Vasoactive prostanoids are generated from arachidonic acid by COX-1 and COX-2 in the mouse

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Baber, Syed R., Weiwen Deng, Jorge Rodriguez, Ryan G. Master, Trinity J. Bivalacqua, Albert L. Hyman, and Philip J. Kadowitz. Vasoactive prostanoids are generated from arachidonic acid by COX-1 and COX-2 in the mouse. Am J Physiol Heart Circ Physiol 289: H1476–H1487, 2005. First published April 29, 2005; doi:10.1152/ajpheart.00195.2005.—Generation of vasoactive prostanoids from arachidonic acid by cyclooxygenase (COX)-1 and COX-2 was investigated in anesthetized mice. Intravenous injections of the prostanoic precursor arachidonic acid increased pulmonary arterial pressure and decreased systemic arterial pressure. Pulmonary pressor and systemic depressor responses were attenuated by SC-560 and nimesulide, inhibitors of COX-1 and COX-2, in doses that did not alter responses to injected prostanoids. Pulmonary pressor responses to arachidonic acid were blocked and a depressor response was unmasked, whereas systemic depressor responses were not altered, by a thromboxane receptor antagonist. Pulmonary and systemic pressor responses to angiotensin II injections and systemic pressor responses to angiotensin II infusion were not modified by COX-1 or COX-2 inhibitors but were attenuated by losartan. Systemic depressor responses to arachidonic acid were smaller in COX-1 and COX-2 knockout mice, whereas responses to angiotensin II, norepinephrine, U-46619, endothelin-1, and PGE1 were not different in COX-1 and COX-2 knockout and wild-type control mice. These results suggest that vasoactive prostanoids with pulmonary pressor and systemic vasodepressor activity are formed by COX-1 and COX-2 and are consistent with Western blot analysis and immunostaining showing the presence of COX-1 and COX-2. These data suggest that thromboxane A2 (TXA2) is formed from the precursor by COX-1 and COX-2 in the lung and are in agreement with immunofluorescence studies showing thromboxane synthase. The present data suggest that COX-1- or COX-2-derived prostanoids do not modulate responses to angiotensin II or other vasoactive agents and that prostanoid responses are similar in CD-1 and C57BL/6 and in male and female mice.

Pulmonary and systemic vascular bed; angiotensin II

METHODS

Experiments in male CD-1 mice. Prior approval for this study was obtained from the animal care committee of Tulane University Medical Center, and all procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee. Male CD-1 mice (20–29 g body wt; Charles Rivers Laboratories, Wilmington, MA) were anesthetized with thiobutabarbital (Inactin, Sigma Chemical, St. Louis, MO) at 60 mg/kg ip, with supplemental doses given as needed to maintain a uniform level of anesthesia. The mice were placed on an isothermal pad to maintain body temperature. The trachea was cannulated with a short segment of PE-90 tubing to maintain a patent airway, and the mice breathed room air enriched with 100% oxygen. A carotid artery was catheterized with PE-10 tubing for measurement of systemic arterial pressure. A jugular vein was catheterized with PE-10 tubing for intravenous administration of drugs. For right-heart catheterization, the mice were placed supine on a fluoroscopic table, and a specially designed single-lumen catheter (0.25 mm OD; NuMed, Hopkington, NY) with a curved tip was passed from the right jugular vein into the right atrium and ventricle and into the right or left pulmonary artery under fluoroscopic (Picker, Cleveland, OH) guidance. Pulmonary and systemic arterial pressures measured with transducers (model P23, Statham, Oxnard, CA) and mean pressures determined by electronic integration were recorded on a polygraph (model 7, Grass Instruments, Quincy, MA). Baseline mean systemic and pulmonary arterial pressures were recorded, and the systemic vascular resistance was calculated. After baseline pressures were recorded, the mice were injected with prostaglandin E1 (PGE1, 0.5 and 1.0 μg/kg ip) or norepinephrine (1.0 μg/kg ip) to determine the baseline prostanoid contribution to the response to these vasoactive agents. The contributions of COX-1 and COX-2 in the pulmonary and systemic vascular beds were studied in COX-1 and COX-2 knockout mice. The roles of COX-1 and COX-2 in the generation of vasoactive prostanoids were determined by the differential modulation of vasoactive prostanoids between COX-1 and COX-2 knockout mice.

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pulmonary arterial pressures were 87 ± 8 and 13 ± 2 mmHg, respectively. The methods for measurement of pulmonary and systemic arterial pressures in the intact-chest mouse have been described previously (5, 8).

