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Role of Microsomal Prostaglandin E Synthase-1 (mPGES1)-Derived PGE_2 in Patency of the Ductus Arteriosus in the Mouse

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ABSTRACT: Prostaglandin E_2 (PGE_2) plays a key role in the ductus arteriosus, prenatally by maintaining patency and postnatally by promoting tissue remodeling for closure. Here, by using near-term mouse fetuses with (wild-type, WT) and without microsomal PGE synthase-1 (mPGES1−/−), we have examined the importance of this enzyme for PGE_2 formation and function. mPGES1−/− ductus, unlike WT ductus, contracted little, or not all, to indomethacin *in vitro*. Coincidentally, as evident from responses to N^G-nitro-L-arginine methyl ester and zinc photoporphyrin, the mutant showed no significant enhancement of nitric oxide (NO)- and carbon monoxide (CO)-based relaxation. mPGES1 suppression differs, therefore, from cyclooxygenase (COX) suppression, whether genetically or pharmacologically induced, where NO is markedly up-regulated. *In vivo*, the ductus was patent, albeit occasionally with a narrowed lumen, in all mPGES1−/− fetuses. Conversely, postnatal closure progressed regularly in mPGES1−/− animals thanks to residual PGE_2 originating via mPGES2. We conclude that mPGES1 is critical for PGE_2 formation in the ductus but its loss does not entail compensatory up-regulation of other relaxing mechanisms. Accordingly, an mPGES1 inhibitor stands out as a prospective better tool, compared with the currently used COX inhibitors, for the management of premature infants with persistent ductus. (***Pediatr Res* 64: 523–527, 2008**)

Prostaglandin (PG) E_2 is viewed as the prime agent for prenatal patency of the ductus arteriosus (1). Conversely, PGI_2 is without a role, notwithstanding its occurrence in the vessel and its known importance for vasoregulation elsewhere (2,3). Ductal PGE_2 is formed primarily through the cyclooxygenase-2 (COX2)/microsomal PGE synthase-1 (mPGES1) complex, while little of the compound originates from the COX1/mPGES1 complex and none at all from cytosolic PGES (cPGES). No information is available on microsomal PGE synthase-2 (mPGES2), but evidence from other vascular districts points to its minor function (4). Several findings support this arrangement in the ductus, namely, immunocytochemical data showing a predominant colocalization of COX2 with mPGES1 (5); the increase in COX2 and mPGES1 expression occurring selectively through development (6); the constrictor action of COX2 inhibitors on the ductus, being close or even equal in magnitude to that of a dual COX1/COX2 inhibitor (7–9); the greater impairment of PGE_2-based relaxation upon COX2 than COX1 deletion (5) along with the marked curtailment of PGE_2 formation after inhibition of either COX2 or mPGES1 (6,7). Consistent with the preferential operation of the COX2/mPGES1 route for PGE_2 synthesis is also evidence from adult blood vessels (10) and the notion that mPGES1 is catalytically most active among the PGES (11).

Despite these facts, previous attempts to prove the actual importance of the COX2/mPGES1 pathway for ductus patency have failed. As shown by others and ourselves (5,12,13), COX2 removal does not result in loss of patency, likely thanks to compensation from COX1-derived PGE_2 and nonPG agents such as nitric oxide (NO) and carbon monoxide (CO). Indeed, COX2 removal, even if limited to a single allele, is followed by selective up-regulation of NO (5,14). An additional complication in addressing this issue is that PGE_2 not only sustains patency of the ductus *in utero*, but also promotes its closure after birth (9,15,16). Hence, COX2/mPGES1 function needs to be considered pre and postnatally.

Our objective here was 2-fold: first, to assess the importance of mPGES1 in ensuring normal patency and closure of the ductus, and second, to ascertain whether mPGES1 removal mimics COX2 removal in up-regulating NO. CO was examined comparatively to verify the specificity of any such effect. An answer to these questions is important insofar as it may provide a better insight into the functional organization of the PGE_2 system and the role of PGE_2 vis-à-vis NO and CO in ductus relaxation. In addition, any advance in this area may lead to better agents, in comparison to the currently used COX inhibitors (17), for the management of the prematurely born infant with patent ductus (PDA).

