Effect of 8-isoprostaglandin $F_{2\alpha}$ on the newborn rat pulmonary arterial muscle and endothelium


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Belik, J., R. P. Jankov, J. Pan, M. Yi, C. R. Pace-Asciak, and A. K. Tanswell. Effect of 8-isoprostaglandin $F_{2\alpha}$ on the newborn rat pulmonary arterial muscle and endothelium. J Appl Physiol 95: 1979–1985, 2003. First published July 11, 2003; 10.1152/japplphysiol.00420.2003.—8-Isoprostaglandin $F_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) is a bioactive lipid peroxidation product that is a vasoconstrictor at high concentrations. Paradoxically, at lower, and possibly physiological, concentrations, it is a pulmonary vascular muscle’s relaxant. Its effects on newborn pulmonary vasculature are unknown. We hypothesized that the pulmonary arterial 8-iso-PGF$_{2\alpha}$ responses may be developmentally regulated. Therefore, the purpose of this study was to evaluate and compare 8-iso-PGF$_{2\alpha}$ effects between 1- and 2-wk-old newborn and adult rat isolated intrapulmonary arteries (100 μm) mounted on a myograph. Force after 8-iso-PGF$_{2\alpha}$ stimulation was greatest in the adult ($P < 0.01$). In newborns, force was significantly increased by the nitric oxide (NO) synthase inhibitor $N^\omega$-nitro-$\omega$-arginine methyl ester (l-NAME) ($P < 0.01$) and was suppressed by blockade of the thromboxane (Tx) A2 receptor. Whereas 8-iso-PGF$_{2\alpha}$ induced a significant dose-dependent relaxation of adult precontracted vessels in the presence of a TxA2 mimetic (U-46619; 1 μM), contraction was observed in the 1-wk-old rat. This 8-iso-PGF$_{2\alpha}$-induced contraction was abolished by endothelium removal and l-NAME and was attenuated by the cyclooxygenase inhibitor ibuprofen. In the presence of a TxA$_2$/prostaglandin H$_2$ receptor blocker, 8-iso-PGF$_{2\alpha}$ induced NO-mediated relaxation, the magnitude of which was greater in the newborn, compared with the adult ($P < 0.01$). When exposed to 8-iso-PGF$_{2\alpha}$ in vitro, only the newborn lung secreted TxB$_2$. We conclude that, in contrast to its relaxant effect in the adult, 8-iso-PGF$_{2\alpha}$ induces contraction of the pulmonary arteries in the early postnatal period, which is likely to be mediated by endothelium-derived TxA$_2$. This phenomenon may contribute to the maintenance of a higher pulmonary vascular resistance in the early postnatal period.

isoprostanes; reactive oxygen species; endothelin-1; antioxidants; cyclooxygenase; 8-isoprostaglandin $F_{2\alpha}$

PEROXIDATION OF LIPID MEMBRANES by oxygen free radicals results in isoprostane production (17). In the adult rat pulmonary artery, 8-isoprostaglandin $F_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) has been described to have a dual effect, inducing vasoconstriction in relaxed vessels and a dose-dependent relaxation in lower doses after thromboxane (Tx) A2 analog stimulation (19).

Despite the fast-growing body of literature addressing isoprostanes and their effects on pulmonary vascular smooth muscle, no data are available regarding their functional effects on the newborn pulmonary vasculature. There are several possible reasons why a distinct 8-iso-PGF$_{2\alpha}$-induced response may be expected in the newborn pulmonary vasculature. First, the transition from liquid to air exposure in the alveoli, and the threefold increase in the arterial oxygen tension after birth, renders the lung more susceptible to oxidant-mediated lipid peroxidation. Second, during the late stages of fetal life and early neonatal period, lung nitric oxide (NO) production is increased, accounting in part for the reduction in vascular resistance after birth (8, 11). This greater NO availability may result in increased 8-iso-PGF$_{2\alpha}$ production in the pulmonary circulation of the newborn, as has been previously shown to occur in human pulmonary arteries in vitro (20). Third, the lung of the newborn produces greater quantities of TxA$_2$ than later in life, which may be stimulated by 8-iso-PGF$_{2\alpha}$ (12, 13).

