Increased Susceptibility to Pulmonary Hypertension in Heterozygous BMPR2-Mutant Mice

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Background—Bone morphogenetic protein receptor-2 (BMPR2)–heterozygous, mutant (BMPR2+/−) mice have a genetic trait similar to that of certain patients with idiopathic pulmonary arterial hypertension (IPAH). To understand the role of BMPR2 in the development of IPAH, we examined the phenotype of BMPR2+/− mice and their response to inflammatory stress.

Methods and Results—BMPR2+/− mice were found to have the same life span, right ventricular systolic pressure (RVSP), and lung histology as those of wild-type mice under unstressed conditions. However, when treated with recombinant adenovirus expressing 5-lipoxygenase (Ad5LO), BMPR2+/− mice exhibited significantly higher RVSP than wild-type mice. The increase of RVSP occurred in the first 2 weeks after Ad5LO delivery. Modest but significant muscularization of distal pulmonary arterioles appeared in BMPR2+/− mice 4 weeks after Ad5LO treatment. Measurement of urinary metabolites of vasoactive molecules showed that cysteinyl leukotrienes, prostacyclin metabolites, and PGE2 were all increased to a similar degree in both BMPR2+/− and wild-type mice during SLO transgene expression, whereas urinary endothelin-1 remained undetectable. Urinary thromboxane A2 metabolites, in contrast, were significantly higher in BMPR2+/− than in wild-type mice and paralleled the increase in RVSP. Platelet activation markers, serotonin, and soluble P-selectin showed a trend toward higher concentrations in BMPR2+/− than wild-type mice. Cell culture studies found that BMP treatment reduced interleukin-1β–stimulated thromboxane A2 production in the pulmonary epithelial cell line A549.

Conclusions—BMPR2+/− mice do not develop pulmonary hypertension spontaneously; however, under inflammatory stress, they are more susceptible to an increase in RVSP, thromboxane A2 production, and vascular remodeling than wild-type mice. (Circulation. 2005;112:553-562.)

Key Words: inflammation ■ hypertension, pulmonary ■ vasoconstriction ■ bone morphogenetic proteins ■ thromboxane
postnatal expression of the mutant BMPR2 gene in smooth muscle leads to a significant increase of right ventricular systolic pressure (RVSP) and relatively modest pulmonary vascular remodeling in mice.

In the present study, we examined the phenotype of BMPR2+/− mice with respect to (1) their survival rate during prenatal development, (2) their RVSP and lung histology in the basal state, and (3) their responses to inflammatory stress resulting from adenovirus-mediated pulmonary overexpression of 5-lipoxygenase (5LO). Inflammation has been previously suggested to be one of the mechanisms involved in the development of PAH (for reviews, see Voelkel et al.,17 Tuder and Voelkel,18 Jeffery and Morrell,19 and Dorfmuller et al.20). 5LO catalyzes leukotriene formation and facilitates an inflammatory process.21 Increased 5LO expression or leukotriene production has been demonstrated in patients with PAH and an animal model of PAH.22-24 Thus, using this inflammatory mediator as a stimulus may yield useful information about the role of inflammation in the development of pulmonary hypertension in individuals with BMPR2 haploinsufficiency.

Methods

Animals
BMPR2-mutant mice were established by Beppu and colleagues15 via targeted gene disruption. Because homozygous BMPR2-mutant mice die in utero, breeding of BMPR2+/− mice was carried out by crossing BMPR2+/− mice with wild-type mice (C57/B16 mice). Genotyping of the offspring was performed after weaning. The mutated BMPR2 allele in the mice was identified by a polymerase chain reaction with primer pairs 5′-GCTAA AGGCG ATGCT CCAGA CTCGCC TTG-3′ and 5′-AGGTG GGCTG GGAAC CTGCTAA AGCGC ATGCT-3′. Amplification conditions were as follows: 1 cycle of 120 seconds at 95°C; 30 cycles of 30 seconds at 95°C, 30 seconds at 63°C, and 90 seconds at 72°C; and 5 minutes at 72°C. The mice were maintained at the Boston University Laboratory Animal Science Center under stress-free conditions, i.e., unlimited food and water access, regular light cycles (12-hour/12-hour light:dark), no forced exercise, and generally pathogen-free. All animals received humane care. The study was approved by the Institutional Animal Care and Use Committee at Boston University.