METHODS

Animals. Experiments were carried out in 129×C57BL/6 mice with mPGES1−/− genotype (18) (litter size, 2–9; mean, 4), and wild-type (WT) C57BL/6 mice (litter size, 1–12; mean, 5) served as a control. In either case, genotype was confirmed in tail specimens by polymerase chain reaction (PCR). Animals were housed in temperature- and humidity-controlled quar-

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Abbreviations: COX, cyclooxygenase; **L-NAME,** N^G-nitro-L-arginine methyl ester; **mPGES1,** microsomal PGE synthase-1; **mPGES2,** microsomal PGE synthase-2; **ONO-11113,** 9, 11-epithio-11, 12-methano-thromboxane A_2; **PDA,** patent ductus arteriosus; **PG,** prostaglandin; **ZnPP,** zinc protoporphyrin

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ters with constant 12:12-h light-dark cycles and were given food and water *ad libitum*. Surgical procedures and experimental protocols were approved by the Animal Care Committee of the Ministry of Health.

In vitro studies. Term fetal mice, both WT and gene-deleted (gestational age, 19 d), were delivered by cesarean section under halothane anesthesia and were killed by cervical dislocation. Body weight varied between 0.9 and 1.4 g, and only one fetus was used in each experiment. The procedure for dissection of the ductus arteriosus, normalization of internal circumference and mechanical record has been described previously (19). In brief, the animal was secured with its left side up in a dissection chamber containing ice-cold Krebs solution gassed with 5% CO_2 in N_2. Through a thoracotomy, the ductus was exposed, separated from the adjoining large blood vessels and then suspended onto 25-μm tungsten wires (Cooner wire, Chatsworth, CA) inside an organ bath. The fluid of the bath was gassed with a mixture containing 2.5% O_2 to mimic the fetal condition, and the same gas mixture was flushed through a hood covering the bath. Preparations were then equilibrated (about 60 min at 37°C) with a minimal stretch being applied (all values in mN mm^{-1}) (WT, 0.09 ± 0.01 (n = 14); mPGES1−/−, 0.08 ± 0.008 (n = 16)) and the attendant internal circumference (C_0) with the related resting dimension (Table 1) served as a reference for choosing the appropriate load. Afterward, tension was applied to attain an operating circumference (*i.e.* C_{50}) coinciding with the condition *in vivo* (WT, 0.45 ± 0.007 (n = 14); mPGES1−/−, 0.45 ± 0.009 (n = 16); values in mN mm^{-1}), and the actual experiment was started after a second, 60- to 150-min period of equilibration.

The study consisted of a single protocol with two test procedures, addressing respectively the effect of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM; n = 13) and zinc protoporphyrin (ZnPP, 10 μM; n = 8) on the basal tone. Indomethacin (2.8 μM; n = 9) was tested comparatively to verify the degree of loss of PGE_2 function upon mPGES1 deletion. In all cases, treatment lasted 60 min or as long as required to achieve a maximal effect, and the contraction to the thromboxane (TX) A_2 analogue (0.1 μM) provided a reference. Only one test procedure was used with each vessel.

In vivo studies. Experiments were carried out in fetuses (gestational age, 19 d) or newborns (3 h after birth) depending on the protocol. In the former case, animals (WT and mPGES1-deleted) were delivered by cesarean section under halothane anesthesia, whereas in the latter they were used at the stated interval after vaginal delivery. No obvious difference in the length of gestation was noted between WT and mutant, and time zero (*i.e.* the time at which the delivery was completed) was assessed for each animal in the litter. Throughout the survival period, newborns were kept with the mother. All animals were killed by cervical dislocation.

Changes in ductus caliber through the transition from the pre to the postnatal condition were assessed by fixing the vessel *in situ* with the whole-body freezing technique (19,20). Briefly, animals were placed with their right side up in a Petri dish and were covered with an embedding medium (Tissue-Tek optimum cutting temperature compound; Sakura Finetek, Torrance, CA). The dish with the animal was then wrapped with aluminum foil and immersed in liquid N_2. Once frozen, specimens were stored at −20°C for further work-up. To prepare a block of tissue with the ductus, the carcass was freed of its embedding in the frozen state. With a razor blade, soft tissues covering the dorsum were removed making certain that the exposed surface would be parallel to the underlying descending aorta, hence perpendicular to the ductus. Several, transversal sections (5 μm thick), progressing from this cut through the whole length of the vessel, were obtained on a Zeiss freezing microtome (model HM500 OM, Welledorf, Germany) and were stained with 1% methylene blue. Care was taken to collect a complete series of ductus sections. Afterward, each section was photographed with a charge-coupled device solid-state camera (COHU, San Diego, CA) and lumen area of the ductus was measured with a MCID-M4 program (Brock University, St. Catharines, Canada). Both maximal and minimal values of this area were retrieved from each series for computation.