**Experiments in knockout mice.** Male and female COX-1 and COX-2 knockout mice (B6.129P2-Ptgs1<sup>tm1Unc</sup> and B6.129P2-Ptgs<sup>2tm1Smi</sup>) and wild-type control mice (20–23 g body wt; Taconic Laboratories, Germantown, NY) were anesthetized and prepared for systemic arterial pressure studies as described for CD-1 mice. Baseline mean systemic arterial pressure was 93 ± 8 and 89 ± 7 mmHg in COX-1 and COX-2 knockout mice, respectively. Baseline mean systemic arterial pressure was 90 ± 11 mmHg in wild-type control mice, which were prepared by mating wild-type COX-1 or COX-2 male and female mice. The COX-1 and COX-2 targeted mutation mice were created in the laboratory of Dr. Robert Langenbach by gene targeting into E14TG2a embryonic stem cells from 129/oLAHsd mice and injected into C57BL/6 mice (22, 24). The COX-1 and COX-2 mice are maintained on a mixed B6.129P2 background. Homozygous and heterozygous knockout mice are produced by mating homozygous male and heterozygous female mice (22, 24).

**Strain and gender comparison experiments.** Male CD-1 and C57BL/6 mice and male and female wild-type controls for the COX knockout mice were used to compare responses to angiotensin II, arachidonic acid, PGE<sub>1</sub>, and U-46619.

**Western blot analysis.** Western blot analysis was carried out in lung from CD-1, COX-1 and COX-2 knockout, and wild-type control mice. Western blot analysis was also carried out in aorta from CD-1 mice. The tissues were removed from anesthetized mice after intracardiac perfusion with cold phosphate-buffered 3% sucrose solution and frozen in liquid nitrogen. Lung tissue and aorta were homogenized in buffer containing 0.32 M sucrose, 10 mM Tris·HCl, pH 7.4, 2 mM EDTA, and 10 μl/ml protease arrest (Protease Inhibitors, Geno Technology, St. Louis, MO). The homogenates were incubated on ice for 30 min. After centrifugation twice at 15,000 g at 4°C for 20 min, the supernatant protein was quantified colorimetrically using the bicinchoninic protein assay (Pierce Biotechnology, Rockford, IL). For Western analysis, 60 μg of tissue homogenate were loaded onto a 4–20% Tris-glycine gel (ICN Biomedicals, Irvine, CA). After electrophoresis, the protein was transferred to a nitrocellulose membrane.
by electroelution. Immunodetection was performed with mouse anti-COX-1 monoclonal antibody (1:500 dilution) and rabbit anti-COX-2 polyclonal antibody (1:2,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies were horseradish peroxidase conjugated to goat anti-mouse IgG, goat anti-rabbit IgG, or donkey anti-goat IgG (all 1:4,000 dilution; Santa Cruz Biotechnology). The nitrocellulose membrane was processed using enhanced chemiluminescence. Western blotting detection reagents were obtained from Amersham Pharmacia Biotech (Piscataway, NJ). COX-1 and COX-2 standards (200 ng; Cayman Chemical, Ann Arbor, MI) were also used as a positive control for COX-1 and COX-2.

**Immunohistochemical studies.** Immunohistochemical studies for the expression of COX-1 and COX-2 in the lung were carried out in CD-1, COX-1 and COX-2 knockout, and wild-type control mice. Immunofluorescence studies were carried out for the localization of thromboxane synthase in lung from CD-1, wild-type control, and COX knockout mice. The localization of COX-1 and COX-2 protein was evaluated by immunohistochemical analysis using specific antibodies (Santa Cruz Biotechnology) and an indirect immunoperoxidase technique as described previously in thick ascending limb cells (31). Lung slices were fixed by immersion in Bouin’s solution for 24 h at room temperature. The tissue was then dehydrated, embedded in Paraplast plus (Monoject Scientific, St. Louis, MO), serially prepared in 5-μm sections with use of a rotary microtome, mounted on glass slides, and stored for immunostaining. Renal slices from the same animals were used as a control for COX-1 and COX-2 immunostaining in the lung.

For immunohistochemical detection of thromboxane synthase in the mouse lung, the mice were anesthetized and, after intracardiac perfusion with cold phosphate-buffered 3% sucrose solution, the lung was removed, fixed with ice-cold 4% paraformaldehyde in PBX (USB, Cleveland, OH) at 4°C overnight, and transferred to 30% sucrose in PBX (Sigma Chemical) at 4°C overnight. The lung tissue was embedded in optimal cutting temperature compound (OCT, Tissue-Tek, Miles Laboratories, Elkhart, IN), frozen in liquid nitrogen, and stored at −70°C. Lung sections (40 μm) were prepared with a cryostat and immunostained with rabbit anti-thromboxane synthase polyclonal antibody (1:200 dilution; Cayman Chemical). Fluorescein isothiocyanate-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) or Alexa Fluor 594-conjugated donkey anti-rabbit IgG (1:200 dilution; Molecular Probes, Eugene, OR) was used as the secondary antibody. The lung sections were then mounted with Vectashield mounting medium for fluorescence with 4′,6-diamidino-2-phenylindole hydrochloride (Vector Laboratories) and viewed under a Leica SP2 AOB confocal microscope.

