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Age-related changes in isoprostane-mediated relaxation of piglet blood vessels

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1. ABSTRACT

We studied the putative relaxant effects of several isoprostanes (8-iso-PGE_1, and 8-iso-PGE_2, 8-iso-PGF_{1alpha}, 8-iso-PGF_{1beta}, 8-iso-PGF_{2alpha}, and 8-iso-PGF_{2beta}) on pulmonary (PA), mesenteric (MA), coronary (CA) arteries and pulmonary veins (PV), from newborn and 2-week-old piglets. Isoprostanes were compared with agonists of the EP (PGE_1, PGE_2, and misoprostol), DP (PGD_2), and IP (iloprost) receptors. Isoprostane-induced relaxation was only observed when TP receptors were occupied (by U46619) or blocked (by SQ 29,548). Under these conditions, 8-iso-PGE_2 induced a relaxation of PA (but not PV or MA) that increased with postnatal age. 8-iso-PGE_1, 8-iso-PGE_2, and 8-iso-PGF_{2alpha} evoked modest relaxations in CA. 8-iso-PGE_2-induced relaxation of PA was impaired by endothelium removal and by the presence of blockers of NO synthase (L-NAME), guanylate cyclase (ODQ), or EP receptor (AH6809). PGE_1, PGE_2, and misoprostol (but not PGD_2 or iloprost) induced a relaxation of PA that increased with age. In conclusion, occupancy or blockade of TP receptors unmasked a relaxant effect of 8-iso-PGE_2 in piglet PA. This relaxation increased with postnatal age, was endothelium-dependent and involved EP receptors and NO.

2. INTRODUCTION

Isoprostanes are prostaglandin (PG) isomers that are produced *in vivo* primarily by a free radical-catalyzed peroxidation of polyunsaturated fatty acids (1, 2). Initially, isoprostanes were recognized as being valuable markers of oxidative stress and numerous pathological conditions have been shown to be associated with increases in urinary, plasma, and tissue levels of isoprostanes. Moreover, few members of the isoprostane family are biologically active and could contribute to the functional consequences of oxidant injury and mediate many of the features of the disease states for which they are used as indicators (1, 2).

There is increasing evidence that isoprostanes exert a wide variety of actions on vascular smooth muscle and endothelial cells. Many have described contractile responses to isoprostanes in a wide variety of vascular tissues, generally via stimulation of thromboxane A_2 receptors (TP receptors) (1-4) . In addition, and depending on the compound and the vascular bed, vasodilatory actions of isoprostanes have been also identified (5-7). These actions were frequently masked by the vasoconstrictive effects of isoprostanes on TP receptors and only were observed when these receptors were blocked by antagonists

(such as SQ 29,548 or ICI 192605) or saturated by an agonist (such as U46619) (5-7).

Newborns and particularly preterm infants are very susceptible to oxidative stress (8, 9). A large growing body of literature is addressing the possible role of isoprostanes in the pathophysiology of several neonatal conditions, such as asphyxia, intraventricular hemorrhage, periventricular leukomalacia, bronchopulmonary dysplasia, pulmonary hypertension and retinopathy of prematurity (10-16). However, the possible contribution of isoprostanes to the normal transition to extra-uterine life remains largely unknown. In a previous study, we reported that several E- and F-ring isoprostanes induced vasoconstriction in pulmonary arteries (PA), pulmonary veins (PV), and mesenteric arteries (MA) from newborn and 2-wk-old piglets (17). This effect was mediated by stimulation of TP receptors and decreased with postnatal age. Interestingly, we observed that 8-iso-PGE_2 presented a dual effect on piglet PA, inducing contraction at low concentrations and relaxation at high concentrations. The relaxant effects of 8-iso-PGE_2 were more marked in the PA from 2-week-old piglets than in the newborns (17). Similarly, Belik *et al.* demonstrated that in PA of adult rats contracted with U46619, 8-iso-$PGF_{2\alpha}$ induced a significant dose-dependent relaxation. In contrast, no effect was observed in arteries from 2-week-old animals, and a significant contraction was noted in PA from 1-wk-old animals (18). Therefore, the relaxant effects of isoprostanes in PA appear to be age-dependent. In the present study, we hypothesized that TP receptor blockade or occupancy unmasked the relaxant effects of several E-ring (8-iso-PGE_1 and 8-iso-PGE_2) and F-ring (8-iso-$PGF_{1\alpha}$, 8-iso-$PGF_{1\beta}$, 8-iso-$PGF_{2\alpha}$, and 8-iso-$PGF_{2\beta}$) isoprostanes in neonatal and 2-week-old piglet pulmonary, mesenteric and coronary vessels.

3. MATERIALS AND METHODS

3.1.Tissue preparation

All experiments were carried out in accordance with the European Animals Act 1986 (Scientific Procedures) and approved by institutional review board. Neonatal piglets aged 12–24 h (n=30) and 2 weeks (n=30), obtained from a local farm, were killed by exsanguination after being anesthetized with sodium pentobarbitone (100 mg/kg). The lungs, heart and intestines were rapidly immersed in cold (4°C) Krebs-Ringer bicarbonate buffer (composition in mmol/L: NaCl, 118.5; KCl, 4.75; $MgSO_4 \cdot 7\ H_2O$, 1.2; KH_2PO4, 1.2; $NaHCO_3$, 25.0; $CaCl_2$, 2.5; glucose, 5.5). PA, PV (third-fourth branch, *in situ* external diameter 1–2 mm), MA with a similar *in situ* external diameter (distal half of the MA trunk) and coronary artery (CA, left anterior descendent) were carefully dissected free of surrounding tissue and cut into rings of 2–3 mm of length under a dissection microscope. Lungs from adult pigs were obtained from a local abattoir and processed in the same way.