Total RNA preparation and quantitative RT-PCR. mPGES2 expression was measured to assess any change resulting from mPGES1 deletion. For this purpose, ductuses were collected from WT and mPGES1 null fetuses and were pooled in three separate groups for each genotype (35–41 specimens/group). RNA was then isolated as described earlier (5), and its yield was measured spectrophotometrically. DNAse I (Roche, Indianapolis, IN)—treated total RNA (2.5 μg) was reverse transcribed with 1 U of Thermoscript RT (Invitrogen, Carlsbad, CA) in the presence of random hexanucleotide primers according to the manufacturer's instructions. QRT-PCR reactions (40 cycles) were performed on an ABI Prism 7700 instrument (Applied Biosystems, Foster City, CA) using the TaqMan Universal PCR Master Mix (Applied Biosystems). Primer sequences for mPGES2 and cyclophilin B (internal standard) were obtained from an online library (Applied Biosystems). Gene expression was quantified in triplicate for each group, using the comparative cycle-threshold method and cyclophilin for normalization.

Solutions and drugs. The Krebs medium had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1, $MgSO_4$ 0.9, dextrose 11.1, and $NaHCO_3$ 25. Depending on the stage of the experiment (see above), the solution was bubbled with gas mixtures containing either no O_2 or 2.5% O_2 plus 5% CO_2 and balance N_2. Po_2 was measured with a Chiron gas analyzer (mod. 248, Halstead, UK) and was 0.95 ± 0.0009 and 2.14 ± 0.02 kPa (pH, 7.41 ± 0.003) when gas mixtures had 0 and 2.5% O_2, respectively.

The following compounds were used: the NO synthase inhibitor, L-NAME (Sigma Chemical Co., St. Louis, MO); the heme oxygenase (HO) inhibitor, ZnPP (Porphyrin Products, Canforth, UK); the dual COX1/COX2 inhibitor, indomethacin (Sigma Chemical Co.); the TXA_2 analogue 9, 11-epithio-11, 12-methano-TXA_2 (ONO-11113, courtesy of ONO Pharmaceutical, Osaka, Japan). Concentrations of the inhibitors were derived from the literature with the aim of combining efficacy with selectivity. In particular, at the stated concentrations, ZnPP exerts a specific effect on the HO enzyme complex in the ductus (21).

ONO-11113 was dissolved in distilled ethanol (5 mg ml^{-1}), and aliquots (stored at −70°C) were diluted with Tris buffer (pH 7.4). Indomethacin was also dissolved in ethanol (10 mg ml^{-1}) before preparation of the final solution in the Krebs medium. Likewise, ZnPP was first prepared as a stock solution in 0.1 M NaOH (1 mM) on the day of the experiment. Ethanol in the fluid bathing isolated ductus preparations did not exceed 0.01% (indomethacin) or 0.001% (ONO-11113). Solutions of ZnPP were protected from light.

Concentrations of compounds are given in molar units and refer to their final value in the bath. Vehicle alone had no effect on vessel tone.

Analysis of data. Baseline contractile tension, which varied with the preparation (see "Results"), refers to the net active tension (*i.e.* total tension minus applied tension) developed by the preparation before any treatment. Contractile responses were measured by the rise in tension over baseline and were expressed in absolute values.

Data are presented as means ± SEM. Comparisons were made using a t test or analysis of variance (ANOVA), followed by the Bonferroni test. Differences are considered significant for $p < 0.05$.

Table 1. *Resting dimension of isolated ductus arteriosus from WT and mPGES1−/− term fetal mice*

Genotype	Internal diameter	Vessel length*		Wall thickness
		Short side	Long side	
WT (n = 14)	132 ± 1	568 ± 19	581 ± 19	20 ± 0.5
mPGES1−/− (n = 16)†	132 ± 2	513 ± 11‡	524 ± 11‡	20 ± 0.5

All values (μm) expressed as mean ± SEM.

* Vessel length of the ductus arteriosus is uneven because of its insertion into aorta at an angle. Short side was taken for normalisation of internal circumference and calculation of tension output (see "Methods").

† One of the fetuses presented a clearly constricted ductus (internal diameter: 107; length, 496 and 488, respectively, for long and short side; wall thickness, 22; all values in μm) on dissection. Otherwise, it behaved normally through the experiment and was included in the computation.