**Drugs.** Arachidonic acid sodium salt, angiotensin II, norepinephrine hydrochloride, and endothelin-1 (Sigma Chemical) were dissolved in 0.9% saline. PGE1 and U-46619 (Cayman Chemical) were dissolved in 95% ethanol, and dilutions were made in 0.9% saline. Nimesulide, SC-560, and NS-398 (Cayman Chemical) were dissolved in 0.5 mM NaCO3 solution, and daltroban (SmithKline Beecham, Philadelphia, PA), losartan (Merck, Rahway, NJ), and CGS-13080 (CIBA-GEIGY, Summit, NJ) were dissolved in Tris buffer, pH 7.4. Doses of SC-560, nimesulide, daltroban, losartan, and CGS-13080...
were determined in pilot studies and are similar to the doses used in previous studies (1, 2, 5, 11, 19, 23, 29). Nimesulide at 3 mg/kg iv does not affect arachidonic acid-induced platelet aggregation and does not alter arachidonic acid-induced platelet aggregation in human subjects (2, 11). The IC_{50} values for nimesulide are 70 and 1.27 nM for human recombinant COX-1 and COX-2, respectively, and 22 and 0.03 nM for ovine COX-1 and COX-2, respectively (3, 20). Previous experiments in the rat show that nimesulide does not alter vascular responses to injected prostanoids (2). Nimesulide, SC-560, NS-398, CGS-13080, daltroban, and losartan were administered by intravenous injection over a 5- to 10-min period and, with the exception of nimesulide, had only small effects on baseline pressures when values were compared before and 10–20 min after administration. Nimesulide produced a significant increase in systemic arterial pressure. The vehicles for the drugs used in these studies had no consistent effect on baseline pressures or vascular responses in the mouse. Agonist injections were made in small volumes (3–10 l) in a random fashion, with time allowed for vascular pressures to return to baseline value. The intravenous angiotensin II infusions were made in small volumes (5–10 l kg^{-1} min^{-1}) with an infusion pump (model 210, KD Scientific, Holliston, MA). The saline vehicle had only small effects on systemic arterial pressure when infused at 5–10 l kg^{-1} min^{-1}.

The hemodynamic data represent peak changes in pressure and are expressed as means ± SE; they were analyzed by using a paired or group t-test or a one-way analysis of variance and Scheffe’s test. P < 0.05 was used as the criterion for statistical significance.

Fig. 3. Effect of the COX-2 inhibitor nimesulide on increases in pulmonary arterial pressure and decreases in systemic arterial pressure in response to intravenous arachidonic acid injections in CD-1 mice (A), increase in pulmonary and systemic arterial pressures in response to intravenous injections of U-46619 (B), and decrease in pulmonary and systemic arterial pressures in response to intravenous injections of PGE_{1} (C). D: effect of the COX-2 inhibitor NS-398 on increase in pulmonary arterial pressure and decrease in systemic arterial pressure in response to intravenous injection of arachidonic acid. n, Number of experiments. *Significantly different from control.

Fig. 4. Effect of the thromboxane receptor antagonist daltroban on increase in pulmonary arterial pressure and decrease in systemic arterial pressure in response to intravenous injections of arachidonic acid (A), pulmonary pressor and systemic pressor responses to intravenous injections of U-46619 (B), and decreases in pulmonary and systemic arterial pressures in response to intravenous injections of PGE_{1} (C) in CD-1 mice. D: effect of the thromboxane synthase inhibitor CGS-13080 on increase in pulmonary arterial pressure and decrease in systemic arterial pressure in response to intravenous injection of arachidonic acid in CD-1 mice. n, Number of experiments. *Significantly different from control.
RESULTS

