were recorded every 10 min for QLPA, MPAP, AoP, LAP, and HR. After baseline measurements were stable for a 30-min period, sertraline (10 mg) was infused into the LPA over 20 min. Hemodynamic measurements were recorded every 10 min for 40 min after the infusion concluded. Arterial blood gas tensions were obtained at baseline and at the conclusion of each infusion.

Protocol 4: effects of ROCK inhibition on the hemodynamic response of exogenous 5-HT administration. The purpose of this protocol was to determine whether in this model of PPHN serotonin induces pulmonary vasoconstriction through ROCK activation. The hemodynamic response of 5-HT (20 µg) infusion alone was compared with the infusion of fasudil (100 µg) alone and the combination of fasudil (100 µg) and 5-HT (20 µg).

Protocol 5: 5-HT production and Tph1 protein content in fetal PAEC isolated from control and PPHN fetal sheep. The purpose of this study was to determine whether 5-HT synthesis is increased in PAEC isolated from fetal sheep with experimental PPHN. Using ELISA, we measured 5-HT levels in the supernatant from control (N = 3) and PPHN (N = 4) PAECs grown to 80–90% confluence in
150-mm dishes. 5-HT content was normalized to cell number. To determine whether Tph1 protein is increased in experimental PPHN, PAEC lysates were collected from 80–90% confluent cells grown between passages 2 and 4 from control (N = 9) animals and animals with experimental PPHN (N = 9). Tph1 protein content was measured using Western blot, and the values were normalized to β-actin.

Protocol 6: expression of 5-HT2A R in whole lung and isolated PASMC from control and PPHN fetal sheep. Whole lung lysates were collected from the ovine fetus (gestational age 136–143 days) with (N = 4) and without (N = 4) experimental PPHN. PASMC cell lysates were collected from 80–90% confluent cells between passages 2 and 4 from control animals (N = 6) and animals with experimental PPHN (N = 6). 5-HT2A R protein expression was measured by Western blot analysis, and values were normalized for β-actin expression.

**Statistical Analysis**

Statistical analysis for hemodynamic variables was performed using SAS version 9.2 (SAS Institute). Hemodynamic variables over time were compared using repeated-measures ANOVA with Fisher’s least-squares difference for individual means comparison. Data measured once were analyzed using unpaired t-tests and paired t-tests. Data are presented as means ± SE. The significance level was set at P < 0.05.

**RESULTS**

**Protocol 1: Pulmonary Hemodynamic Effects of Brief Serotonin Infusion in Experimental PPHN**

Brief intrapulmonary infusions of 5-HT (12–20 µg) had no effect on MPAP, 67 ± 2.7 mmHg (baseline) to 67 ± 2.8 mmHg, or AoP, 42 ± 2.9 mmHg (baseline) to 44 ± 2.2 mmHg (treatment). Infusions of 5-HT decreased pulmonary blood flow (QLPA) by 15% (67 ± 4 vs. 57 ± 5 ml/min, P < 0.05) and increased PVR by 40% from baseline (1.0 ± 0.07 vs. 1.4 ± 0.22 mmHg/ml per min, P < 0.05) (Fig. 1). The effects of 5-HT were short-lived as QLPA and PVR returned to baseline shortly after the end of the infusion. HR decreased from 176 ± 4 (baseline) to 159 ± 4 beats per minute (bpm) at the end of the infusion (P < 0.05), and pH decreased from 7.37 ± 0.02 to 7.36 ± 0.02, P < 0.05. PaO2 and PaCO2 did not change in response to 5-HT.

**Protocol 2: Pulmonary Hemodynamic Effects of the 5-HT2A Receptor Blocker, Ketanserin**

Brief intrapulmonary infusions of ketanserin (20 mg), a 5-HT2A receptor antagonist, decreased MPAP from 67 ± 2.7 to 65 ± 3.2 mmHg, P < 0.05 (Fig. 2). AoP decreased during the infusion (from 38 ± 2.3 to 34 ± 2.5 mmHg, P < 0.05) but returned to baseline values for the remainder of the study. Ketanserin increased pulmonary blood flow (QLPA) by 27% (64 ± 7 vs. 81 ± 10 ml/min, P < 0.05, Fig. 2) and decreased PVR by 26%, from 1.1 ± 0.15 at baseline to 0.82 ± 0.09 mmHg/ml per min (P = 0.05 Fig. 2). Ketanserin did not change HR or arterial blood gas tensions.