Our laboratory has previously demonstrated that chronic oxygen exposure in the immediate neonatal period results in pulmonary hypertension and lung parenchymal changes associated with an increase in lung 8-iso-PGF$_{2\alpha}$ in the rat (14). The pulmonary vascular smooth muscle response to 8-iso-PGF$_{2\alpha}$ may vary according to age. In pig cerebral microvessels, the degree of 8-iso-PGF$_{2\alpha}$-mediated constriction decreased with age and was greatest in the fetus (13). This developmental difference was likely related to a greater endothelial cell TxA$_2$ release in response to 8-iso-PGF$_{2\alpha}$ stimulation (13).

Therefore, the purpose of this study was to compare the effect of 8-iso-PGF$_{2\alpha}$ stimulation on pulmonary vessels from both newborn and adult animals. We hypothesized that the 1-wk-old pulmonary arterial smooth muscle force generation after 8-iso-PGF$_{2\alpha}$ is significantly greater than later in life. We further hypothesized that the increased response to 8-iso-PGF$_{2\alpha}$ early in life is directly mediated by reactive oxygen species.

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METHODS

Animal Preparation. Sprague-Dawley rats (Charles River, ON, Canada) were studied. Preliminary evaluation indicated that, by 3 wk of age, the rat pulmonary arterial muscle response to 8-iso-PGF$_{2\alpha}$ was similar to the adult. For this reason we chose to evaluate the response in 3–7 days (1 wk old), 13–15 days (2 wk old), and adult animals.

Institutional review. All procedures involving animals were conducted according to criteria established by the Canadian Council for Animal Care. Approval for the study was obtained from the Animal Care Review Committee of the Hospital for Sick Children Research Institute.

Organ bath studies. Fourth-generation or fifth-generation (adult) left lung intralobar pulmonary artery ring segments (average diameter = 100 μm and length = 2 mm) were dissected free and mounted in a wire myograph (Danish Myo Technology, Aarhus, Denmark). Isometric changes were digitized and recorded online (Myodaq, Danish Myo Technology). Tissues were bathed in Krebs-Henseleit buffer (in mM: 15 NaCl, 25 NaHCO$_3$, 1.38 NaHPO$_4$, 2.51 KCl, 2.46 MgSO$_4$, 7 H$_2$O, 1.91 CaCl$_2$, and 5.56 dextrose) bubbled with 94% air-6% CO$_2$ and maintained at 37°C. Endothelial denudation was by intraluminal friction by using a human hair. Effective elimination of the endothelium was confirmed by the absence of acetylcholine relaxation after precontraction with U-46619.

After 1 h of equilibration, the optimal resting tension of the tissue was determined by repeated stimulation with 128 mM KCl until maximum active tension was reached. All subsequent force measurements were obtained at optimal resting tension.

Pulmonary vascular muscle force generation was evaluated by stimulation with 8-iso-PGF$_{2\alpha}$, 128 mM KCl, and the TxA$_2$ mimetic U-46619. Contractile responses were normalized to the tissue cross-sectional area as follows: (width × diameter) × 2 (expressed as mm/mm$^2$). The relaxant response to 8-iso-PGF$_{2\alpha}$ was determined by preconstriction with U-46619 (10$^{-6}$ M) or 128 mM KCl. In preliminary experiments, we determined that a dose of 128 mM of KCl results in maintenance of stable pulmonary arterial muscle force in the 1-wk-old, 2-wk-old, and adult tissue for up to 30 min. The TxA$_2$/prostaglandin H$_2$ (TP) receptor blocker L-670596 (6 × 10$^{-6}$ M), combined endothelin-A and -B receptor antagonist SB-217242 (10$^{-5}$ M), the antioxidant Trolox (10$^{-4}$ M), the NO synthase inhibitor N$^\omega$-(nitro-l-arginine methyl ester (L-NAME) (10$^{-5}$ M) and the cyclooxygenase inhibitor ibuprofen (10$^{-5}$ M) were used.