3.2 Isometric force measurement

After dissection, two L-shaped stainless steel wires were inserted into the arterial lumen and the rings were introduced in Allhin organ chambers filled with Krebs solution at 37°C, gassed with 95% O_2/5% CO_2. One wire was attached to the chamber and the other to an isometric force-displacement transducer (model PRE 206-4, Cibertec, Madrid, Spain). The isometric force signal was amplified, A/D converted (PowerLab, ADInstruments Pty., Castle Hill, Australia), and recorded (Chart v3.4, ADInstruments Pty.). An optimal resting tension of 0.3 g (PA of 12 to 24 h-old animals), 0.5 g (PA of 2-week-old animals and PV of both groups), 1 g (MA of 12- to 24 h-old animals and CA), or 2 g (MA of 2-week-old animals, PA of adults) was applied to the vascular segments, as determined from previous experiments (19-22). Tissues were allowed to equilibrate for 60–90 min. During this period, they were restretched and washed every 30 min with warm Krebs solution.

Concentration-response curves to isoprostanes: 8-iso-PGE_1 (15-E_{1t}-IsoP in the nomenclature of Taber *et al.*) (23), 8-iso-PGE_2 (15-E_{2t}-IsoP), 8-iso-$PGF_{1\alpha}$ (15-F_{1t}-IsoP), 8-iso-$PGF_{1\beta}$ (9-epi-15-F_{1t}-IsoP), 8-iso-$PGF_{2\alpha}$ (15-F_{2t}-IsoP), and 8-iso-$PGF_{2\beta}$ (9-epi-15-F_{2t}-IsoP) and prostanoids (misoprostol, PGE_1, PGE_2, PGD_2, and iloprost) were carried out in rings pre-contracted with U46619 (0.1-1μM) or endothelin-1 (ET-1, 10nM). In some experiments, the vascular effects of isoprostanes were tested in endothelium-denuded arteries or in the presence of the TP receptor antagonist SQ 29,548 (0.1μM), the non-selective EP receptor antagonist AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid; 10 μM), the cyclooxygenase (COX) inhibitor indomethacin (10μM), the non-selective NO synthase inhibitor L-NAME (0.1mM), or the soluble guanylate cyclase inhibitor ODQ (10μM). At the end of the experiments, papaverine (0.1mM) was added to determine the maximum relaxation of each vessel.

3.3 Drugs and solutions

Isoprostanes, U46619 (9,11-dideoxy-11$_\alpha$,9 $_\alpha$-epoxymethanoprostaglandin $F_{2\alpha}$ methyl acetate solution), misoprostol, PGE_1, PGE_2, PGD_2, iloprost, and AH6809 were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Isoprostanes and ODQ were dissolved initially in DMSO. Indomethacin and prostaglandins were dissolved initially in ethanol. All other chemicals were dissolved initially in distilled deionized water. The maximal bath concentration of DMSO and ethanol did not exceed 0.1%, which was found to have no effect on mechanical activity.

3.4 Analysis of data

Results are expressed as mean ± SEM and *n* reflects the number of animals from which the rings were obtained. Contractions are expressed in terms of active wall tension (mN), while the relaxant responses are expressed as the percentage of the maximum relaxation induced by papaverine in each individual vessel. The maximal effect (E_{max}) produced with the highest concentration (10 μM) of an agonist and the half-maximum effective concentration (EC_{50}) value were interpolated from the individual concentration-effect curves. The pD_2 values were calculated as the negative log of EC_{50} values. Statistically significant differences between groups were calculated by

Table 1. Contractions induced by U46619 and ET-1 in porcine pulmonary arteries

Agonist	Concentration (μM)	Age	Tension (mN)	*n*
U46619	0.1	NB	4.88±0.51*†	24
U46619	0.1	2 wk	6.85±0.64	26
U46619	1	2 wk	9.16 ±0.88*	8
U46619	0.1 +AH	2 wk	4.51±0.52*	8
U46619	0.1	adult	17.73±2.95*	12
ET-1	0.01	NB	6.70±0.70	24
ET-1	0.01	2 wk	8.83±0.82	26
ET-1	0.01 +SQ	2 wk	8.47±0.94	26

Values are means ± SEM of n animals. NB= newborn; AH= AH6809; SQ= SQ29,548; *P<0.05 vs. U46619/0.1 μM/2 wk ; †P<0.05 vs. adult.

Student's test for unpaired observations or for multiple comparisons by one-way ANOVA followed by Bonferroni's test. P<0.05 was considered statistically significant.

4. RESULTS

The contractions induced by U46619 and ET-1 under the different experimental conditions are summarized in Table 1. In endothelium intact PA pre-contracted with U46619 (0.1 μM), 8-iso-PGE_2 induced a concentration-dependent relaxation (Figures 1A and 1B) with higher efficacy and potency in the 2-wk-old (E_{max} 76.37± 5.09%, pD_2 5.95±0.13, *n*=8) than in the neonatal vessels (E_{max} 22.06 ±2.92%, pD_2 5.51±0.01, *n*=5, P<0.05 vs. 2-week-old) (Figures 1 and 2). No significant differences in the relaxant effects of 8-iso-PGE_2 were observed when the PA were pre-contracted with U46619 1μM instead of 0.1μM (Figure 2). The other isoprostanes (8-iso-PGE_1, 8-iso-$PGF_{1\alpha}$, 8-iso-$PGF_{1\beta}$, 8-iso-$PGF_{2\alpha}$, and 8-iso-$PGF_{2\beta}$) did not induce significant relaxation of U46619-contracted PA from newborn and 2-wk-old animals (an example of 8-iso-$PGF_{2\alpha}$ is shown in Figure 1C), and none of the isoprostanes relaxed PV (an example is shown in Figure 1D). In adult PA, 8-iso-PGE_1, and 8-iso-$PGF_{2\alpha}$ induced modest relaxations and 8-iso-PGE_2 evoked a relaxation of 56.71 ± 8.91% (pD_2 6.29 ±0.12, n=6, P<0.05 vs. newborn) (Figure 2C).