Expression of COX-1, COX-2, and thromboxane synthase. The expression of COX-1 and COX-2 was investigated in CD-1 mice, and the results are shown in Fig. 1. Western blot analysis showed expression of COX-1 and COX-2 protein in lung tissue from CD-1 mice (Fig. 1A). The expression of COX-1 protein in aorta from CD-1 mice was high, whereas the expression of COX-2 protein was low (Fig. 1A). Immunostaining of COX-1 and COX-2 was observed in airway epithelial cells from lung of CD-1 mice (Fig. 1A). Immunofluorescent studies showed prominent staining for thromboxane synthase in airway epithelial cells from lung of CD-1 mice (Fig. 1B). For comparison and cross-reactivity studies, kidney slices were immunostained under the same conditions as lung tissue, and the results are shown in Fig. 1C. COX-2 immunostaining was observed in thick ascending limb cells (Fig. 1C). COX-1 immunostaining was mainly observed in medullary collecting ducts, where expression was very high (Fig. 1C). The antibodies were tested for cross-reactivity using preadsorption of COX-1 and COX-2 protein. For COX-2 antibodies, no cross-reactivity for COX-1 was detected. However, a small amount of cross-reactivity for COX-1 antibody was detected against COX-2 with very low antibody dilutions.

Role of COX-1 and COX-2 in mediating responses to arachidonic acid. The role of COX-1 and COX-2 in mediating hemodynamic responses to the prostanoid precursor arachidonic acid was investigated in CD-1 mice, and the results are summarized in Fig. 2. Arachidonic acid (0.1–1 mg/kg iv) caused dose-related increases in pulmonary arterial pressure and decreases in systemic arterial pressure (Fig. 2A). Responses were rapid in onset, and pressures returned to baseline over 1.5–6 min. The increases in pulmonary arterial pressure and decreases in systemic arterial pressure in response to intravenous injections of arachidonic acid were reduced significantly after administration of the COX-1 inhibitor SC-560 (10 mg/kg iv; Fig. 2A). The area under the curve for increases in pulmonary arterial pressure and decreases in systemic arterial pressure in response to arachidonic acid was reduced significantly after administration of SC-560 (data not shown). The administration of SC-560 had no significant effect on increases in pulmonary and systemic arterial pressures in response to intravenous injections of the thromboxane A2 (TxA2) mimic U-46619, and the COX-1 inhibitor had no significant effect on decreases in pulmonary and systemic arterial pressure in response to intravenous injections of PGE1 (Fig. 2, B and C).

The role of COX-2 in mediating hemodynamic responses to arachidonic acid was also investigated in CD-1 mice. The increases in pulmonary arterial pressure and decreases in systemic arterial pressure in response to intravenous arachidonic acid injections were also significantly reduced after administration of the COX-2 inhibitor nimesulide (3 mg/kg iv; Fig. 3A). The area under the curve for the pulmonary and systemic responses to arachidonic acid was reduced significantly after treatment with nimesulide (data not shown). Nimesulide had no significant effect on the increases in pulmonary and systemic arterial pressures in response to intravenous injections of U-46619 or the decreases in pulmonary and systemic arterial pressures in response to intravenous injections of PGE1 (Fig. 3). The effect of another COX-2 inhibitor on responses to intravenous injections of arachidonic acid were investigated in CD-1 mice, and the data are summarized in Fig. 3D. The increase in pulmonary arterial pressure and decrease in systemic arterial pressure in response to arachidonic acid were significantly attenuated after administration of NS-398 (3 mg/kg iv; Fig. 3D).

Role of thromboxane. The role of TxA2 in mediating the pulmonary pressor response to the precursor and in modulating vasodepressor responses to the substrate in the systemic vascular bed was investigated with a thromboxane receptor antagonist. The effects of the TxA2 receptor antagonist daltroban on responses to arachidonic acid are summarized in Fig. 4. The increases in pulmonary arterial pressure in response to intravenous injections of arachidonic acid were abolished, and a significant decrease in pulmonary arterial pressure was unmasked in response to the precursor after administration of daltroban (5 mg/kg iv; Fig. 4A). After administration of daltroban, the decreases in systemic arterial pressure in response to intravenous injections of arachidonic acid were not changed (Fig. 4A). To assess the effectiveness of the thromboxane receptor blockade, the effect of daltroban on pulmonary and systemic pressor responses to U-46619 was investigated. After administration of the thromboxane receptor blocking agent (5 mg/kg iv), increases in pulmonary and systemic arterial pressures in response to intravenous injections of the TxA2 mimic U-46619 were decreased significantly (Fig. 5).