Right Heart Catheterization
Mice underwent right heart catheterization at various time points during the study. Before catheterization, mice were anesthetized with ketamine (90 mg/kg)/xylazine (6 mg/kg) and given 1% lidocaine by local injection into the midline neck region. Surgical exposure of the trachea was then performed through a midline incision and subsequent blunt dissection to the level of the trachea. A 29-gauge needle bent at a 60° angle was inserted into the trachea, and 2×10^6 plaque forming units (PFU) of Ad5LO was slowly instilled into the trachea. After instillation, the neck incision was closed with No. 4 silk surgical suture. Buprenorphine, 0.1 mg/kg sc, was given every 8 to 12 hours after the procedure for 3 doses, and animals were monitored until full recovery from anesthesia before returning them to their regular cages.

RNA Isolation and Northern Blotting
Mouse lungs were perfused with saline and homogenized immediately in 10 mL TRIzol reagent (Invitrogen) followed by chloroform extraction for total RNA. Northern blotting was carried out by electrophoresing 20 μg RNA in 1.2% agarose gels containing 4% formaldehyde. The RNA was transferred onto nitrocellulose membranes and hybridized with an [α-32P]dCTP-labeled 5LO probe that encompasses nucleotides 45 to 290 of human 5LO cDNA (GenBank accession No. J03600). Hybridization was carried out at 68°C overnight in MiracleHyb solution (Stratagene) containing 10 μg/mL sheared salmon sperm DNA. The membrane was washed with 2× saline sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) at room temperature for 15 minutes and then with 0.1× SSC/0.1% SDS at 68°C for 20 minutes before exposure to X-ray film. The membrane was then washed in 0.1× SSC/0.1% SDS at 100°C twice for 15 minutes to strip the bound 5LO probe and hybridized with a [α-32P]dCTP-labeled mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe (Ambion) to estimate total RNA loading.

Urine Collection and Measurement of Urinary Creatinine
Urine samples were collected from the mice 2 days before Ad5LO treatment (day −2) and on days 3, 7, 12, and 21 after treatment. During the collection, mice were maintained in metabolic cages with water supplied but food withheld for 16 hours. Urine samples were collected during the 16-hour period, and the mice were returned to their regular cages after collection. The urine samples were centrifuged at 20,000g for 5 minutes, and the supernatants were stored in aliquots at −80°C.
Urinary creatinine concentration was determined by the picric acid assay and creatinine assay reagents from Teco Diagnostics. In this assay, 0.01 mL creatinine standards (1.25, 2.5, 5, and 10 mg/mL) or urine samples (diluted 5- to 15-fold in water) were added to a 96-well plate and mixed with 0.2 mL of creatinine picric acid reagent and creatinine buffer reagent (mixed before assay at 1:1, v/v). The plate was incubated at 37°C for 40 minutes and read at 490 nm. The absorbance from this reading was registered as the total absorbance. Acetic acid (0.01 mL) was then added to each well, and the plate incubated at room temperature (22°C to 25°C) for 15 minutes. The plate was read again at 490 nm, and the absorbance from the second reading (nonspecific absorbance) was subtracted from the first reading (total absorbance). The absorbance difference (specific absorbance) was used to construct the standard curve and calculate the sample urinary creatinine concentration.