Materials. Trolox was obtained from Hoffman-La Roche (Basel, Switzerland). SB-217242 was generously provided by Dr. Douglas Hay (SmithKline Beecham Pharmaceuticals, King of Prussia, PA). L-670596 was obtained from Merck Frosst (Kirkland, PQ, Canada). TxB$_2$ standard and 8-iso-PGF$_{2\alpha}$ were obtained from Cayman Chemical (Ann Arbor, MI). All other chemicals were obtained from Sigma Chemical (Oakville, ON, Canada) and dissolved in Krebs-Henseleit buffer.

Tx measurements. Fresh newborn (1 wk old) or adult lung tissue was incubated for 24 h in Krebs-Henseleit solution (same composition as previously described) at 37°C in 95% air-5% CO$_2$ with or without 8-iso-PGF$_{2\alpha}$. TxB$_2$, a stable hydrolysis product of TxA$_2$, was measured in lung tissue with a Scieix API4000 tandem mass spectrometry (MS)-MS in the electrospray ionization negative-ion mode with TurboIon-Spray. Lung tissue was spiked with 1 ng of a mixture of deuterated analogs of TxB$_2$, acidified to pH 4 with 1 N HCl, and extracted three times with ethyl acetate. After centrifugation, the ethyl acetate phase was separated and washed to neutrality with water. The organic phase was evaporated to dryness and transferred to siliconized minivials for analysis by MS-MS. Quantitation was carried out by comparing the deuterium-to-proton ratio of TxB$_2$ in the sample with standard lines generated from authentic TxB$_2$.

An Agilent HPLC 1100 was at the front end, equipped with a short Zorbax SB phenyl column (3.0 × 50 mm, 3.5-μm spherical size). The MS source temperature was maintained at 500°C and the ion source voltage at 4,500 V. Compounds were separated on HPLC with a direct inlet to the MS source. HPLC solvents were acetonitrile and water each containing 2 μl propionic acid/l mixed according to the following program: 80:20 water-acetonitrile (vol/vol) at sample injection and maintained for 2 min, 75:25 (vol/vol) for 0.5 min, 50:50 (vol/vol) by 5 min, 45:55 (vol/vol) by 6.2 min, and 0:100 (vol/vol) by 11 min, where this was maintained for 1.5 min. The solvent was then recycled to 80:20 (vol/vol) for the next run. The flow rate was at 400 μl/min.

MS-MS parameters were established through infusion (20 μl/min) of each authentic standard separately. The Q1 spectrum was first obtained, followed by selection of the M-1 fragment ion and recording of a Q3 spectrum after collision-induced decomposition (CID). Optimization of the parameters was carried out either manually or by running the quantitative optimization program to establish conditions for use in the analysis by the metabolic rate monitor. The CID gas was nitrogen.

Authentic standards in appropriate dilutions (1 ng deuterated species containing from 1 ng to 10 pg of undeuterated species) were prepared and standards concentrations of TxB$_2$ were analyzed at the same time as the samples containing unknown amounts of the compound. Typically, 1 ng of deuterated standard was added to each unknown sample, and 20% (vol/vol) of the sample was injected for analysis.

Data analysis. Data were evaluated by the Student's t-test or two-way ANOVA with multiple comparisons obtained by the Tukey-Kramer test when appropriate. Statistical significance was accepted if $P < 0.05$. All statistical analysis was performed with the Number Cruncher Statistical System (Kaysville, UT). Data are presented as means ± SE.

RESULTS

Significant developmental differences ($P < 0.01$) in 8-iso-PGF$_{2\alpha}$-induced force were observed. Force development was both age and concentration dependent for 8-iso-PGF$_{2\alpha}$, as well as the for TxA$_2$ mimetic U-46619 and KCl (Fig. 1). 8-iso-PGF$_{2\alpha}$-induced force was significantly increased by the NO synthase inhibitor $N^\omega$-(nitro-l-arginine methyl ester (l-NAME) in the pulmonary arteries from 1- and 2-wk-old rats ($P < 0.01$) but not from adults (Fig. 2). 8-iso-PGF$_{2\alpha}$-induced force was completely inhibited by the TP receptor blocker L-670596 (Fig. 1).