‡ p = 0.01 *vs* WT (ANOVA with Bonferroni *post hoc* test).

RESULTS

mRNA analysis. The mPGES2 transcript was barely detectable in the WT ductus (reading at 36–38 cycles), indicating a minor contribution of this enzyme to PGE_2 synthesis. Its level of expression remained marginal upon mPGES1 deletion and, consequently, no importance can be attached to any apparent variation, either upward (n = 2) or downward (n = 1), relative to the WT value.

In vitro studies. The isolated ductus arteriosus was slightly shorter in mPGES1−/− than WT and, in one case, it also showed the signs of a prior constriction *in utero* (Table 1). With either genotype, preparations developed a variable degree of tension during equilibration (WT, 0.26 ± 0.06; mPGES1−/− 0.18 ± 0.04, all values in mN mm^{-1}), which, most of the times, was maintained throughout the experiment. In addition, the baseline was often interrupted by transient

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fluctuations of uneven magnitude (0.1–0.7 mN mm^{-1}) and by low-amplitude, fast discharges in variable combination. However, despite a similar behavior at rest, the two genotypes differed in their contraction to the reference TXA_2 analogue (ONO-11113, 0.1 μM), with mPGES1−/− being lesser responsive than WT (0.71 ± 0.04 *versus* 1.25 ± 0.03 mN mm^{-1}, n = 11 and 7; $p < 0.001$). Furthermore, as expected from available data (see "Introduction"), the mPGES1−/− ductus presented a markedly curtailed response to indomethacin (2.8 μM) with steady tension being in the whole minimal compared with WT (Fig. 1). In particular, only one mutant developed a partially sustained contraction (0.43 and 0.21 mN mm^{-1}, respectively for peak and steady tension), whereas the other showed some transient contraction at random (peak