Urinary Vasoactive Metabolites

The production of cysteinyl leukotrienes, prostacyclin, prostaglandin (PG) E₂, endothelin-1, and thromboxane (Tx) A₂ in mice was determined by analyzing the urinary concentration of the products or their metabolites by ELISA. For cysteinyl leukotriene measurements, an ELISA kit detecting leukotrienes C₄, D₄, and E₄ from Assay Designs (No. 900-070) was used. Urine samples were diluted a minimum 20-fold for the assay to avoid nonspecific binding. For urinary prostacyclin measurements, an ELISA kit detecting 6-keto-PGF₁α and 2,3-dinor-6-keto-PGF₁α, 2 major metabolites of prostacyclin in urine, was used (Assay Designs No. 901-025). Urine samples were diluted a minimum of 40-fold for these assays. For PGE₂ measurements, a high-sensitivity ELISA kit detecting PGE₂ from Assay Designs (No. 930-001) was used. Urine samples were diluted a minimum of 20-fold for the assay. For endothelin-1 measurements, an ELISA kit detecting endothelin-1, -2, and -3 was used (No. 583151, Cayman Chemical). Urine samples were diluted a minimum of 40-fold for these assays. For PGF₁α measurement, cells were washed with Dulbecco’s phosphate buffered saline (D-PBS) and incubated with 0.01 mmol/L arachidonic acid in D-PBS for 15 minutes at 37°C. The released TXB₂ in the assay medium was analyzed by ELISA as described earlier. Total cell protein concentration was determined with DC protein assay reagents from Bio-Rad.

Statistics

Data are presented as the mean±SEM. Statistical analysis was performed by χ² analysis (the Table), Student t test (Figures 4, 10, and 11), or 2-way ANOVA (Figures 5 and 7–9) with the SigmaStat program. P<0.05 indicated statistical significance.

Results

BMPR2+/− Mice Breeding

BMPR2+/− mice were bred by crossing BMPR2+/− with wild-type mice (C57/BL6 mice). The genotypes of the offspring were determined after weaning. The distribution of BMPR2+/− and BMPR2+/+ genotypes in the offspring was significantly different, 498 and 618, respectively (χ²= 12.9, P<0.001). No statistical difference exists between female and male distribution in either BMPR2+/− or BMPR2+/+ offspring.

Lung Structure and RVSP in BMPR2+/− Mice

To assess lung development in the BMPR2+/− mice, lung tissue sections from BMPR2+/− and wild-type mice were prepared and stained with hematoxylin and eosin. As shown in Figure 1, no apparent gross abnormalities were observed in the sections from either mouse type, although a minor increase in adhesion of leukocytes to the vessel wall was noted in the BMPR2+/− mice (Figure 1B).

The pulmonary artery pressure in the BMPR2+/− mice was assessed by measuring RVSP via right heart catheterization. In the absence of pulmonary valve disease, RVSP is equivalent to the pulmonary artery systolic pressure. As shown in Figure 2, the RVSP in BMPR2+/− mice was similar to that of wild-type mice from 2 to 18 months of age, indicating that BMPR2+/− mice did not develop pulmonary hypertension spontaneously under unstressed conditions.

Expression of 5LO in Lungs of Mice

To examine the responses of BMPR2+/− mice to pulmonary inflammation, recombinant human 5LO was expressed in the lungs of mice via adenovirus-mediated gene transfer. Ap-
proximately 2×10⁶ PFU of replication-deficient adenovirus expressing recombinant human 5LO (Ad5LO) was administered to the mice via intratracheal instillation. The time course of transgene expression was examined by Northern blotting. As shown in Figure 3, expression of 5LO peaked at day 7 after Ad5LO delivery and decreased significantly by day 21. No difference in transgene expression was found between wild-type and BMPR2⁻/⁻ mice.