When the pulmonary vessels were pre-contracted with ET-1 (10 nM) none of the isoprostanes induced a significant relaxation. As shown in Figure 2A, high concentrations of 8-iso-PGE_2 even increased the tone induced by ET-1 and this effect was reversed by adding the TP receptor antagonist SQ 29,548 (0.1μM). As shown in Figure 2B, when SQ 29,548 was present in combination with ET-1, 8-iso-PGE_2 relaxed the PA (but not PV). The maximal relaxation induced by iso-8-iso-PGE_2 in 2-wk-old PA incubated with SQ 29,548 and pre-contracted with ET-1 was 35.70 ± 12.02 (Figure 2A). The presence of SQ 29,548 did not unmask a relaxant effect of the other isoprostanes tested in PA or veins (data not shown).

As shown in Figures 3A and 3B, the relaxation induced by 8-iso-PGE_2 in the PA was impaired (P<0.05 vs. control) by endothelium removal (E_{max} 28.08 ± 7.06%), or by the presence of NO synthase inhibitor L-NAME (E_{max} 42.79± 7.06%), the guanylate cyclase inhibitor ODQ (E_{max} 27.72± 4.65%), or the EP receptor antagonist AH6809 (E_{max} 44.50 ± 6.04%) In contrast, the presence of the COX inhibitor indomethacin did not significantly affect 8-iso-PGE_2–evoked relaxation of PA (Figure 3B).

In order to asses the functionality of prostanoid receptors in the 2-wk-old piglet PA, we examined the relaxant effects of the EP receptor agonists PGE_1, PGE_2, and misoprostol, the DP-receptor agonist PGD_2, and the IP receptor agonist iloprost and we observed that only the EP receptor agonists relaxed U46619-contracted arteries (Figure 4). In contrast, the IP and EP receptor agonists did not produce any relaxation under the same experimental conditions. As shown in Figure 5 and Table 2, the relaxant efficacy of PGE_1, PGE_2, and misoprostol, as well as the sensitivity of the PA to the adenylate cyclase stimulator forskolin increased with postnatal age.

None of the isoprostanes tested induced relaxation of MA pre-contracted with U46619. In contrast, 8-iso-PGE_1, 8-iso-PGE_2, and 8-iso-$PGF_{2\alpha}$ relaxed U46619-contracted CA in a concentration-dependent-manner (Figure 3C). The maximal relaxations obtained (with an isoprostane concentration of 10 μM) were 19.85 ± 6.54%, 29.56 ± 7.89%, and 10.27 ± 5.78% (for 8-iso-PGE_1, 8-iso-PGE_2, and 8-iso-$PGF_{2\alpha}$, respectively). Endothelium removal did not affect isoprostane-induced relaxation in CA (data not shown). The other isoprostanes tested (i.e. 8-iso-$PGF_{1\alpha}$, 8-iso-$PGF_{1\beta}$, and 8-iso-$PGF_{2\beta}$) did not induce significant relaxations in CA.

5. DISCUSSION

In a previous work we demonstrated that several E- and F-ring isoprostanes induced TP receptor-mediated contraction of PA, PV, and MA form newborn and 2-week-old piglets (17). Herein, we report that occupancy (with the agonist U46619) or blockade (with the antagonist SQ 29,548) of TP receptors unmasked a relaxant effect of 8-iso-PGE_2 in the PA. This effect was not observed for the other E- or F-ring isoprostanes tested. 8-iso-PGE_2 - mediated PA relaxation increased with postnatal age, was partially endothelium-dependent and appeared to involve stimulation of EP receptors and production of NO.

Several studies demonstrated that when their effects through TP receptors are blocked, certain isoprostanes can elicit a relaxant response in pulmonary vessels. However, the relaxant potency and efficacy of isoprostanes in pulmonary vascular smooth muscle is very low when compared with airway smooth muscle (1, 2, 24). 8-iso-$PGF_{2\alpha}$ induced relaxation of rat PA by a non-TP receptor mechanism that involves the release of NO (5, 18). 8-iso-PGE_1 evoked dose-dependent relaxations in human and canine PV, and in canine PA, but not in the human PA (25). 8-iso-PGE_2, and 8-iso-$PGF_{1\alpha}$ also induced modest relaxations of human PV (25). In the present study, we observed that 8-iso-PGE_2 was the isoprostane with the highest relaxing efficacy in porcine PA. 8-iso-PGE_1 and $PGF_{2\alpha}$ elicited modest relaxations in the adult PA, which were not found in the vessels from newborn or 2-week-old