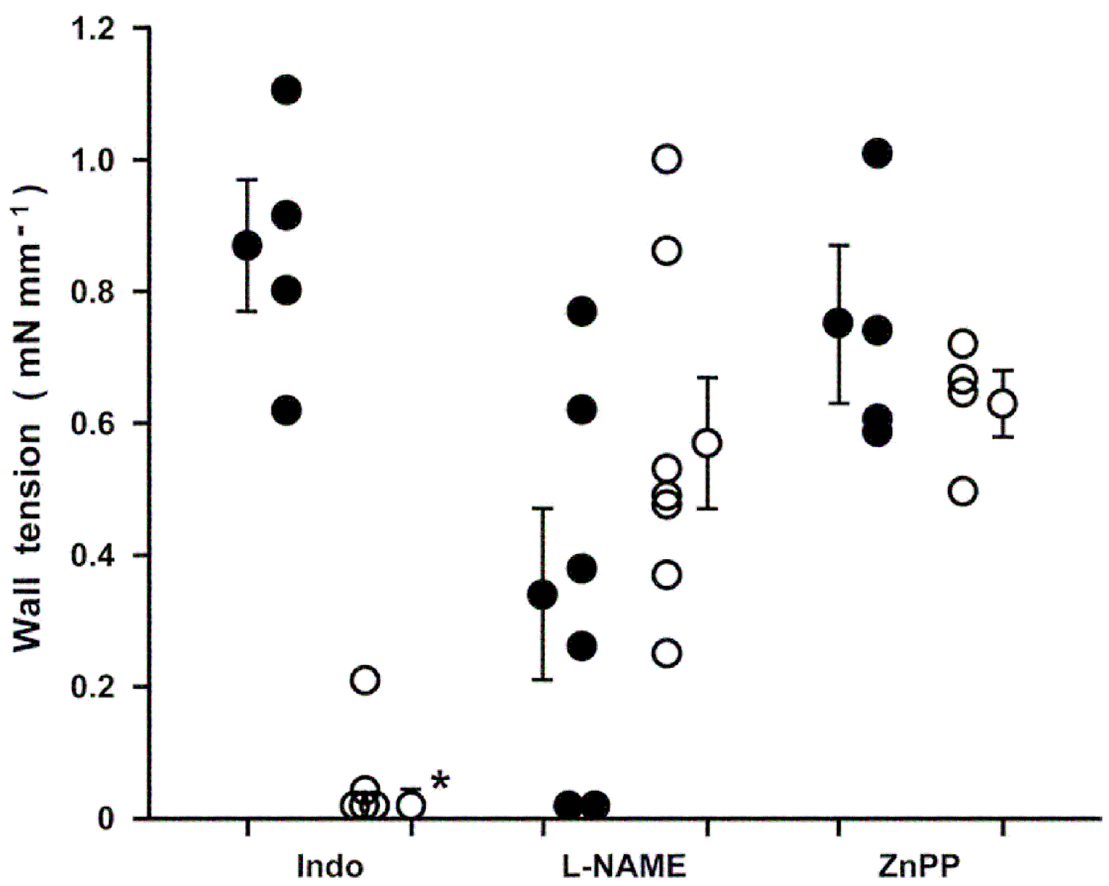

Figure 1. Isolated ductus arteriosus from fetal mouse. Comparison of contractile responses to indomethacin (Indo, 2.8 μM), L-NAME (100 μM) and ZNPP (10 μM) in WT (●) *vs* mPGES1−/− (○). Values apply to steady tension, with individual experiments and mean ± SE being given for each group. Baseline wall tension (mN mm^{-1}) before treatment was (WT/mPGES1−/−) 0.23 ± 0.08/0.07 ± 0.06, 0.11 ± 0.03, 0.14 ± 0.04, and 0.52 ± 0.14/0.27 ± 0.1, respectively, for the indomethacin, L-NAME, and ZNPP groups. Note that peak tension was 0.67 and 0.90 mN mm^{-1} with the two WT preparations not presenting a sustained response to L-NAME. *$p < 0.0001$ *vs* WT.

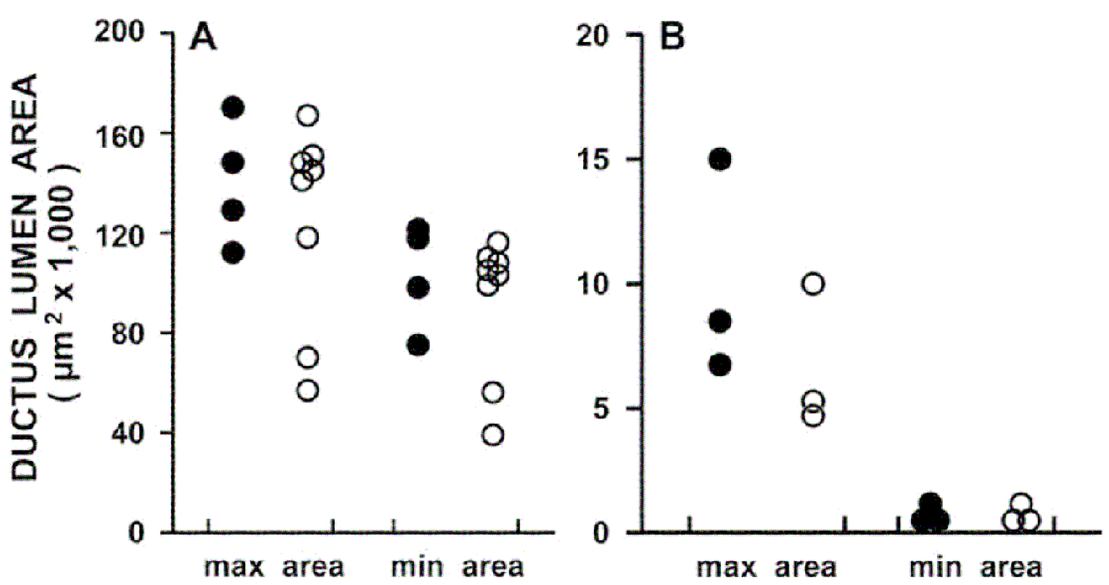

Figure 2. Mouse ductus arteriosus. Maximal and minimal cross-sectional area for WT (●) *vs* mPGES1−/− (○) with each point representing a single experiment. *A*, Fetus at 19 d gestation. *B*, Newborn at 3 h of age. The two genotypes showed no significant difference under any condition. Note, however, that two mPGES1−/− fetuses differed from the remainder of the group in presenting a smaller ductus caliber.

between 0.21 and 0.47 mN mm^{-1}, n = 3) or did not contract at all (n = 1). In contrast, the response of the WT ductus was consistently high and persistent.

The contraction of the WT ductus to L-NAME (100 μM) started after a delay (3–22 min, mean 11) and progressed with variable speed (12–106 min, mean 48) to the peak (Fig. 1). The resulting tone was maintained in all but two experiments in which a complete reversal was seen despite the continued treatment. Upon mPGES1 deletion, this contraction became faster in onset (3–11 min, mean 5) and always attained a steady plateau. The response also tended to be greater, but its final value did not reach significance (Fig. 1). Conversely, no difference at all was noted between the two genotypes in either magnitude (Fig. 1) or time course of the ZnPP contraction, the latter being uniformly slow (see Ref. 14).