**RVSP of Mice After Receiving Ad5LO**

The RVSP of wild-type and BMPR2⁻/⁻ mice was examined initially at day 12 after administration of Ad5LO and compared with that of mice that received adenovirus-expressing green fluorescence protein (AdGFP). The choice of time point was based on a previous study, which showed that rats receiving Ad5LO and monocrotaline treatment developed pulmonary hypertension 10 days later.²⁶ As shown in Figure 4, RVSP was significantly increased in Ad5LO-treated BMPR2⁻/⁻ mice compared with those with no treatment (23 ± 1 mm Hg) and became normal at day 12 (13 ± 1 mm Hg). The RVSP response of BMPR2⁻/⁻ mice was not absolutely specific for exogenous 5LO expression, because the mice that received AdGFP had an elevated RVSP as well, though to a significantly milder degree (Figure 4). Adenovirus-mediated gene transfer is known to cause inflammation. Thus, the increase in RVSP found in BMPR2⁻/⁻ mice likely reflects a general response to inflammation, which is intensified by 5LO overexpression. Wild-type mice exhibited little increase in RVSP under either Ad5LO or AdGFP treatment, suggesting that BMPR2⁻/⁻ mice are more sensitive to an inflammation-mediated RVSP increase than are wild-type mice.

To investigate the time course of change of RVSP in the mice, BMPR2⁻/⁻ or wild-type mice were treated with Ad5LO and examined by right heart catheterization at days 0, 3, 7, 10, 12, 14, or 21 after Ad5LO delivery. As shown in Figure 5, the increase of RVSP in BMPR2⁻/⁻ mice peaked at day 7 (33 ± 3 mm Hg) and returned to near normal by day 21 (14 ± 2 mm Hg, compared with day 0, 12 ± 1 mm Hg). The RVSP of wild-type mice increased mildly at day 7 (18 ± 2 mm Hg) and became normal at day 12 (13 ± 1 mm Hg). The increase of RVSP in the BMPR2⁻/⁻ mice was consistent with the time course of transgene expression (shown in Figure 3) and was significantly higher than that of wild-type mice.

To determine whether the change in RVSP was associated with pulmonary vascular remodeling, immunohistochemical staining for smooth muscle α-actin was carried out in lung tissue sections prepared from wild-type and BMPR2⁻/⁻ mice after Ad5LO delivery. As shown in Figure 6, lung sections obtained at day 7 after Ad5LO delivery showed no sign of vascular remodeling in either type of mouse, although significant inflammatory cell infiltration was present. Increased muscularization of distal pulmonary arterioles was found in lung sections obtained at day 28 after Ad5LO treatment. Counting the α-actin-stained distal pulmonary arterioles (vessels that are located distal to terminal bronchioles, adjacent to the alveolar duct, noncollapsed, and with diameters <40 μm) showed that BMPR2⁻/⁻ mouse lungs had a significantly greater number of muscularized vessels than lungs from wild-type mice (36 ± 3 versus 7 ± 1) (Figure 6G). The muscularized distal arterioles accounted for ≈10% of total distal arterioles in the BMPR2⁻/⁻ lungs. The degree of muscularization (thickness of the muscle layer), however, was mild. Because this increase in muscularization occurred after the maximal increase in RVSP, the increased RVSP observed in BMPR2⁻/⁻ mice in the first 2 weeks after Ad5LO delivery was mild.
delivery was not due to pulmonary vascular remodeling but rather to enhanced pulmonary vasoconstriction.

**Generation of Vasoactive Molecules During 5LO Overexpression**

To understand the molecular basis of the enhanced pulmonary vasoconstriction in BMPR2+/− mice during 5LO expression, urine samples were collected from the mice 2 days before (day −2) and 3, 7, 12, and 21 days after Ad5LO delivery. Urinary levels of several vasoactive molecules, including cysteinyl leukotrienes, prostacyclin metabolites, PGE2, TxB2, and endothelin-1, were determined. As shown in Figure 7, the concentration of cysteinyl leukotrienes in urine was undetectable before Ad5LO delivery (day −2) but was markedly increased after 5LO expression. The production of cysteinyl leukotrienes peaked at day 7 (≈20 000 pg/mg cysteinyl leukotrienes/urinary creatinine) and diminished by day 21. There was no difference between wild-type and BMPR2+/− mice in cysteinyl leukotriene production.