**Protocol 3: Pulmonary Hemodynamic Effects of the SSRI Sertraline in Experimental PPHN**

Pulmonary artery pressure (67 ± 5.4 to 75 ± 4 mmHg; P < 0.05) and systemic pressures (39 ± 3.1 to 44 ± 3.1 mmHg; P < 0.05) were significantly increased following the infusion of sertraline (Fig. 3). Infusions of sertraline decreased QLPA by 7% (65 ± 5.9 vs. 61 ± 4.6 ml/min, P < 0.05) (Fig. 3) and increased PVR by 30% (Fig. 3) from 1.08 ± 0.17 (baseline) to 1.40 ± 0.17 mmHg/ml per min (40 min), P < 0.05. The pulmonary vasoconstrictor response to sertraline was sustained for at least 80 min after the completion of the infusion. Acute infusions of sertraline did not alter HR (169 ± 9 at baseline vs. 187 ± 13 bpm). Direct intrapulmonary infusions of sertraline decreased pH (7.4 ± 0.008 vs. 7.2 ± 0.016; P < 0.001) and increased PCO2 (48 ± 2 vs. 55 ± 2.2 Torr; P < 0.05). Acute infusions of sertraline did not alter Po2 (18 ± 1.4 vs. 16 ± 1.1 Torr).

**Protocol 4: Effects of ROCK Inhibition on the Hemodynamic Response of Exogenous 5-HT Administration**

The hemodynamic response of 5-HT (20 µg) infusion alone was compared with the infusion of fasudil (100 µg) alone and the combination of fasudil (100 µg) and 5-HT (20 µg). The infusion of 5-HT alone increased PVR by 30% (Fig. 4).
infusion of fasudil alone decreased PVR by 30% (Fig. 4). However, in combination with 5-HT, fasudil had no effect on PVR. Acute intrapulmonary infusions of 5-HT in combination with fasudil resulted in a 38% increase in PVR (Fig. 4).

Protocol 5: Tph1 Expression and 5-HT Content in Fetal PAECs Isolated from Normal and PPHN Sheep

Compared with controls, PAECs from fetal sheep with PPHN exhibited increased expression of Tph1 and increased production of 5-HT. Western blot analysis of PAEC lysates from normal and PPHN fetal sheep demonstrated a doubling of Tph1 protein in PAECs from PPHN animals ($P < 0.05$; Fig. 5A). As determined by ELISA, 5-HT content in the media of PAECs grown to 80–90% confluence was increased 56% in PPHN PAECs compared with control PAECs ($P < 0.05$; Fig. 5B).

Protocol 6: Expression of 5-HT 2A R in Whole Lung Homogenates and Isolated PASMC from Control and PPHN Fetal Sheep

Compared with those isolated from control fetal sheep, whole lung lysates (Fig. 6A; $P < 0.01$) and PASMC lysates (Fig. 6B; $P < 0.01$) isolated from PPHN fetal sheep had increased expression of the 5-HT 2A R by 35% and 32%, respectively.
DISCUSSION

Maternal SSRI use is associated with an increased risk for the development of PPHN, yet SSRIs protect against the development of pulmonary hypertension in adults (9, 33, 35, 37, 63). The mechanisms through which altered 5-HT signaling causes PPHN remain unclear, and little is known about the role of serotonin in the developing lung. Based on our previously published findings that serotonin and SSRIs are potent pulmonary vasoconstrictors in the normal fetus and that serotonin contributes to the development of PAH in adults, we hypothesized that 5-HT and SSRIs increase fetal PVR and that blockade of the 5-HT2A receptor may increase pulmonary blood flow in fetal sheep with experimental PPHN. We further hypothesized that increased pulmonary serotonin production and activity contributes to the pathogenesis of PPHN in this model. We report that acute intrapulmonary infusions of serotonin and SSRIs further increase fetal PVR and that blockade of the 5-HT2A receptor causes pulmonary vasodilation in experimental PPHN. In contrast to our findings in control animals, infusions of the ROCK inhibitor, fasudil, did not attenuate 5-HT-mediated pulmonary vasoconstriction. In addition, we found that PAEC synthesis of serotonin and SSRIs further increase fetal PVR and that blockade of the 5-HT2A receptor causes pulmonary vasodilation in experimental PPHN. In contrast to our findings in control animals, infusions of the ROCK inhibitor, fasudil, did not attenuate 5-HT-mediated pulmonary vasodilatation. In addition, we found that PAEC synthesis of serotonin and SSRIs further increase fetal PVR and that blockade of the 5-HT2A receptor causes pulmonary vasodilation in experimental PPHN. In contrast to our findings in control animals, infusions of the ROCK inhibitor, fasudil, did not attenuate 5-HT-mediated pulmonary vasodilation.