In vivo studies. The ductus was patent in all fetuses, with no significant difference in lumen area between WT (max area: 139674 ± 12415 μm^2; min area: 103312 ± 10910 μm^2) and mPGES1−/− (max area: 124821 ± 14358 μm^2; min area: 92174 ± 10012 μm^2) (Fig. 2*A*). However, two mutants departed from the group for their smaller vessel (Fig. 2*A*), which is indicative of a preexisting constriction *in utero*. Postnatally, both WT and mPGES1−/− animals closed their duct rapidly (Fig. 2*B*), with narrowing of the lumen being uniform over the entire length or more prominent in the centro-pulmonary portion.

DISCUSSION

In agreement with the prime role being assigned to mPGES1 for PGE_2 formation, the ductus lacking this particular enzyme contracted little or not at all to indomethacin. Furthermore, any such contraction was not sustained, indicating that the companion enzyme, mPGES2, is unable to maintain an adequate rate of PGE_2 synthesis for steady relaxation. Hence, mPGES1−/− differs sharply from the null mutant for either COX in which indomethacin could still produce a full-fledged effect (5). However, despite its greater impact, mPGES1 removal did not interfere with ductus patency, the only evidence of PGE_2 loss being the lesser caliber of few preparations (see "Results").

How is, then, patency of the ductus maintained in the face of a marked curtailment of the PGE_2 mechanism? Compensation by other intramural prostanoids, in particular PGI_2 being up-regulated by mPGES1 deletion (10,22,23), is unlikely. PGI_2 is far less potent than PGE_2 in dilating the vessel (1) and, moreover, its involvement in tone regulation is ruled out by findings in animals lacking the receptor (2). More plausibly, other local agents, specifically NO and CO, come into play even though their function is not overtly enhanced as a result of mPGES1 suppression. Furthermore, a relaxant influence may derive from blood-borne PGE_2 being formed elsewhere, particularly in the placenta, through alternative PGE synthases (24,25). Conversely, no contribution is expected from the endothelium—derived hyperpolarizing factor (EDHF) because its action unfolds only in the absence of NO and CO (14). Whatever its mechanism(s), the intervening compensation is expectedly limited and, in fact, appears inadequate in the few mPGES1−/− fetuses showing a constricted ductus.

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The mPGES1−/− fetus differs from fetuses lacking COX1 or COX2 whose duct presents a marked up-regulation of NO (5). Not fortuitously, the latter mutants have always an open ductus without any sign of constriction (5). The question, then, can be raised on why is the NO system unevenly affected by mPGES1 *versus* COX deletion, with the lesser activation occurring somewhat paradoxically in a situation (*i.e.* the mPGES1 mutation) where a compensation for PGE_2 loss would be more acutely needed. No firm explanation can be provided with the available data. However, by considering the results in mutants along with work documenting an enhanced NO function upon indomethacin treatment (26), one may conclude that the up-regulatory drive is linked specifically to interference with COX. Extending this reasoning further, one could then assume that COX suppression is coupled with a rebound of parallel pathways in arachidonic acid metabolism (lipoxygenase, monooxygenase) wherefrom a stimulatory signal is generated for the NO system and, particularly for eNOS (*i.e.* the main synthetic enzyme; see Refs. 5,14). Among the many potential candidates for this messenger role, products from 12S-lipoxygenase are most promising in view of the prominence of this pathway in the ductus (27). 12-hydroxytetraenoic acid (12-HETE), for example, could stimulate eNOS not only through its transcription factors SP1 and AP2 acting on specific recognition sites in the promoter (28; see http://www.genomatix.de), but also through changes in phosphorylating kinases and free Ca^{2+} favoring catalytic activity (29,30).

Contrary to animals lacking both COXs or the prime PGE_2 receptor, EP_4, the mPGES1 mutant closed its duct normally after birth. The former mutants present instead a persistent duct, which is incompatible with extra-uterine life (13,15). These findings raise important questions, both conceptual and methodological. Persistency of the ductus upon complete removal of PGE_2 may be linked to two conditions: NO up-regulation expectedly occurring with the double COX knockout but not with the EP_4 knockout, and interference with the remodeling process being shared by both mutants. Ductus closure requires, in fact, PGE_2 for its smooth progression (9,15,16). No such events take place in the mPGES1−/− ductus where, conversely, NO function is practically normal and some PGE_2 may still be produced via the COX/mPGES2 pathway (31), both locally (this study) and systemically (24,25). Hence, this mutant not only affords a better insight into the operation of the ductus, but also represents a useful model for the study of PGE_2 function without the limitations of the double COX and EP_4 mutants.

