Role of Microsomal Prostaglandin E Synthase-1 (mPGES1)-Derived PGE₂ in Patency of the Ductus Arteriosus in the Mouse

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ABSTRACT: Prostaglandin E₂ (PGE₂) 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 PGE₂ formation and function. mPGES1−/− ductus, unlike WT ductus, contracted little, or not at all, to indomethacin in vitro. Coincidentally, as evident from responses to N⁵-nitro-L-arginine methyl ester and zinc photophorphyrin, 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 pharmaco-logically 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 PGE₂ originating via mPGES2. We conclude that mPGES1 is critical for PGE₂ 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) E₂ is viewed as the prime agent for prenatal patency of the ductus arteriosus (1). Conversely, PGI₂ is without a role, notwithstanding its occurrence in the vessel and its known importance for vasoregulation elsewhere (2,3). Ductal PGE₂ 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 PGE₂-based relaxation upon COX2 than COX1 deletion (5) along with the marked curtailment of PGE₂ formation after inhibition of either COX2 or mPGES1 (6,7). Consistent with the preferential operation of the COX2/mPGES1 route for PGE₂ 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 PGE₂ 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 PGE₂ 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 PGE₂ system and the role of PGE₂ 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 129X/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-

Abbreviations: COX, cyclooxygenase; t-NAME, N⁵-nitro-L-arginine methyl ester; mPGES1; microsomal PGE synthase-1; mPGES2, microsomal PGE synthase-2; ONO-11113, 9, 11-epithio-11, 12-methano-thromboxane A₂; PDA, patent ductus arteriosus; PG, prostaglandin; ZnP', zinc protoporphyrin
In vitro studies. Term fetal mice, both WT and gene-deleted (gestational age, 19.0 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% CO2 in N2. 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% O2 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⁻²) (WT, 0.09 ± 0.01; mPGES1⁻/⁻, 0.08 ± 0.008 (n = 16)) and the attendant internal circumference (C₀) with the related resting dimension (Table 1) served as a reference for choosing the appropriate load. Afterward, tension was applied to maintain an operating circumference (i.e., C₄₀) coinciding with the condition in vivo (WT, 0.45 ± 0.007 (n = 14); mPGES1⁻/⁻, 0.45 ± 0.009 (n = 16); values in mN mm⁻²), 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 N²-nitro-L-arginine methyl ester (NAME, 100 μM; n = 13) and zinc protoporphyrin (ZnP, 10 μM; n = 8) on the basal tone. Indomethacin (2.8 μM; n = 9) was tested comparatively to verify the degree of loss of PGF₂α function upon mPGES1 deletion. In all cases, treatment lasted 60 min as or long as required to achieve a maximal effect, and the contraction to the thromboxane (TX) A₂ analogue (0.1 μM) provided a reference. Only 1 procedure was used in the experiments.

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 femoral clot 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 N₂. Once frozen, specimens were stored at −20°C for further work-up. To prepare a block of tissue with the ductus, the carcase 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, Welleslde, 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.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Internal diameter</th>
<th>Vessel length*</th>
<th>Wall thickness</th>
</tr>
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<tbody>
<tr>
<td>WT (n = 14)</td>
<td>132 ± 1</td>
<td>568 ± 19</td>
<td>581 ± 19</td>
</tr>
<tr>
<td></td>
<td>132 ± 2</td>
<td>513 ± 111</td>
<td>524 ± 111</td>
</tr>
<tr>
<td>mPGES1⁻/⁻</td>
<td>132 ± 2</td>
<td>513 ± 111</td>
<td>524 ± 111</td>
</tr>
</tbody>
</table>

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 normalization 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 PGF₂α 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⁻²), which, most of the times, was maintained throughout the experiment. In addition, the baseline was often interrupted by transient
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 \(\mu\)M), with mPGES1\(^{-/-}\) being less responsive than WT (0.71 ± 0.04 \textit{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 \(\mu\)M) 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 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 \(\text{L-NAME}\) (100 \(\mu\)M) 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).

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

\section*{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 prostanooids, 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 overly 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 syntheses (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.
The mPGES1−/− fetus differs from fetuses lacking COX1 or COX2 whose ducts 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 PGE2 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 (lipoxigenase, monoxygenase) 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 most potential candidates for this messenger role, products from 12S-lipoxygenase are most promising in view of the prominence of this pathway in the duct (27), 12-hydroxytetraenoic acid (12-HETE), for example, could stimulate eNOS not only through its transcription factors SPI 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 Ca2+ favoring catalytic activity (29,30).

Contrary to animals lacking both COXs or the prime PGE2 receptor, EP4, 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 PGE2 may be linked to two conditions: NO up-regulation expectedly occurring with the double COX knockout but not with the EP4 knockout, and interference with the remodeling process being shared by both mutants. Ductus closure requires, in fact, PGE2 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 PGE2 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 PGE2 function without the limitations of the double COX and EP4 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 PGJ2 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 PGE2 synthesis for the orderly progression of the remodeling process within the closing ductus.

In conclusion, we have demonstrated that mPGES1 is critical for PGE2 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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