These findings are interesting, as this is the first study to show that acute and selective pulmonary infusions of the SSRI sertraline cause potent and sustained pulmonary vasoconstriction in animals with experimental PPHN. The novel finding that fetal PAECs synthesize serotonin and that serotonin synthesis is increased in experimental PPHN suggests that serotonin signaling may contribute to altered pulmonary vascular tone seen in this model of experimental PPHN.

Serotonin is a signaling molecule with a number of diverse biological functions. Although it is mainly studied for its role in the central nervous system, the majority of serotonin is located in the systemic circulation, where it has numerous physiological roles including the regulation of intestinal motility, vascular tone, and platelet aggregation. The serotonin receptors are classified into seven different subfamilies, which have many different subtypes, accounting for the diverse physiological effects of serotonin (34). In addition to the 5-HT receptors, the SERT is also known to contribute to the physiological effects of 5-HT (25, 45, 50). Previous studies have shown that the 5-HT receptors 1B, 2A, and 2B as well as the SERT mediate serotonin signaling in the adult lung (26, 36, 40, 44, 45, 49).

Previous laboratory and clinical studies have strongly implicated 5-HT in the pathogenesis of adult pulmonary hypertension (15, 16, 26, 45). Specifically, clinical studies demonstrate that patients with iPAH have increased plasma levels of 5-HT (29) and that 5-HT production and Tph1 expression are increased in experimental PPHN (26, 36). Western blot analysis of PAEC lysates from control and PPHN PAECs demonstrated a doubling in Tph1 protein in PAECs from PPHN lambs (Fig. 5A; P < 0.05). As determined by ELISA, 5-HT synthesis was increased 56% in PPHN PAECs (P < 0.05; B). NML, normal.

![Fig. 4. Fasudil does not inhibit 5-HT-induced pulmonary vasoconstriction. Pulmonary vascular response to the infusion of 5-HT alone, fasudil (Rho kinase inhibitor) alone, or 5-HT in combination with fasudil, expressed as percentage of change from baseline PVR. Infusions of 5-HT resulted in a 30% increase in PVR; this was not attenuated by the coadministration of fasudil.](http://ajplung.physiology.org/)

![Fig. 5. Tryptophan hydroxylase 1 (Tph1) expression and 5-HT content are increased in experimental PPHN. Compared with controls, pulmonary artery epithelial cells (PAECs) from PPHN lambs exhibited increased expression of Tph1 and increased production of 5-HT. Western blot analysis of PAEC lysates from control and PPHN PAECs demonstrated a doubling in Tph1 protein in PAECs from PPHN lambs (P < 0.05; A). As determined by ELISA, 5-HT synthesis was increased 56% in PPHN PAECs (P < 0.05; B). NML, normal.](http://ajplung.physiology.org/)
intrapulmonary infusions of SSRIs result in profound pulmonary hypertension (9, 33, 37). We have previously shown that acute treatment with SSRIs, particularly when ingested during the second half of gestation (9, 33, 37), results in a sixfold increase in the incidence of PPHN in infants exposed to SSRIs. Indeed, clinical studies at the Mayo Clinic have reported that treatment of pregnant rats results in increased mortality in rat pups and pulmonary vascular remodeling. In this study, we found that, in contrast to findings in adult models of PAH, treatment with SSRIs in a model of PPHN results in further increases in pulmonary vascular tone, with no evidence for vasodilation.