The present study has significant implications for the clinic inasmuch as it may lead to a better tool for the management of premature infants with PDA. The currently used COX inhibitors carry, in fact, a high incidence of failures due largely, in our view, to the rebound activation of nonPG relaxing mechanisms, NO in particular (26). No such complication is envisaged with an mPGES1 inhibitor. In addition, this inhibitor would be more selective and, specifically, would preserve the synthesis of PGI_2 in vascular districts critically dependent on this compound for their normal function. Significant is also the fact that an mPGES1 inhibitor may be particularly useful with PDA complicated by an infectious state (see Ref. 18) and may also preserve some PGE_2 synthesis for the orderly progression of the remodeling process within the closing ductus.

In conclusion, we have demonstrated that mPGES1 is critical for PGE_2 synthesis in the ductus. mPGES1 deletion, unlike COX inhibition or deletion, is not associated with a major compensation from NO. Based on these findings, an mPGES1 inhibitor stands out as a prospective new tool, superior to the currently used COX inhibitors, for the management of the premature infant with PDA.

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REFERENCES

1. Smith GC 1998 The pharmacology of the ductus arteriosus. Pharmacol Rev 50:35–58
2. Narumiya S, Sugimoto Y, Ushikubi F 1999 Prostanoid receptors: structures, properties, and functions. Physiol Rev 79:1193–1226
3. Kobayashi T, Narumiya S 2002 Function of prostanoid receptors: studies on knockout mice. Prostaglandins Other Lipid Mediat 68–69:557–573
4. Radi ZA, Ostroski R 2007 Pulmonary and cardiorenal cyclooxygenase-1 (COX-1), -2 (COX-2), and microsomal prostaglandin E synthase-1 (mPGES-1) and -2 (mPGES-2) expression in a hypertension model. Mediators Inflamm 2007:85091
5. Baragatti B, Brizzi F, Ackerley C, Barogi S, Ballou LR, Coceani F 2003 Cyclooxygenase-1 and cyclooxygenase-2 in the mouse ductus arteriosus: individual activity and functional coupling with nitric oxide synthase. Br J Pharmacol 139:1505–1515
6. Bouayad A, Fouron J-C, Hou X, Beauchamp M, Quiniou C, Abran D, Peri K, Clyman RI, Varma DR, Chemtob S 2004 Developmental regulation of prostaglandin E_2 synthase in porcine ductus arteriosus. Am J Physiol Regul Integr Comp Physiol 286:R903–R909
7. Takahashi Y, Roman C, Chemtob S, Tse MM, Lin E, Heymann MA, Clyman RI 2000 Cyclooxygenase-2 inhibitors constrict the fetal lamb ductus arteriosus both in vitro and vivo. Am J Physiol Regul Integr Comp Physiol 278:R1496–R1505
8. Coceani F, Ackerley C, Seidlitz E, Kelsey L 2001 Function of cyclooxygenase-1 and cyclooxygenase-2 in the ductus arteriosus from foetal lamb: differential development and change by oxygen and endotoxin. Br J Pharmacol 132:241–251
9. Reese J, Anderson JD, Brown N, Roman C, Clyman RI 2006 Inhibition of cyclooxygenase isoforms in late-but not midgestation decreases contractility of the ductus arteriosus and prevents postnatal closure in mice. Am J Physiol Regul Integr Comp Physiol 291:R1717–R1723
10. Cheng Y, Wang M, Yu Y, Lawson J, Funk CD, FitzGerald GA 2006 Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. J Clin Invest 116:1391–1399
11. Thorén S, Weinander R, Saha S, Jegerschöld C, Pettersson PL, Samuelsson B, Hebert H, Hamberg M, Morgenstern R, Jakobsson P-J 2003 Human microsomal prostaglandin E synthase-1: purification, functional characterization, and projection structure determination. J Biol Chem 278:22199–22209
12. Reese J, Paria BC, Brown N, Zhao X, Morrow JD, Dey SK 2000 Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. Proc Natl Acad Sci U S A 97:9759–9764
13. Loftin CD, Trivedi DB, Tiano HF, Clark JA, Lee CA, Epstein JA, Morham SG, Breyer MD, Nguyen MT, Hawkins BM, Goulet JL, Smithies O, Koller BH, Langenbach R 2001 Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. Proc Natl Acad Sci U S A 98:1059–1064
14. Baragatti B, Brizzi F, Barogi S, Laubach VE, Sodini D, Shesely EG, Regan RF, Coceani F 2007 Interactions between NO, CO and an endothelium-derived hyperpolarizing factor (EDHF) in maintaining patency of the ductus arteriosus in the mouse. Br J Pharmacol 151:54–62
15. Nguyen MT, Camenisch T, Snouwaert JN, Hicks E, Coffman TM, Anderson PA, Malouf NN, Koller BH 1997 The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. Nature 390:78–81
16. Yokoyama U, Minamisawa S, Quan H, Ghatak S, Akaike T, Segi-Nishida E, Iwasaki S, Iwamoto M, Misra S, Tamura K, Hori H, Yokota S, Toole BP, Sugimoto Y, Ishikawa Y 2006 Chronic activation of the prostaglandin receptor EP4 promotes hyaluronan-mediated neointimal formation in the ductus arteriosus. J Clin Invest 116:3026–3034
17. Ohlsson A, Walia R, Shah S 2003 Ibuprofen for the treatment of patent ductus arteriosus in preterm and/or low birth weight infants. Cochrane Database Syst Rev:CD003481
18. Uematsu S, Matsumoto M, Takeda K, Akira S 2002 Lipopolysaccharide-dependent prostaglandin E_2 production is regulated by the glutathione-dependent prostaglandin E_2 synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. J Immunol 168:5811–5816
19. Coceani F, Liu Y-A, Seidlitz E, Kelsey L, Kuwaki T, Ackerley C, Yanagisawa M 1999 Endothelin A receptor is necessary for O_2 constriction but not closure of ductus arteriosus. Am J Physiol 277:H1521–H1531