Urinary levels of prostacyclin metabolites, 6-keto-PGF1α and 2,3-dinor-6-keto-PGF1α, were next measured. As shown in Figure 8A, the basal level of urinary prostacyclin metabolites in BMPR2+/− and wild-type mice was similar, ≈10 000 pg/mg (prostacyclin metabolites/urinary creatinine). The prostacyclin concentration was increased by 3- to 3.5-fold at days 3 and 7 after Ad5LO treatment and returned to the basal level by day 12. The increase was similar in BMPR2+/− and wild-type mice.

Urinary levels of PGE2, a pulmonary vasodilator, in BMPR2+/− and wild-type mice are shown in Figure 8B. The basal level of the compound in the 2 types of mice was 2200 to 4500 pg/mg (PGE2/urinary creatinine). During 5LO overexpression in the mice, the concentration increased to 16 000 to 20 000 pg/mg at days 3 and 7 and returned to basal level by day 12. There was no significant difference in PGE2 production between the wild-type and BMPR2+/− mice. Interestingly, the pattern of PGE2 production in the mice was comparable to that of prostacyclin, which suggests that the increased production of these compounds was due to activa-
tion of an upstream enzyme(s) in PG synthesis, such as cyclooxygenase-2.27

To determine whether the increased RVSP in the BMPR2+/− mice was due to overproduction of vasoconstrictive molecules, the generation of endothelin-1 and TxA2 in mice during 5LO expression was examined. Results showed that urinary endothelin-1 was undetectable in these mice either before or after Ad5LO delivery, indicating no marked increase in the production of this vasoactive peptide in these mice.

TxA2 production in the mice was assessed by measuring urinary concentrations of TxB2 and 11-dehydro-TxB2, metabolites of TxA2. As shown in Figure 9A, the basal level of urinary TxB2 in both wild-type and BMPR2+/− mice was ≈30 000 pg/mg (TxB2/creatinine). During 5LO expression, TxB2 concentration in the BMPR2+/− mice increased to 71 000, 116 000, 91 000, and 59 000 pg/mg at days 3, 7, 12, and 21, respectively. The change in Tx production in wild-type mice, by contrast, was less striking and did not reach statistical significance compared with pretreatment controls, with values of 47 000, 60 000, 39 000, and 24 000 pg/mg on days 3, 7, 12, and 21, respectively. Urinary 11-dehydro-TxB2 concentration in the BMPR2+/− mice increased to 71 000, 116 000, 91 000, and 59 000 pg/mg at days 3, 7, 12, and 21, respectively. The change in Tx production in wild-type mice, by contrast, was less striking and did not reach statistical significance compared with pretreatment controls, with values of 47 000, 60 000, 39 000, and 24 000 pg/mg on days 3, 7, 12, and 21, respectively. Urinary 11-dehydro-TxB2 concentration in the BMPR2+/− mice was found only in TxA2 production. The time course of the change in TxB2 level was comparable to that of the RVSP change in the mice.

Platelet Activation

Platelet activation is the major source of Tx production in vivo,28 and urinary Tx metabolites have been used as markers for platelet activation. To determine whether the increased TxA2 production in BMPR2+/− mice under inflammatory stress was due to augmented platelet activation, we examined 2 other platelet activation markers, plasma sP-selectin and urinary serotonin, in the mice. As shown in Figure 10A, untreated wild-type and BMPR2+/− mice had similar plasma sP-selectin levels, 725 ± 26 and 751 ± 99 ng/mL, respectively. Ad5LO treatment increased the plasma sP-selectin concentration to 1326 ± 100 and 1791 ± 187 ng/mL in wild-type and BMPR2+/− mice, respectively. BMPR2+/− mice tended toward a higher sP-selectin level than did wild-type mice, but the difference did not reach statistical significance. A similar trend was found in urinary serotonin concentrations in the 2 types of mice. As shown in Figure 10B, before Ad5LO delivery, plasma sP-selectin levels were determined by ELISA and picric acid assay, respectively. *P<0.05 vs day −2 group; #P<0.05 BMPR2+/− vs wild-type group at same time point. Arrows indicate partially (D) and fully (E and F) muscularized distal pulmonary vessels. Bars = 50 μm.