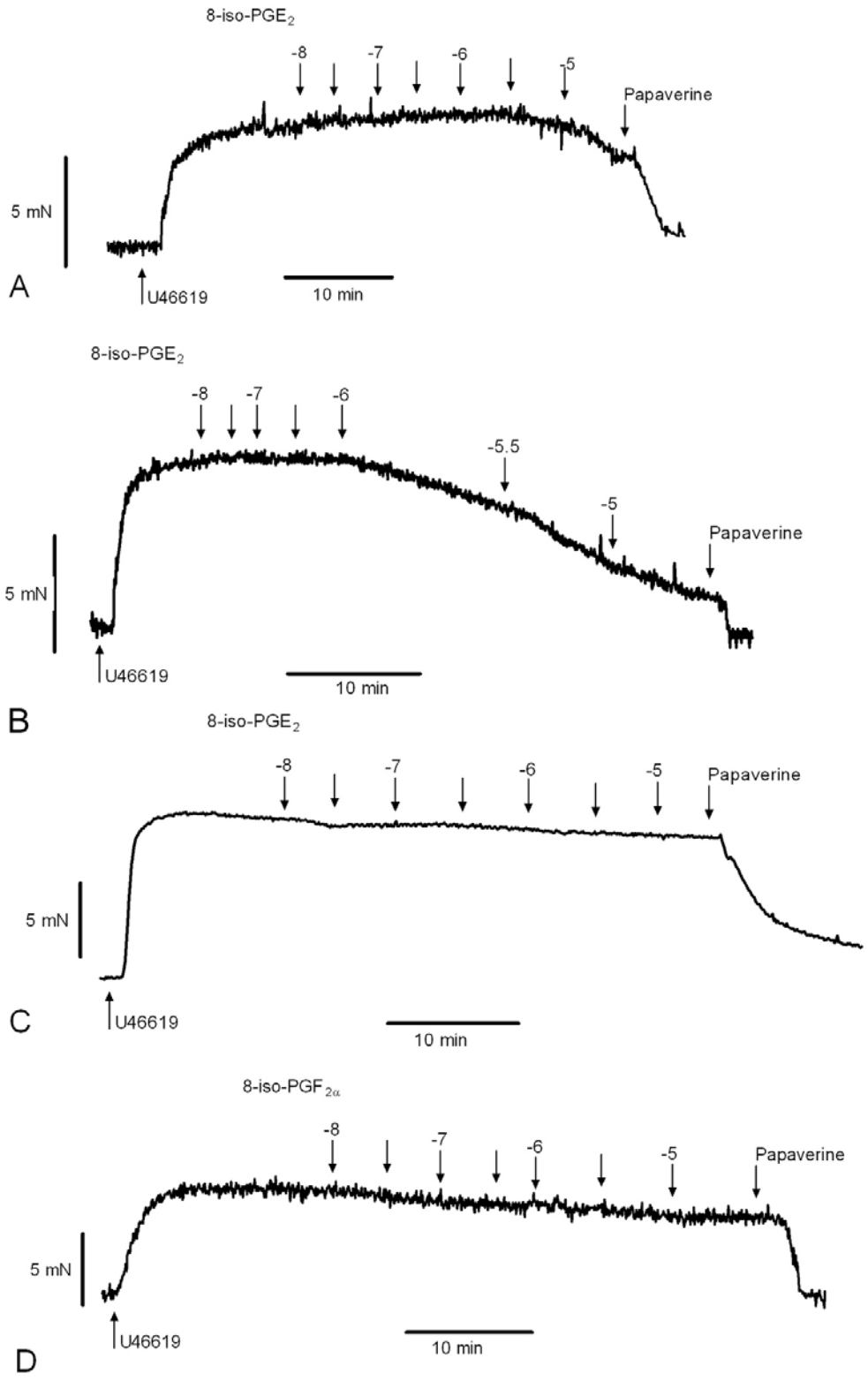

Figure 1. Relaxant effects of cumulative half-logarithmic concentrations of isoprostanes in piglet pulmonary vessels. Typical original recordings of tension development over time in pulmonary artery (A-C) and vein (D) rings from newborn (A) and 2-wk-old (C-D) piglets. Vessels were pre-contracted with U46619 (0.1 μM) and, at the end of the experiment, papaverine (0.1mM) was added to determine the maximum relaxation.