Although we have demonstrated that SSRIs induce pulmonary vasoconstriction in an experimental model of PPHN, the mechanisms underlying these findings are not clear. Investigators have proposed that SSRIs induce ductal constriction, resulting in PPHN; our previous findings, however, do not support this hypothesis (12). SSRIs do increase extracellular levels of serotonin, and we have previously reported that pretreatment with ketanserin abolishes SSRI-mediated vasoconstriction, leading us to speculate that SSRI-induced vasoconstriction occurs via increased extracellular serotonin and activation of the 5-HT<sub>2A</sub> receptor (12).

Pharmacological blockade of the 5-HT<sub>2A</sub> receptor blocks the development of PAH in rodents with monocrotaline-induced pulmonary hypertension (30). The use of the 5-HT<sub>2A</sub> receptor antagonist, ketanserin, in adults with pulmonary hypertension had modest effects on lowering PVR, but this use has been limited by systemic hypotension (47). Few studies have been performed in the fetus or neonate to elucidate which serotonin receptor subtype is involved in serotonin-mediated vasoconstriction at this developmental stage. In 1998, Morecroft et al. (51) reported that the predominant receptor subtype that mediates vasoconstriction in isolated rabbit pulmonary arteries is the 5-HT<sub>2A</sub> receptor. Recently Goyal (22) reported that the 5-HT<sub>2A</sub> receptor is the predominant 5-HT receptor involved in 5-HT-induced constriction in isolated ovine fetal pulmonary arteries exposed to maternal long-term hypoxia. Our findings, as well as findings by MacLean and Goyal, support that the 5-HT<sub>2A</sub> receptor is the predominant receptor that regulates serotonin-induced vasoconstriction in the perinatal circulation.

Serotonin induces pulmonary vasoconstriction and vascular remodeling via activation of several downstream cellular targets, including MAP Kinase 67, the generation of reactive oxygen species, and ROCK activation (23, 31, 43, 27, 46). We have previously shown that ROCK inhibition attenuates 5-HT-mediated pulmonary vasoconstriction in the normal fetus. However, in this model of PPHN, fasudil did not block 5-HT-induced vasoconstriction. However, when infused alone, fasudil did decrease basal PVR by 30%. We speculate that, although ROCK activation contributes to high PVR in PPHN, 5-HT-induced constriction is not mediated through ROCK in this experimental model of PPHN. This may be due to already enhanced 5-HT-ROCK activity or other mechanisms that will be addressed in future studies.

One potential limitation of this study is the use of fetal PAsECs harvested from proximal vessels and the possibility that the behavior of these cells may differ from PAsECs harvested from more distal vessels. Future studies are needed to evaluate serotonin production and signaling in the pulmonary microvascular circulation.

In conclusion, 5-HT and SSRIs further increase PVR in an ovine model of PPHN, and blockade of the 5-HT<sub>2A</sub> receptor decreases PVR in this model. In addition, experimental PPHN results in increased pulmonary endothelial cell production of serotonin and increased pulmonary expression of Tph1 and the

**Fig. 6.** 5-HT<sub>2A</sub> receptor expression is increased in PPHN. Increased protein expression of the 5-HT<sub>2A</sub> receptor in whole lung lysates (A; p < 0.05) and pulmonary artery smooth muscle cells (PASMCs) (B; p < 0.01) isolated from fetal sheep with experimental PPHN.

Increased 5-HT<sub>2A</sub> receptor expression is associated with a reduction in the risk of death in infants exposed to SSRIs, particularly when ingested during the second half of gestation (9, 33, 37). We have previously shown that acute intrapulmonary infusions of SSRIs result in profound pulmonary hypertension, and Fornaro et al. (12, 18) have reported that treatment of pregnant rats results in increased mortality in rat pups and pulmonary vascular remodeling. In this study, we found that, in contrast to findings in adult models of PAH, treatment with SSRIs in a model of PPHN results in further increases in pulmonary vascular tone, with no evidence for vasodilation.

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In conclusion, 5-HT and SSRIs further increase PVR in an ovine model of PPHN, and blockade of the 5-HT<sub>2A</sub> receptor decreases PVR in this model. In addition, experimental PPHN results in increased pulmonary endothelial cell production of serotonin and increased pulmonary expression of Tph1 and the
5-HT$_{2A}$ R. We speculate that altered serotonin signaling contributes to the pathogenesis of PPHN and that manipulation of serotonin signaling may be an attractive therapeutic target for the treatment of newborns with pulmonary hypertension.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


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