Fig. 5. Effect of the AT1 receptor antagonist losartan (A) and the thromboxane receptor antagonist daltroban (B) on increases in pulmonary and systemic arterial pressures in response to intravenous injections of angiotensin II in CD-1 mice. n, Number of experiments. *Significantly different from control.
Administration of the thromboxane receptor antagonist had no significant effect on decreases in pulmonary and systemic arterial pressures in response to intravenous injections of PGE₁ (Fig. 4C). The effects of the thromboxane synthase inhibitor CGS-13080 on responses to arachidonic acid were investigated in CD-1 mice, and the data are summarized in Fig. 4D. The increase in pulmonary arterial pressure in response to intravenous injection of arachidonic acid was reduced significantly after administration of CGS-13080 (10 mg/kg iv), whereas the decrease in systemic arterial pressure in response to the precursor was not changed significantly (Fig. 4D).

Role of COX-1 and COX-2 in modulating responses to angiotensin II in CD-1 mice. The role of COX-1 and COX-2 in modulating responses to the vasoactive peptide in the pulmonary and systemic vascular beds was investigated, and the data are summarized in Figs. 5 and 6. Angiotensin II (0.1–1 μg/kg iv) caused dose-related increases in pulmonary and systemic arterial pressures in CD-1 mice. The increases in pulmonary and systemic arterial pressures in response to angiotensin II were decreased significantly after administration of the angiotensin (AT₁) receptor antagonist losartan (1 mg/kg iv; Fig. 5A). The increases in pulmonary and systemic arterial pressures in response to intravenous injections of angiotensin II were not altered after administration of the thromboxane receptor antagonist daltroban at 5 mg/kg iv, a dose that attenuated pulmonary and systemic pressor responses to U-46619 (Fig. 5, A and B).

The effect of the COX-1 and COX-2 inhibitors on pressor responses to angiotensin II was investigated, and the data are summarized in Fig. 6. After administration of SC-560 (10 mg/kg iv) or nimesulide (3 mg/kg iv) in two groups of CD-1 mice, the increases in pulmonary and systemic arterial pressures in response to intravenous injections of angiotensin II were not altered (Fig. 6, A and B). The area under the curve of the pressor responses to intravenous injections of angiotensin II was not different before and after administration of SC-560 or nimesulide (data not shown). The increase in systemic arterial pressure in response to an intravenous infusion of angiotensin II (10 ng·kg⁻¹·min⁻¹) for 30 min was not altered by SC-560 or nimesulide in two groups of CD-1 mice (Fig. 6C).

Studies in COX-1 and COX-2 knockout mice. COX-1 and COX-2 protein expression and COX-1 and COX-2 immunostaining were investigated in lung tissue from COX-1 and COX-2 knockout and wild-type control mice, and the results are shown in Fig. 7. Western blot analysis showed expression of COX-2 protein in lung from COX-1 knockout and wild-type control mice, whereas the expression of COX-1 was not altered.
detected in COX-1 knockout mouse lung (Fig. 7A). Western blot analysis showed the expression of COX-1 protein in lung from COX-2 knockout and wild-type control mice, whereas the expression of COX-2 protein was not detected in lung from COX-2 knockout mice (Fig. 7A). COX-1 immunostaining was observed in airway epithelial cells from wild-type control and COX-2 knockout mice, whereas COX-2 immunostaining was not detected in COX-2 knockout mouse lung (Fig. 7B). COX-2 immunostaining was detected in airway epithelial cells from wild-type control and COX-1 knockout mice, whereas COX-1 immunostaining was not detected in COX-1 knockout mouse lung (Fig. 7B). The absence of immunostaining for COX-1 in COX-1 knockout mice and for COX-2 in COX-2 knockout mice suggests that there is little cross-reactivity between COX-1 and COX-2 antibodies (Fig. 7B). To examine the histochemical localization of α-actin, an irrelevant antibody, in these experiments in COX-1 and COX-2 knockout mice, lung sections from COX-1 and COX-2 knockout mice were immunostained for α-actin (Fig. 7C). α-Actin immunostaining was observed in smooth muscle cells around the bronchiolar epithelial cells (Fig. 7C). In the control section, the lung tissue was not stained with primary antibody (Fig. 7C). No protein immunostaining was detected in cells around the bronchiole in the section stained without primary antibody (Fig. 7C).

**Hemodynamic responses in COX-1 and COX-2 knockout mice.** Peak hemodynamic responses to intravenous injections of arachidonic acid and vasoactive agents were compared in COX-1 and COX-2 knockout and wild-type control mice, and the data are summarized in Figs. 8 and 9. The decreases in systemic arterial pressure in response to intravenous injections of arachidonic acid were significantly smaller in COX-1 and COX-2 knockout than in wild-type control mice (Fig. 8A). The decreases in systemic arterial pressure in response to intravenous injection of PGE1 and the increases in systemic arterial pressure in response to intravenous injections of U-46619 in COX-1 and COX-2 knockout mice were not significantly different from the responses in wild-type control mice (Fig. 8, B and C). The decreases in systemic arterial pressure in response to intravenous injection of PGE1 and the increases in systemic arterial pressure in response to intravenous injections of U-46619 in COX-1 and COX-2 knockout mice were not significantly changed after administration of the COX-1 inhibitor SC-560 (10 mg/kg iv; Fig. 8D). The decreases in systemic arterial pressure in response to arachidonic acid in COX-2 knockout mice were not significantly altered after administration of the COX-2 inhibitor nimesulide (3 mg/kg iv; Fig. 8E).
Baseline systemic arterial pressure was not significantly different in COX-1 and COX-2 knockout and wild-type control mice.