Figure 7. Time course of cysteinyl leukotriene production in Ad5LO-treated mice. Wild-type (white column) and BMPR2+/− (gray column) mice were treated with 2 × 10⁶ PFU Ad5LO via intratracheal instillation at day 0. Urine samples were collected from mice 2 days before (day −2) and 3, 7, 12, and 21 days after treatment. Urinary cysteinyl leukotrienes (CysLT) and creatinine were determined by ELISA and picric acid assay, respectively. *P<0.05 vs day −2 group. Urinary CysLT concentration in either type of mouse was undetectable at days −2 and 21; no statistical difference existed between BMPR2+/− and wild-type mice at any time point. Data are presented as mean±SEM; n=6 to 10 mice per group. Statistical analysis was performed by 2-way ANOVA.
treatment, the urinary serotonin concentrations in wild-type and BMPR2+/−/− mice were similar, 73/9 and 73/8 pg/mg urinary creatinine, respectively. Seven days after Ad5LO treatment, urinary serotonin concentration increased to 173/13 and 215/25 pg/mg in wild-type and BMPR2+/−/− mice, respectively. The difference between wild-type and BMPR2+/−/− mice did not reach statistical significance. The relatively small difference in sP-selectin and serotonin levels found between wild-type and BMPR2+/−/− mice after Ad5LO treatment suggested that platelet activation was not the primary source of the increased TxA2 production in the Ad5LO-treated BMPR2+/−/− mice.

Effect of BMP on TxA2 Production in A549 Cells

Pulmonary epithelial cells are another source of TxA2 production in vivo.28,29 We therefore examined the effect of BMPs on TxA2 production in a human pulmonary epithelial cell line, A549. As shown in Figure 11, untreated A549 cells released a small amount of TxB2 (207±19 pg/mg total cell protein) in the basal state, and IL-1β stimulated TxB2 production by >10-fold (2822±41 pg/mg). BMP-2 did not affect basal level of TxB2 production (208±11 pg/mg) but reduced IL-1β–stimulated production by ≈20% (2215±186 pg/mg). BMP-6 had an effect (2339±41 pg/mg) similar to BMP-2 in the assay. These data indicated that BMP signaling did not directly affect TxA2 production in A549 cells but reduced the stimulating effect of IL-1β on the synthesis of this prostanoid.

Discussion

To understand the relation between BMPR2 haploinsufficiency and pulmonary hypertension, this study examined BMPR2+/−/− mice and explored 3 issues: (1) whether BMPR2 haploinsufficiency affects the development of the mice; (2) whether haploinsufficiency alone leads to pulmonary hypertension; and (3) whether haploinsufficiency increases the susceptibility to pulmonary hypertension in the setting of inflammation. The results showed that BMPR2 heterozygosity caused ≈20% fatality in mice during embryonic development but did not lead to pulmonary hypertension under unstressed conditions. The study also showed that BMPR2+/−/− mice were more sensitive to inflammation-induced pulmonary hypertension than wild-type mice.