In pulmonary arteries of adult rats prestimulated with a TxA$_2$ mimetic (U-46619), 8-iso-PGF$_{2\alpha}$ induced a significant ($P < 0.05$) dose-dependent relaxation (Fig. 3). In contrast, no effect was observed in arteries from 2-wk-old animals, and a significant ($P < 0.01$) contraction was noted in pulmonary arteries from 1-wk-old animals (Fig. 3). After KCl stimulation, 8-iso-PGF$_{2\alpha}$-induced a dose-dependent relaxation in the 1-wk-old ($P < 0.01$) and adult ($P < 0.05$) arteries. In the 2-wk-old animals, a significant ($P < 0.01$) 8-iso-PGF$_{2\alpha}$-induced contraction was observed (Fig. 4).
To determine the mechanism(s) responsible for the 8-iso-PGF$_{2\alpha}$/H9251-induced contraction in the 1-wk-old pulmonary arteries, we variously inhibited the cyclooxygenase, endothelin, NO, and oxygen free radical pathways. We found that 8-iso-PGF$_{2\alpha}$/H9251-induced contraction was significantly reduced ($P < 0.01$) by the cyclooxygenase inhibitor ibuprofen but that it was unaffected by the combined endothelin-A and -B receptor blocker SB-217242 or by the antioxidant Trolox (Fig. 5). Furthermore, neither l-NAME nor removal of the endothelium completely abolished the 8-iso-PGF$_{2\alpha}$/H9251-induced contraction in the TxA$_2$ mimetic-prestimulated arteries of the 1-wk-old animals (Fig. 6).

To evaluate the mechanism of 8-iso-PGF$_{2\alpha}$/H9251-induced relaxation after KCl stimulation, we blocked the TP receptor blocker L-670596 [TP (–)]. Values are means ± SE. **$P < 0.01$ compared with control values (by 2-way ANOVA).

Fig. 1. A: 8-isoprostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) dose-response curves for 1-wk-old ($n = 17$), 2-wk-old ($n = 7$), and adult ($n = 8$) pulmonary arteries. Similar data are also shown for U-46619 (1-wk-old ($n = 17$), 2-wk-old ($n = 10$), and adult ($n = 12$) animals; $B$) and KCl (1-wk-old ($n = 4$), 2-wk-old ($n = 6$), and adult animals ($n = 7$); $C$). Pulmonary arteries (adult, $n = 4$; 1-wk, $n = 4$) were stimulated with 8-iso-PGF$_{2\alpha}$ in the presence of the thromboxane (Tx) A$_2$/prostaglandin H$_2$ (TP) receptor blocker L-670596 [TP (–)]. Values are means ± SE. **$P < 0.01$ compared with values from 1-wk-old and 2-wk-old animals (by 2-way ANOVA).

Fig. 2. 8-iso-PGF$_{2\alpha}$ dose-response curves in the presence and absence (control) of the nitric oxide (NO) synthase inhibitor N$\textsuperscript{G}$-nitro-l-arginine methyl ester (l-NAME) for 1-wk-old ($n = 4$; $A$), 2-wk-old ($n = 8$; $B$), and adult ($n = 8$; $C$) pulmonary arteries. Values are means ± SE. **$P < 0.01$ compared with control values (by 2-way ANOVA).

Fig. 3. 8-iso-PGF$_{2\alpha}$ dose-response curves for 1-wk-old ($n = 33$), 2-wk-old ($n = 7$), and adult ($n = 8$) pulmonary arteries precontracted with U-46619 (10$^{-6}$ M). Values are means ± SE. **$P < 0.01$ compared with values from 2-wk-old and adult animals (by 2-way ANOVA). †$P < 0.05$ and ††$P < 0.01$ compared with prestimulation values (by 2-way ANOVA).
response to 8-iso-PGF$_{2\alpha}$ was observed in the 1-wk-old rat, but not the adult.