The increases in systemic arterial pressure in response to intravenous injections and intravenous infusions of angiotensin II were compared in COX-1 and COX-2 knockout and wild-type control mice. The peak increases in systemic arterial pressure in response to intravenous injections of angiotensin II (0.1–1 μg/kg) were not significantly different in COX-1 and COX-2 knockout and wild-type control mice (Fig. 9A). The increases in systemic arterial pressure in response to a 30-min intravenous infusion of angiotensin II at 10 ng·kg⁻¹·min⁻¹ in COX knockout and wild-type control mice are shown in Fig. 9B. The increases in systemic arterial pressure in response to intravenous infusion of angiotensin II were not significantly different in COX-1 and COX-2 knockout and wild-type control mice but were decreased significantly by the administration of losartan (1 mg/kg iv) in wild-type control mice (Fig. 9B). The increases in systemic arterial pressure in response to other pressor agents were compared in COX-1 and COX-2 knockout and wild-type control mice, and the increases in systemic arterial pressure in response to intravenous injections of nor-epinephrine and endothelin-1 were not different in the three groups of mice (Fig. 9, C and D).

Role of mouse strain and gender. Responses to the prostanoids arachidonic acid and angiotensin II were compared in male CD-1 and C57BL/6 and male and female wild-type control mice, which were prepared by mating wild-type COX-1 or COX-2 knockout male and female mice (Taconic Laboratories) (22, 24). The results of these experiments are summarized in Fig. 10, which shows that increases in systemic arterial pressure in response to intravenous injections of angiotensin II and U-46619 were similar and decreases in systemic arterial pressure in response to intravenous injections of arachidonic acid and PGE₁ were similar in the four groups of mice.

DISCUSSION

The results of the present study show that COX-1 and COX-2 are expressed in the normal mouse lung and that injections of the prostaglandin precursor arachidonic acid increased pulmonary arterial pressure and decreased systemic arterial pressure in CD-1 mice. The pulmonary pressor and systemic vasodepressor responses to the precursor were attenuated by selective COX-1 and COX-2 inhibitors in doses that did not alter responses to injected prostanoids. These data suggest that vasoactive prostanoids are formed from their precursor by a constitutively active COX-1 and COX-2 pathway in the mouse. The role of TxA₂ in mediating the pulmonary pressor response to the precursor was examined in experiments with a thromboxane receptor antagonist. In these experiments, the pulmonary pressor response to the precursor was abolished and a decrease in pulmonary arterial pressure was uncovered when the TxA₂ receptor antagonist was administered in a dose that attenuated the pulmonary pressor response to the thromboxane mimic.
The increase in pulmonary arterial pressure in response to arachidonic acid was significantly reduced after treatment with the thromboxane synthase inhibitor CGS-13080. These data are consistent with the hypothesis that the pulmonary pressor response to the precursor is mediated by TxA2 formation and correlates with the results of immunofluorescence studies showing the presence of thromboxane synthase in the lung. The pulmonary localization of COX-1 and COX-2 and thromboxane synthase is consistent with the observation that pulmonary pressor responses to the precursor are attenuated by COX-1 and COX-2 inhibitors and by a thromboxane receptor antagonist or thromboxane synthase inhibitor. These data support the hypothesis that TxA2 is a major product formed from substrate by COX-1 and COX-2 in the mouse lung.

The results showing that the pulmonary pressor response to the precursor is converted to a vasodepressor response after thromboxane receptor blockade are consistent with the hypothesis that vasodepressor prostanoids are also formed from the substrate but that their activity is masked by the potent pressor effects of TxA2. These data are consistent with results in the cerebral vascular bed of the mouse, where COX-1 has been shown to have a dominant role in the formation of vasoactive prostanoids (1, 2, 12, 13, 25). The present results and previous studies in the mouse cerebral vascular bed suggest that the role of COX-1 and COX-2 in the generation of vasoactive prostanoids may depend on the vascular bed studied in this species (1, 2, 12, 13, 25).