BMP signaling plays an important role in embryonic development and is involved in gastrulation, mesoderm formation, neural patterning, skeletal development, and organogenesis (for reviews, see Hogan5 and Zhao30). Homozy-
Inflammation has been suggested to be an important mechanism in the development of PAH. Evidence supporting the hypothesis includes the following: (1) Inflammatory cells are found in the vicinity of remodeled pulmonary vessels with plexiform lesion; (2) proinflammatory cytokines and chemokines are increased in patients with PAH; (3) PAH is a common complication of autoimmune diseases involving systemic inflammation, such as scleroderma and systemic lupus erythematosus; and (4) in a rat model of PAH, monocrotaline administration causes pulmonary endothelial injury and inflammation, which is followed by pulmonary vascular remodeling (for reviews, see Voelkel et al., Tuder and Voelkel, Jeffery and Morrell, and Dorfmuller et al). In the present study, we examined the effect of inflammation on the development of pulmonary hypertension in BMPR2<sup>-/-</sup> mice. Adenovirus-mediated overexpression of 5LO in the lung was used as the inflammatory stress because 5LO expression is increased in the human disease. The results showed that BMPR2<sup>-/-</sup> mice responded to the inflammation with an immediate marked increase of RVSP (peaking at day 7) and delayed muscularization of distal pulmonary arterioles (28 days after Ad5LO treatment). The early increase of RVSP is related to enhanced pulmonary TxA2 production is significantly enhanced in patients with PAH. Mechanistically, however, this finding raises the question of how BMPR2 deficiency leads to increased TxA2.

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BMPR2<sup>-/-</sup> mice were found to produce significantly higher amounts of TxA2 than wild-type mice during 5LO overexpression. This observation is consistent with previous reports that TxA2 production is significantly enhanced in patients with PAH. Mechanistically, however, this finding raises the question of how BMPR2 deficiency leads to increased TxA2.

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**Figure 11.** Effect of BMPs and IL-1β on TxB2 production in A549 cells. A549 cells were treated with 0.5 ng/mL IL-1β and/or 100 ng/mL BMP-2 or BMP-6 for 20 hours. Cells were then washed and incubated with 0.01 mmol/L arachidonic acid in D-PBS for 10 minutes. TxB2 released in assay medium was analyzed by ELISA. *P<0.05 vs untreated cells; #P<0.05 vs cells treated with IL-1β alone. Data are presented as mean±SEM of 3 measurements, each performed in duplicate. Statistical analysis was performed by Student t test.

**Figure 10.** Plasma sP-selectin and urinary serotonin concentrations. Plasma samples were prepared from wild-type (white column) and BMPR2<sup>-/-</sup> (gray column) mice with no treatment (No Tx) or treated with 2×10<sup>5</sup> PFU Ad5LO 7 days previously (day 7). sP-selectin was measured by ELISA (A). Urinary serotonin analysis was performed on same samples as used for measurements depicted in Figure 7. *P<0.05 vs No Tx group (sP-selectin) or day –2 group (serotonin) in same type of mouse. Difference between BMPR2<sup>-/-</sup> and wild-type mice in both sP-selectin and serotonin measurement did not reach statistical significance. Data are presented as mean±SEM; n=6 to 10 per group. Statistical analysis was performed by Student t test.
production. No report in the literature has linked BMP signaling to TxA2 production. Because the TxA2 levels in the wild-type and BMPR2+/− mice were the same before Ad5LO delivery, the difference in TxA2 production in these mice resides in the different responses to 5LO overexpression or inflammation. We therefore examined platelet activation in vivo and pulmonary epithelial cell activation in cell culture, because Tx synthase is most abundantly expressed in platelets (2187 ng/mg protein), and the lung has the highest content of the enzyme, 765 ng/mg, among solid-organ tissues. The cells in lung that express Tx synthase are mainly bronchial epithelial cells and alveolar macrophages in humans and also small pulmonary artery smooth muscle cells in rats. Examining plasma sP-selectin and platelet aggregation under in vivo and pulmonary epithelial cell line A549 was found to produce a very small amount of TxA2 under basal conditions, but the production of this prostanoid was stimulated during 5LO expression. The pulmonary epithelial cell line A549 is therefore examined platelet activation in vivo and pulmonary arterial hypertension: current understanding and future directions. J Am Coll Cardiol 2004;43:33S–39S.


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