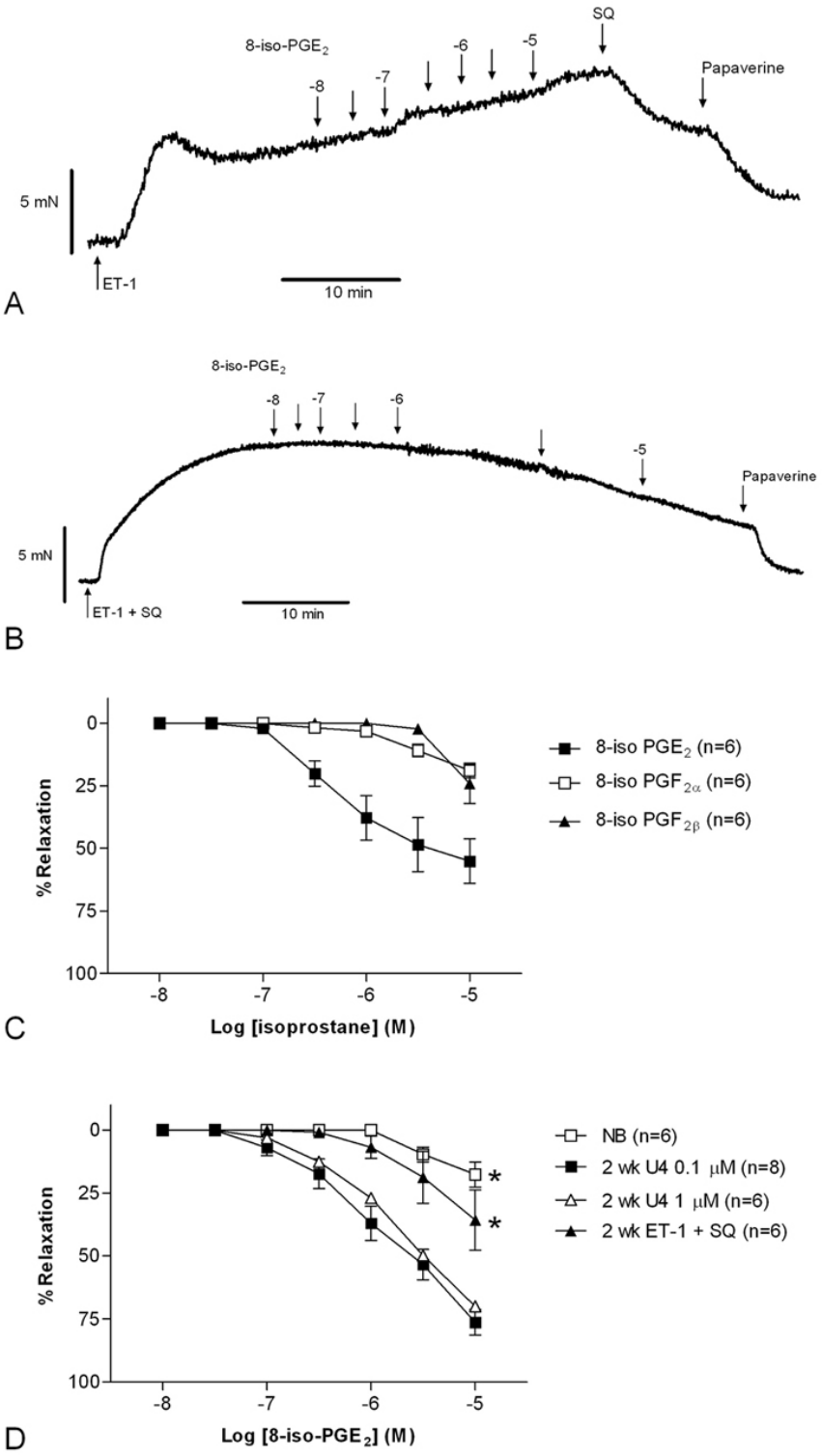

Figure 2. Relaxant effects of isoprostanes in porcine pulmonary vessels. A, B: Typical original recordings of tension development over time in pulmonary artery rings from 2-wk-old piglets pre-contracted with endothelin-1 (ET-1, 10 nM). Observe (A) that 8-iso-PGE_2 increased the tone induced by ET-1 and this effect was reversed by adding the TP receptor antagonist SQ 29,548 (0.1μM). However, when SQ 29,548 was present in combination with ET-1 (B), 8-iso-PGE_2 relaxed the pulmonary artery. At the end of the experiment, papaverine (0.1mM) was added to determine the maximum relaxation. C, D: Summary of the relaxant effects of isoprostanes in PA from newborn (C), 2-wk-old, and adult pigs.