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20. Hörnblad PY, Larsson KS 1967 Studies on closure of the ductus arteriosus I whole-body freezing as improvement of fixation procedures. Cardiologia 51:231–241
21. Coceani F, Kelsey L, Seidlitz E, Marks GS, McLaughlin BE, Vreman HJ, Stevenson DK, Rabinovitch M, Ackerley C 1997 Carbon monoxide formation in the ductus arteriosus in the lamb: implications for the regulation of muscle tone. Br J Pharmacol 120:599–608
22. Trebino CE, Eskra JD, Wachtmann TS, Perez JR, Carty TJ, Audoly LP 2005 Redirection of eicosanoid metabolism in mPGES-1-deficient macrophages. J Biol Chem 280:16579–16585
23. Wang M, Zukas AM, Hui Y, Ricciotti E, Puré E, FitzGerald GA 2006 Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. Proc Natl Acad Sci U S A 103:14507–14512
24. Tanikawa N, Ohmiya Y, Ohkubo H, Hashimoto K, Kangawa K, Kojima M, Ito S, Watanabe K 2002 Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem Biophys Res Commun 291:884–889
25. Meadows JW, Pitzer B, Brockman DE, Myatt L 2004 Differential localization of prostaglandin E synthase isoforms in human placental cell types. Placenta 25:259–265
26. Sodini D, Baragatti B, Barogi S, Laubach VE, Coceani F 2008 Indomethacin promotes nitric oxide function in the ductus arteriosus in the mouse. Br J Pharmacol 153:1631–1640
27. Costa M, Barogi S, Socci ND, Angeloni D, Maffei M, Baragatti B, Chiellini C, Grasso E, Coceani F 2006 Gene expression in ductus arteriosus and aorta: comparison of birth and oxygen effects. Physiol Genomics 25:250–262
28. Nie D, Krishnamoorthy S, Jin R, Tang K, Chen YC, Qiao Y, Zacharek A, Guo Y, Milanini J, Pages G, Honn KV 2006 Mechanisms regulating tumor angiogenesis by 12-lipoxygenase in prostate cancer cells. J Biol Chem 281:18601–18609
29. Bauersachs J, Popp R, Fleming I, Busse R 1997 Nitric oxide and endothelium-derived hyperpolarizing factor: formation and interactions. Prostaglandins Leukot Essent Fatty Acids 57:439–446
30. Brash AR 1999 Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem 274:23679–23682
31. Murakami M, Kudo I 2004 Recent advances in molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway. Prog Lipid Res 43:3–35

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