COX-2 expression is increased in lung cancers, and the severity of lung injury in COX-2 knockout mice correlates with reduced prostaglandin formation (4, 18). However, the role of COX-2 in regulating vascular function in the mouse lung is uncertain. It has been reported that angiotensin II increases prostaglandin formation, and it has been postulated that angiotensin II-stimulated prostaglandin release may modulate pressor responses to the vasoactive peptide (6, 28, 33). The results of studies with nonselective COX inhibitors on pressor responses to angiotensin II have not been consistent (6, 10, 14, 28, 33). It is possible that the effect of a COX inhibitor on the response to angiotensin II may depend on the distribution and activity of COX-1 and COX-2 in an organ system. To circumvent problems related to different activity of COX-1 and COX-2 in the systemic vascular bed of the rat and the dog coronary vascular bed, where COX-1 and COX-2 have been shown to have a role in generating vasoactive prostanoids but where TxA2 formation does not appear to have a major role in the regulation of vascular tone (2, 17).

The demonstration that COX-1 and COX-2 expression is high in the mouse lung and that vasoactive prostanoids can be generated by both pathways is different from results in the cerebral vascular bed of the mouse, where COX-1 has been shown to have a dominant role in the formation of vasoactive prostanoids (1, 2, 12, 13, 25). The present results and previous studies in the mouse cerebral vascular bed suggest that the role of COX-1 and COX-2 in the generation of vasoactive prostanoids may depend on the vascular bed studied in this species (1, 2, 12, 13, 25).
COX-2 and to ascertain the role of COX-1 and COX-2 in modulating responses to angiotensin II and other pressor agents, the effects of selective COX inhibitors and COX-1 and COX-2 gene knockouts on vascular responses were investigated. It has been reported that COX-1 and COX-2 have different roles in modulating systemic pressor responses to angiotensin II in the mouse (27). However, little, if anything, is known about responses to angiotensin II or the role of COX-1 and COX-2 in modulating responses to the peptide hormone in the pulmonary vascular bed of the mouse. In the present study, intravenous injections of angiotensin II produced dose-related increases in pulmonary arterial pressure that were attenuated by the AT1 receptor antagonist losartan. Pressor responses to angiotensin II were not altered by a thromboxane receptor antagonist in a dose that antagonized pulmonary pressor responses to the thromboxane mimic U-46619. These data suggest that the pulmonary pressor response to angiotensin II is not dependent on activation of thromboxane receptors. The hypothesis that pulmonary pressor responses to angiotensin II are differentially modulated by COX-1 and COX-2 was investigated, and the results of these experiments, which indicate that pressor responses to the vasoactive peptide are not modulated by COX-1 or COX-2 because they are not altered by COX-1 or COX-2 inhibitors in the pulmonary vascular bed of the mouse, are in agreement with studies in the rat (2).

The effect of the selective COX inhibitors on systemic pressor responses to angiotensin II was also investigated in CD-1 mice, and the results of these studies show that increases in systemic arterial pressure in response to intravenous angiotensin II injections and infusions were not modified by selective COX-1 or COX-2 inhibitors but were blocked by the AT1 receptor antagonist losartan. The results of the present study in male CD-1 mice are not in agreement with results of previous studies in female C57BL/6 mice (27). The reason for differences in results is uncertain but may be related to the strain or the gender of the mice or the COX inhibitors used in the study.

The role of COX-1 and COX-2 in modulating responses to angiotensin II was further explored in COX-1 and COX-2 knockout mice. The decreases in systemic arterial pressure in response to intravenous injections of arachidonic acid were significantly smaller in COX-1 and COX-2 knockout than in wild-type control mice. The COX-1 inhibitor SC-560 had no significant effect on the response to arachidonic acid in COX-1 knockout mice, and the COX-2 inhibitor nimesulide had no significant effect on the response to arachidonic acid in COX-2 knockout mice, suggesting that the COX inhibitors in the doses used were selective. These data are consistent with the hypothesis that vasoactive prostanoids are generated from the precursor via a constitutively active COX-1 and COX-2 pathway in the mouse and that genetic deletion of COX-1 or COX-2