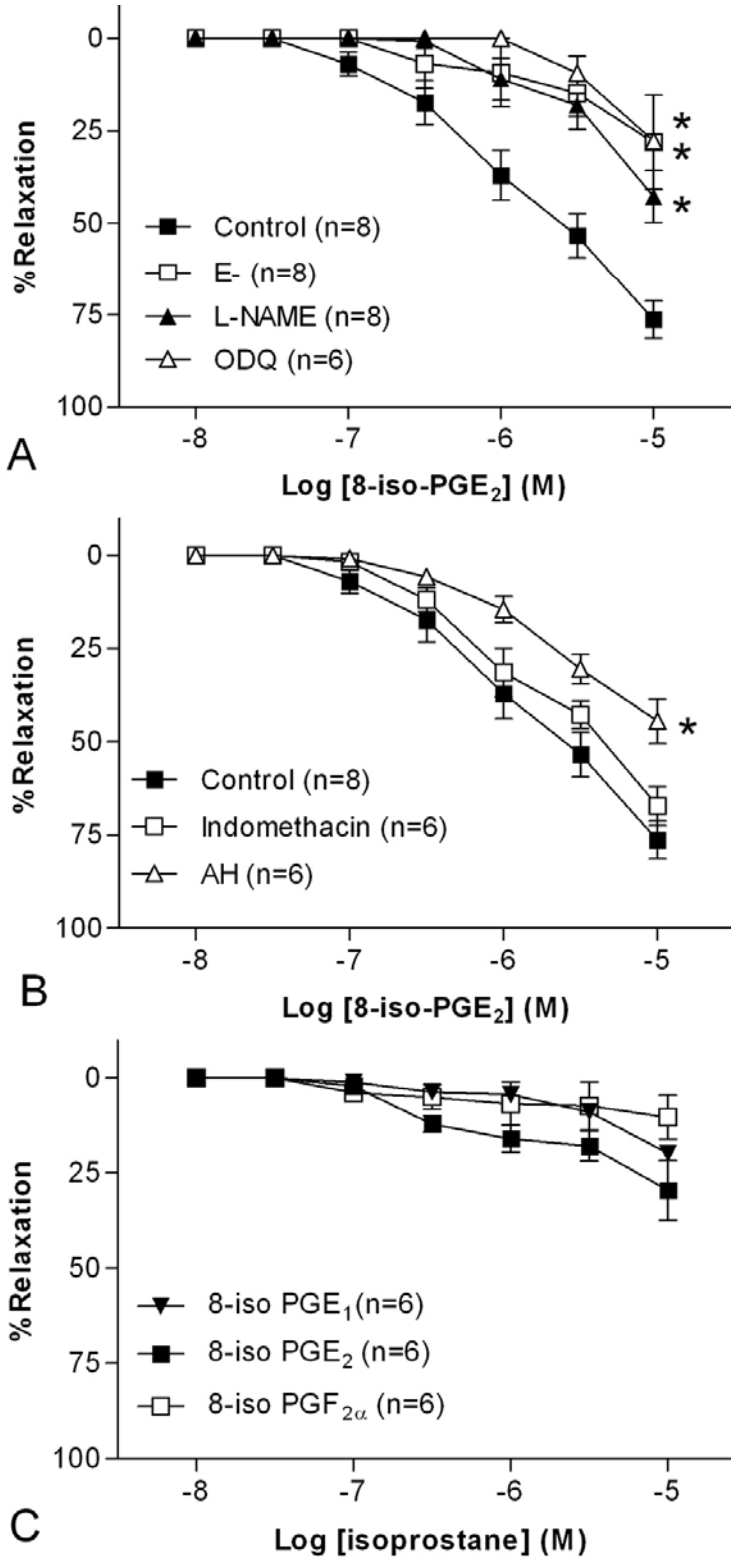

Figure 3. A, B: Effects of endothelium removal (E-), the non-selective NO synthase inhibitor L-NAME (0.1mM), the soluble guanylate cyclase inhibitor ODQ (10μM), the COX inhibitor indomethacin (10μM), and the non-selective EP receptor antagonist AH6809 (10 μM) on the relaxations induced by 8-iso-PGE_2 in pulmonary arteries form 2-wk-old piglets. For clarity, the control group is repeated in panels A and B. C: Relaxant effects of isoprostanes in coronary artery rings from 2-wk-old piglets. All the vessels were pre-contracted with U46619 (0.1 μM). *P<0.05 for difference in E_{max} from control.

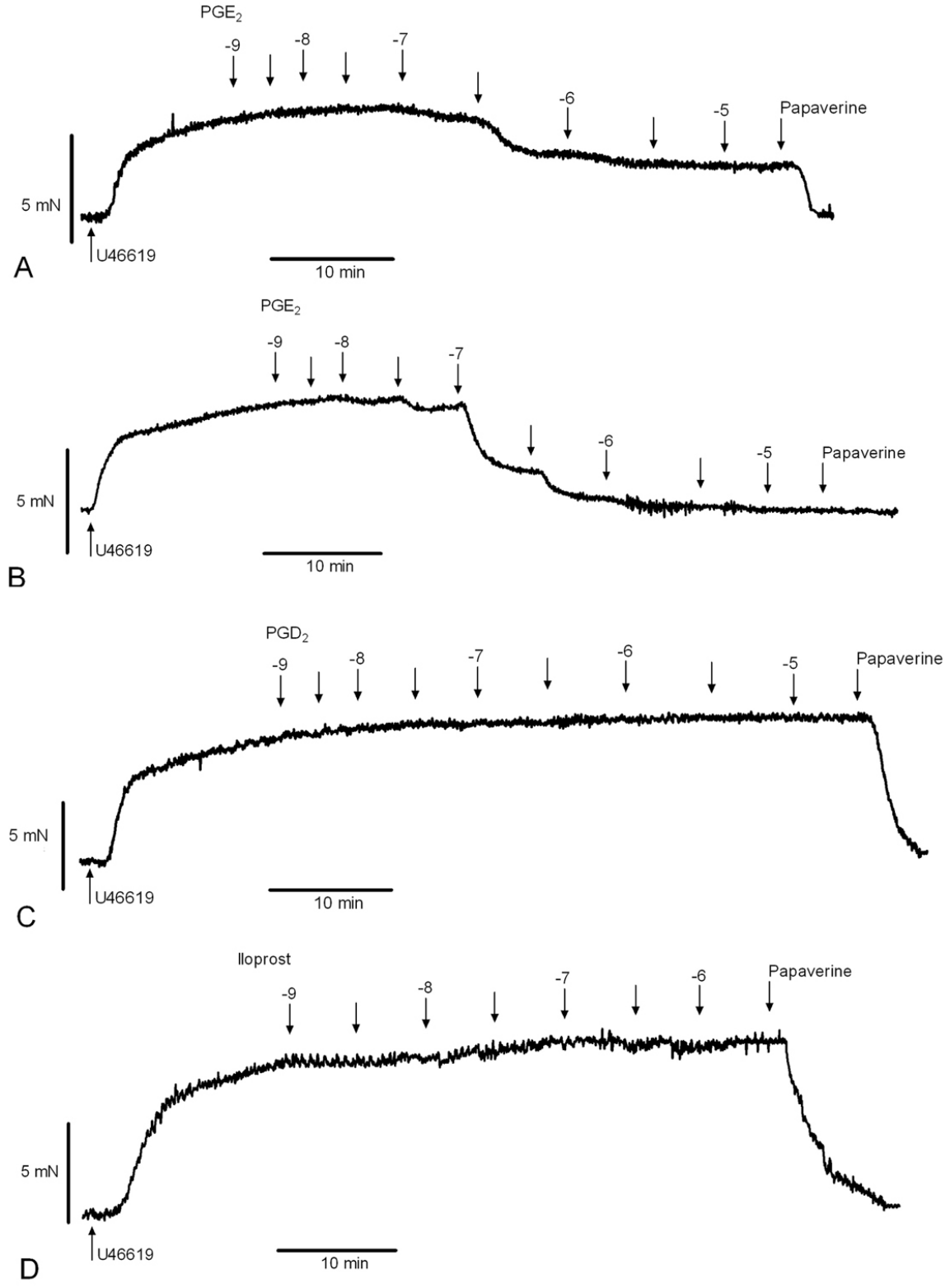

Figure 4. Relaxant effects of cumulative half-logarithmic concentrations of prostanoids in pulmonary arteries from 2-wk-old piglets. Typical original recordings of tension development over time. Vessels were pre-contracted with U46619 (0.1 μM) and, at the end of the experiment, papaverine (0.1mM) was added to determine the maximum relaxation.