Reactive oxygen species induce cell membrane lipid peroxidation, which stimulates the production of isoprostanes. Of the many isomers of isoprostanes, 8-iso-PGF$_{2\alpha}$ is the most studied (16). This isoprostane has a very short half-life (1 min) when injected in the adult rat circulation, and it induces a dose-dependent smooth muscle contraction of the pulmonary arteries mediated via TP receptor stimulation (17, 18). In the endothelium, 8-iso-PGF$_{2\alpha}$ induces endothelin-1 expression, TxA$_2$ synthesis, inositol 1,4,5-trisphosphate formation, and NO release via unknown pathways (10, 13, 19, 22, 26).

We documented that 8-iso-PGF$_{2\alpha}$ stimulation resulted in dose-dependent contraction of the rat pulmonary arterial muscle. The lesser force development of the newborn pulmonary arterial muscle after 8-iso-PGF$_{2\alpha}$ stimulation reflects the decreased force potential of this muscle in response to a number of stimuli. This age-dependent difference in force development is similar to the pattern previously described after stim-

DISCUSSION

Significant developmental differences in the pulmonary arterial smooth muscle response to 8-iso-PGF$_{2\alpha}$ were found. Force tension developed by the pulmonary arteries in response to 8-iso-PGF$_{2\alpha}$-mediated stimulation increased with age, being greatest in the adult. In contrast to the relaxation in TxA$_2$ mimetic-precontracted arteries observed in the adult, 8-iso-PGF$_{2\alpha}$ induced a dose-dependent contraction in the 1-wk-old rat. This 8-iso-PGF$_{2\alpha}$-induced contraction was attenuated by removal of the endothelium or by NO synthase blockade, suggesting involvement of the endothelial NO pathway. Cyclooxygenase inhibition with ibuprofen partially suppressed the 8-iso-PGF$_{2\alpha}$-induced contraction, suggesting that this response was at least partially mediated by the Tx or prostaglandin pathway. When the 1-wk-old pulmonary vessels were pre-stimulated with the nonspecific KCl agonist, 8-iso-PGF$_{2\alpha}$ induced a significant relaxation via NO release that was enhanced after TP receptor blockade with L-670596. Increased lung tissue TxB$_2$ release in re-
ulation with other agonists, including the TxA2 analog U-46619, which appears to act via the same receptor as 8-iso-PGF2α (5).

The factor(s) responsible for the 8-iso-PGF2α-induced contraction and its unique occurrence in the newborn rat is of particular interest given the putative role for the vasoconstrictor isoprostanes as participants in the dramatic changes in pulmonary vascular resistance during the early postnatal period. In this study, we investigated three potential mediators of the 8-iso-PGF2α-induced contraction in the TxA2 mimetic-stimulated pulmonary arteries of the newborn: TxA2, endothelin-1, and reactive oxygen species.

TxA2 is a potent inducer of pulmonary vascular smooth muscle contraction. In the rabbit, the production by the lung of TxA2 is highest at birth and decreases over the first 2 wk of life (12). Fetal onset pulmonary hypertension is associated with an increase in the TxA2 lung content in sheep (1). TxA2 production is also significantly increased in newborns and young children with congenital heart disease (2, 3).

Evidence is emerging to indicate a link between TxA2 and isoprostane production in the lung. In vitro, 8-iso-PGF2α induced TxA2 production in endothelial...
cells (22). With in vivo experiments, our laboratory has previously shown that lung TxB2 content of newborn pups exposed to 60% O2 for 14 days is increased (14, 15). In immature pig periventricular brain microvessels, 8-iso-PGF2α, induced TxA2 release, which was blocked by the specific TxA2 synthase inhibitor CGS-12970, as well as the nonselective cyclooxygenase inhibitor ibuprofen (13).

On the basis of the above evidence, we investigated whether TxA2 was the mediator of the 8-iso-PGF2α-induced contraction in prestimulated arteries of the newborn. In the presence of ibuprofen, we observed a 50% reduction in the 8-iso-PGF2α-induced contraction, implicating cyclooxygenase-derived TxA2 as the dominant agonist. The lack of total suppression of the response with ibuprofen is in keeping with the previous observations of Wagner et al. (25), whereby 8-iso-PGF2α-induced contraction in nonstimulated rat aorta and pulmonary artery was reduced by only 50% in the presence of indomethacin.