Fig. 10. Changes in systemic arterial pressure in response to intravenous injections of angiotensin II, U-46619, arachidonic acid (AA), and PGE1 in male CD-1 (A; n = 18), male C57BL/6 (B; n = 16), male wild-type control (C; n = 11), and female wild-type control (D; n = 9) mice.
C57BL/6 mice and male and female wild-type controls for the determining responses to the prostanoids and angiotensin II, in previous studies in COX-1 and COX-2 knockout mice is evident in mouse strain or gender does not play a major role in determining responses to the prostanoid precursor. Western blot analysis showed that COX-1 protein was not detected in COX-1 knockout mice, whereas there was significant expression of COX-2. Furthermore, COX-2 knockout mice showed significant expression of COX-1 protein, and COX-2 protein was not detected in the lung from COX-2 knockout mice. Responses to angiotensin II, norepinephrine, and endothelin-1 in COX-1 and COX-2 knockout mice show that increases in systemic arterial pressure in response to intravenous injections and infusions of angiotensin II were similar in COX-1 and COX-2 knockout and wild-type control mice. Similar data were obtained in male and female COX-1 and COX-2 knockout mice and male and female wild-type control mice. The explanation for the difference in results in the present study and in previous studies in COX-1 and COX-2 knockout mice is uncertain.

To determine whether mouse strain and gender play a role in determining responses to the prostanoids and angiotensin II, responses to these agents were compared in male CD-1 and C57BL/6 mice and male and female wild-type controls for the COX knockout mice. The responses to arachidonic acid, PGE₁, U-46619, and angiotensin II are similar in the four groups of mice, suggesting that responses to the prostanoids and angiotensin II are similar in the two strains of male mice and in male and female wild-type controls for the COX knockout mice. The results of these studies suggest that mouse strain or gender does not play a major role in determining responses to the prostanoid precursor, the prostanoids, or angiotensin II.

The results of studies with COX-1 and COX-2 inhibitors in CD-1 mice support the hypothesis that vasoactive prostanoids are generated by both COX pathways and are consistent with results in knockout mice showing that responses to arachidonic acid are significantly smaller in COX-1 and COX-2 knockout mice than the decrease in systemic arterial pressure in response to the precursor in wild-type control mice. Although responses to the precursor were smaller in COX-1 and COX-2 knockout mice, responses to U-46619 and PGE₁ were not different from responses in wild-type control mice. These results suggest that prostanoid receptor-mediated responses in the systemic vascular bed are not modified by COX-1 and COX-2 gene deletion. The results with angiotensin II and the data comparing responses to norepinephrine and endothelin-1 in COX-1 and COX-2 knockout mice and in wild-type control mice suggest that COX-1 and COX-2 gene deletion does not alter responses to vasoconstrictor agents.

Although it was once thought that COX-2 is primarily an inducible enzyme upregulated by inflammatory stimuli, the results of the present investigation and previous studies show that COX-1 and COX-2 are constitutively expressed and generate vasoactive prostanoids in the rodent (2, 3, 15, 16, 20–23, 29). It has been reported that COX-2 expression is upregulated in lung cancer and in pulmonary fibrosis; however, the role of COX-2 in the generation of vasoactive prostanoids in the mouse is unknown (4, 18). The results of the present study show that COX-1 and COX-2 can generate vasoactive prostanoids from precursor; however, the role of COX-1- and COX-2-generated prostanoids in regulation of the pulmonary vascular bed has not been determined. The results of Western blot analysis and immunohistochemical studies may suggest that COX-2 is upregulated in COX-1 knockout mice. However, more experiments are needed to confirm this observation.

In summary, the results of the present study provide evidence in support of the hypothesis that vasoactive prostanoids can be generated from arachidonic acid by COX-1 and COX-2 in the mouse. The hemodynamic data are consistent with Western blot analysis showing high COX-1 and COX-2 expression in the normal mouse lung. The vasoactive prostanoids formed by COX-1 and COX-2 have pulmonary pressor and systemic vasodepressor activity. The pulmonary pressor response is, in large part, mediated by ThA2 and correlates with immunofluorescence studies in lung, whereas ThA2 does not play a major role in modulating the systemic vasodepressor response to the precursor, although ThA2 receptors are present in the systemic vascular bed. The data for selective COX inhibitors suggest that although COX-1 and COX-2 generate vasoactive prostanoids, these pathways are not involved in modulating pressor responses to angiotensin II. The results with the selective COX inhibitors are consistent with results in COX-1 and COX-2 knockout mice, in which responses to angiotensin II are not different from responses in wild-type control mice. The decreases in systemic arterial pressure in response to arachidonic acid injection are smaller in COX-1 and COX-2 knockout than in wild-type control mice, whereas responses to U-46619 and PGE₁ are not different, suggesting that the genetic deletion of COX-1 or COX-2 does not change prostanoid-mediated vascular responses. These data, along with results showing that responses to norepinephrine and endothelin-1 are similar in COX knockout and wild-type control mice, suggest that COX-1 or COX-2 gene deletion does not have a major effect on vascular function in the mouse.

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