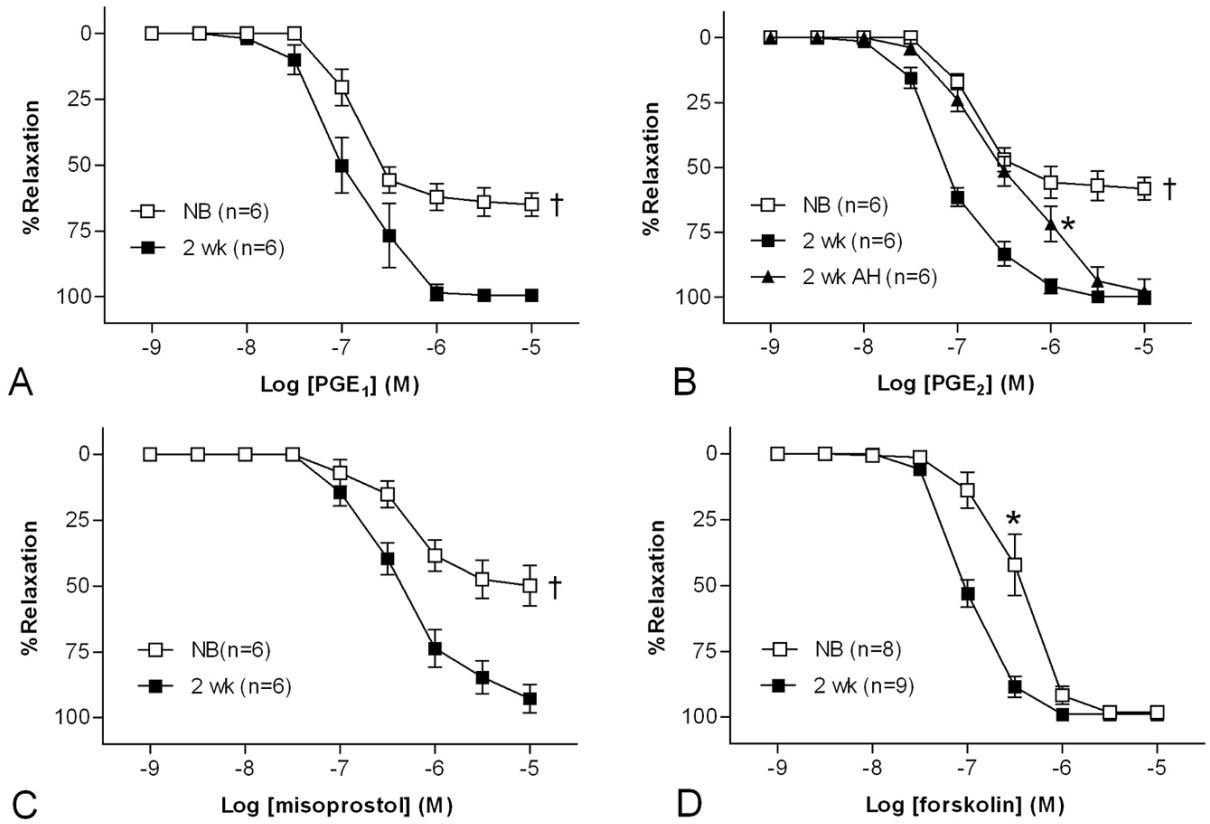

Figure 5. Relaxant effects of the EP receptor agonists prostaglandin (PG)E1 (A), PGE2 (B), and misoprostol and the adenylate cyclase stimulator forskolin (D) in pulmonary arteries form newborn (NB) and 2-wk-old piglets. Vessels were pre-contracted with U46619 (0.1 μM). The effects of the non-selective EP receptor antagonist AH6809 (10 μM) on the relaxation induced by PGE_2 are shown in panel B. *P<0.05 for difference in pD_2 from control. †P<0.05 for difference in E_{max} from control.

animals. Therefore, the vascular relaxant effects of isoprostanes appear to be species-, tissue-, and –age dependent. Accordingly, Belik *et al.* found significant developmental differences in the NO-dependent, rat pulmonary arterial relaxant response to 8-iso-$PGF_{2\alpha}$ (18).