In this study, 8-iso-PGF2α-induced contraction of the prestimulated newborn arteries was suppressed by endothelial removal and L-NAME, implicating endothelial-derived NO in the mechanism of TxA2 release. This speculation is supported by published reports linking the NO synthase and cyclooxygenases. NO is known to activate cyclooxygenase-1 leading to an increase in eicosanoids production in pulmonary fibroblasts (7). In lung epithelial cells, L-NAME decreases the human meconium-induced TxA2 release (21).

Our laboratory has previously shown that endothelin-1 is increased in the newborn rat with chronic hyperoxia-induced pulmonary hypertension and that such an increase is possibly related to the high 8-iso-PGF2α lung content in these animals (15). In addition, there is evidence for 8-iso-PGF2α-induced endothelin-1 release in vascular tissue (9, 10, 26). On this basis, we evaluated whether endothelin-1 mediated the unique contractile response observed in the newborn pulmonary artery. Addition of the endothelin-A and -B receptor blocker SB-217242 did not alter the 8-iso-PGF2α response. This finding does not exclude a role for endothelin-1 in this response because the A receptor mediates vasoconstriction and the B1 receptor mediates vasodilation. Further studies utilizing more specific receptor blockers are warranted.

Last, we tested whether the 8-iso-PGF2α-mediated responses in the 1-wk-old prestimulated pulmonary vessels were mediated by reactive oxygen species. Such speculation was based on the fact that lung nitrotyrosine content of newborn rats exposed to 60% O2 for 14 days is increased (15), possibly reflecting 8-iso-PGF2α-induced peroxynitrite formation. Isoprostane is known to induce NO release (17) via a non-TP receptor (19), which is likely to be localized to the endothelium. Pulmonary vascular tissue is known to produce the peroxynitrite anion, which is rapidly formed when NO and superoxide react (4).

Troxol is a water-soluble analog of the free radical scavenger α-tocopherol that has been shown to decrease H2O2-mediated oxygen toxicity (23). Stimulation of the 1-wk-old pulmonary arteries with 8-iso-PGF2α in the presence of Trolox had no effect on the contractile response. Thus it is unlikely that oxygen free radicals acting directly on the smooth muscle, or via peroxynitrite formation, account for the unique 8-iso-PGF2α response of the newborn.

In this study, we demonstrated that the 8-iso-PGF2α-induced contraction observed in the newborn precontracted artery was only present after TxA2 mimetic stimulation. This suggests that the mechanism responsible for 8-iso-PGF2α-induced contractions depends on “priming” of the smooth muscle TP receptor with TxA2. The additional contractile effect of reactive oxygen species after priming of the adult rat pulmonary arterial smooth muscle has been previously reported by others (24), and it likely accounts for the observed responses in the present study. A schematic representation of the mechanisms accounting for our findings is presented in Fig. 9.

During the process of transition from fetal to postnatal life, marked changes in the pulmonary circulation take place, characterized by a marked decrease in pulmonary vascular resistance within minutes from birth. The resistance in the pulmonary circulation, however, remains elevated during the immediate neonatal period, and the pulmonary vascular muscle has distinct mechanical properties compared with the adult (6). Physiologically low pulmonary tissue concentrations of 8-iso-PGF2α may result in TxA2 release, as shown in this study, and possibly contribute to the higher pulmonary vascular resistance observed in the immediate neonatal period. Our laboratory is currently involved in further testing this hypothesis.

In conclusion, we have demonstrated that at lower concentrations 8-iso-PGF2α induces further contraction of the 1-wk-old rat pulmonary artery prestimulated with a TxA2 mimetic. This response contrasts with the relaxation observed in the adult pulmonary artery under the same conditions. The mechanism of contraction in the newborn involves the endothelial NO pathway, acting via TP receptors in the smooth muscle cells, and...
is TxA2 mediated. This newly described pathway may have physiological significance in the regulation of pulmonary vascular resistance in the immediate neonatal period.

DISCLOSURES

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