Isoprostanes and prostanoids share a similar structure, differing only in the orientation of their aliphatic side chains (1, 2). Thus, it is reasonable to assume that isoprostanes are capable of forming similar binding interactions with classic prostanoid receptors (1, 2, 26), four of which (DP, IP, EP_2, and EP_4) mediate relaxation through a cyclic AMP-dependent mechanism (27). A possible explanation for the modest isoprostane-induced relaxations that we found might be a low functionality of prostanoid receptors in our vascular preparations. However, we observed that the EP receptor agonists PGE_1 (present work and 28), PGE_2, and misoprostol were potent relaxants of the porcine PA. In contrast, the DP-receptor agonist PGD_2, and the IP-receptor agonist iloprost did not evoke relaxation of these vessels. To the best of our knowledge, the prostanoid receptor population expressed in porcine PA has not been characterized. The main receptor responsible for PGE_2-induced ductus arteriosus relaxation in fetal pigs is EP_2, but the cAMP-inhibiting EP_3 receptor is also present, modulating the dilating effect of PGE_2 (29). E-ring isoprostanes evoke EP-mediated relaxations in porcine airway smooth muscle (26). We found that PGE_2- and 8-iso-PGE_2-induced relaxation of piglet PA was impaired by AH6809 suggesting the involvement of EP receptors. However, AH6809 is a non selective EP receptor antagonist, which does not allow us to discriminate between EP_2 and EP_4 receptors. Moreover, AH6809 may also block DP and TP receptors (30, 31). Accordingly, we observed that AH6809 impaired the contraction induced by U46619. Interestingly, a recent study in murine aorta demonstrated that PGE_2 elicits relaxation through EP_4 receptor-mediated formation of cAMP by the endothelium, which stimulates endothelial NOS activity (32). With our present results we can only speculate about the putative participation of this mechanism in the endothelium-dependent relaxation evoked by 8-iso-PGE_2. Future functional experiments with more selective EP antagonists (32), as well as investigations addressing the expression and binding activity of prostanoid receptors in porcine pulmonary vessels are, therefore, warranted.

Depending on the species and the vascular tissue, the relaxations evoked by isoprostanes has been reported to be endothelium-dependent (5, 18) or –independent (7). Isoprostanes have been shown to induce endothelial NO, TxA_2, ET-1 and inositol 1,4,5-trisphosphate formation (10,

Table 2. Concentration-response parameters for prostanoids and forskolin in piglet pulmonary arteries

Agonist	Newborn			2-wk-old		
	E_{max}	pD_2	*n*	E_{max}	pD_2	*n*
PGE_1	64.9 ± 4.4	6.82 ± 0.23	6	99.3 ± 2.2*	6.94 ± 0.19	6
PGE_2	58.2 ± 4.2	6.78 ± 0.14	6	99.8 ± 0.5*	7.08 ± 0.12	6
Misoprostol	49.8 ± 7.1	6.24 ± 0.14	6	92.7 ± 5.3*	6.38 ± 0.12	6
Forskolin	98.2 ± 2.2	6.42 ± 0.11	6	98.9 ± 0.7	7.0 ± 0.11*	6

Values are means ± SEM of n animals. *P<0.05 vs. newborn..

18, 33). We observed that 8-iso-PGE_2 –induced relaxation of PA (but not CA) was impaired by endothelium removal as well as by NOS and sGC inhibition, suggesting a role for endothelium-derived NO. The age-dependent increase that was observed for 8-iso-PGE_2 –elicited relaxation, is a common finding for other vasodilators acting through the NO-sGC-cGMP pathway in piglet PA and has been extensively discussed elsewhere (19-21, 34-37). Developmental changes in the expression and/or activity of NO synthases, sGC, phosphodiesterases, and reactive oxygen species-producing enzymes may account for this postnatal increase in responsiveness (19-21, 34-36). Herein, we found that the relaxant efficacy of PGE_1, PGE_2, and misoprostol, as well as the potency of the adenylate cyclase stimulator forskolin, also increased with age. This suggests that also the adenylate cyclase/cAMP pathway undergoes significant alterations during the first days of postnatal life. Alternatively, as discussed below, the developmental changes in relaxation might be related to alterations in the responsiveness to U46619, the TP receptor agonist used to contract the arteries in our experiments (34).

Cogolludo *et al.* (34) have shown that two pathways are involved in TP-receptor induced vasoconstriction in piglet PA. First, the activation of protein kinase C (PKC) zeta leads to voltage-gated K^+ channels channel blockade, membrane depolarization, L-type Ca^{2+} channel activation, and increase in $[Ca^{2+}]_i$. Second, a RhoA/Rho kinase-mediated Ca^{2+} sensitization pathway also mediate U46619-induced contraction. This second pathway contributes more importantly to TP-mediated contraction in the 2-week-old animals than in the newborns (34). Ca^{2+}-desensitization is mediated by both cGMP- and cAMP-dependent protein kinases (38). Therefore, the maturational shift from PKC and voltage-gated K^+ channels to RhoA/Rho kinase-mediated Ca^{2+} sensitization in TP receptor-elicited pulmonary vasoconstriction might be partially responsible for the developmental increase in cGMP- and cAMP-mediated relaxation.

In conclusion, our results contribute to reinforce the idea that isoprostanes are not only markers but also mediators of the biological effects of oxidative stress. Part of the vascular effects of isoprostanes are based on their capacity to stimulate contractile and relaxant receptors (1, 2, 17, 18, 25) but another part is based on their stimulation of the production and release of vasoconstrictors and vasodilators (1, 2, 10, 18, 33). In addition, reactive oxygen species induce the release of isoprostanes and other vasoactive mediators but can also interact with these mediators (21, 36) and may even be produced by them as part of their transduction mechanisms (37, 39). The interactions of these processes achieve a particular relevance in the perinatal period, which is characterized by abrupt changes in the oxidant status (8, 9).

5. ACKNOWLEDGEMENTS

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Abbreviations: PG: prostaglandin, PA: pulmonary arteries, PV: pulmonary veins, MA: mesenteric arteries, CA: coronary arteries, NO: nitric oxide, NOS: NO synthase, COX: cyclooxygenase, Tx: thromboxane, sGC: soluble guanylate cyclase, PKC: protein kinase C

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