

The Multiple Roles of Prostaglandin E₂ in the Regulation of the Ductus Arteriosus

35

Utako Yokoyama, Susumu Minamisawa, and Yoshihiro Ishikawa

Abstract

The ductus arteriosus (DA) is a shunt vessel between the aorta and the pulmonary artery during the fetal period. It is well recognized that prostaglandin E₂ (PGE₂) dilates the DA through activation of its receptor EP4 and subsequent cyclic AMP (cAMP) production during the fetal period and that oxygen constricts the DA by inhibiting potassium channels immediately after birth. In addition to the regulation of vascular tone, morphological remodeling of the DA throughout the perinatal period, such as prominent intimal thickening and poor elastogenesis, has been demonstrated.

We recently identified the molecular mechanisms of the acquisition of unique morphological remodeling in the DA during development. During the fetal period, PGE₂-EP4 signaling decreases elastic fiber formation through degradation of the cross-linking enzyme lysyl oxidase (LOX) and increases hyaluronan-mediated intimal thickening in the DA. This remodeling is mediated by activation of the EP4 receptor via diverse downstream intracellular signaling pathways. Hyaluronan-mediated intimal thickening was induced by the EP4-Gs protein-cyclic AMP-protein kinase A pathway. The attenuation of elastogenesis is mediated through a non-cyclic AMP signaling pathway, such as c-src-phospholipase C (PLC). These data suggest that placental PGE₂-mediated vascular remodeling via different signaling pathways orchestrates the subsequent luminal DA reorganization, leading to complete obliteration of the DA.

U. Yokoyama (✉) • Y. Ishikawa
Cardiovascular Research Institute, Graduate School of Medicine, Yokohama City University,
Yokohama, Japan
e-mail: utako@yokohama-cu.ac.jp

S. Minamisawa
Department of Cell Physiology, School of Medicine, Jikei University, Tokyo, Japan

Keywords

Ductus arteriosus • Prostaglandin E • Intimal thickening • Smooth muscle • Elastic fiber

35.1 Introduction

The ductus arteriosus (DA) normally closes immediately after birth. Although the DA is a normal and essential fetal structure, it becomes abnormal if it remains patent after birth. DA closure occurs in two phases: functional closure of the lumen in the first hours after birth by smooth muscle constriction and anatomic occlusion of the lumen over the next several days due to extensive neointimal thickening in human DA [1–3]. There are several events that promote DA constriction immediately after birth. Increasing oxygen tension and a dramatic decrease in circulating PGE₂ promote muscular constriction of the DA. In addition, DA remodeling is also necessary for its complete closure. Remodeling is characterized by (a) an area of subendothelial deposition of extracellular matrix [4], (b) the disassembly of the internal elastic lamina and loss of elastic fiber in the medial layer [5], and (c) migration into the subendothelial space of undifferentiated medial smooth muscle cells (SMCs). Some of these changes begin about halfway through gestation, and some occur after functional closure of the DA in the neonate [3, 6]. In addition to the well-known vasodilatory role of PGE₂, our findings revealed the role of PGE₂ in the anatomical closure of the DA.

35.2 The Molecular Mechanisms of Intimal Thickening of the Ductus Arteriosus

35.2.1 Hyaluronan-Mediated Intimal Thickening

PGE₂ plays a primary role in maintaining the patency of the DA via its receptor EP4. However, previous studies have demonstrated that genetic disruption of the PGE receptor EP4 paradoxically results in fatal patent DA in mice [7, 8]. In addition, double mutant mice in which cyclooxygenase (COX)-1 and COX-2 are disrupted also exhibit patent DA [9]. We found that intimal thickening was completely absent in the DA of EP4-disrupted neonatal mice [3]. Moreover, a marked reduction in hyaluronan production was found in EP4-disrupted DA, whereas a thick layer of hyaluronan deposit was present in wild-type DA. PGE₂-EP4-cyclic AMP (cAMP)-protein kinase A (PKA) signaling upregulates hyaluronan synthase type 2 mRNA, which increases hyaluronan production in the DA. Accumulation of hyaluronan then promotes SMC migration into the subendothelial layer to form intimal thickening [3].

EP4 is a Gs protein-coupled receptor that increases intracellular cAMP by adenylyl cyclases (ACs) consisting of nine different isoforms of membrane-bound forms of ACs (AC1 through AC9). We found that AC2 and AC6 are more

highly expressed in rat DA than in the aorta during the perinatal period [10]. Our data using AC subtype-targeted siRNAs and AC6-deficient mice suggest that AC6 is responsible for hyaluronan-mediated intimal thickening of the DA, whereas AC2 inhibits AC6-induced hyaluronan production. The activation of both AC2 and AC6 induces vasodilation.

35.2.2 Epac-Mediated SMC Migration

In addition to PKA, a new target of cAMP that is an exchange protein activated by cAMP has recently been discovered; it is called Epac [11]. Epac is a guanine nucleotide exchange protein that regulates the activity of small G proteins and has been known to exhibit a distinct cAMP signaling pathway that is independent of PKA [12]. There are two variants: Epac1 is expressed in most tissues, including the heart and blood vessels, whereas Epac2 is expressed in the adrenal gland and the brain. Although both Epac1 and Epac2 are upregulated during the perinatal period, Epac1, but not Epac2, acutely promotes SMC migration and thus intimal thickening in the DA [13]. Since Epac stimulation does not increase hyaluronan production, the effect of Epac1 on SMC migration is independent of that of hyaluronan accumulation, which operates through a mechanism different from that underlying PKA stimulation.

35.2.3 Regulation of Elastogenesis

Elastic fiber formation begins in mid-gestation and increases dramatically during the last trimester in the great arteries. However, the DA exhibits lower levels of elastic fiber formation [5], which may contribute to vascular collapse and subsequent closure of the DA after birth. We found that EP4 significantly inhibited elastogenesis and decreased lysyl oxidase (LOX) protein, which catalyzes elastin cross-links in DA SMCs but not in aortic SMCs. In EP4-knockout mice, electron microscopic examination showed that the DA acquired an elastic phenotype that was similar to the neighboring aorta. More importantly, human DA and aorta tissues from seven patients showed a negative correlation between elastic fiber formation and EP4 expression, as well as between EP4 and LOX expression. Together with *in vitro* experiments, these data suggest that PGE₂-EP4 signaling inhibits elastogenesis in the DA by degrading LOX protein. The EP4-cSrc-PLC- γ -signaling pathway, a signaling pathway that has not previously been recognized, most likely promoted the lysosomal degradation of LOX [14, 15].

35.3 Future Direction and Clinical Implications

The persistently patent DA after birth is a major cause of morbidity and mortality, especially in premature infants, that can lead to severe complications, including pulmonary hypertension, right ventricular dysfunction, postnatal infections, and

respiratory failure [16]. The incidence of DA patency has been estimated to be 1 in 500 in term newborns [17]. In preterm babies with birth weights <1,500 g, the incidence of patent DA exceeds 30 % [18]. Therefore, it is important to improve current pharmacological therapy through understanding the precise mechanisms of the regulation of the DA. Since both vascular contraction and remodeling are required for complete DA closure, pharmacological therapies that promote vasoconstriction and remodeling would be ideal for premature infants with persistently patent DAs. On the other hand, vasodilation and inhibition of intimal thickening are required for DA-dependent congenital heart diseases.

Our data suggest that PGE₂-EP4-cAMP signaling promotes hyaluronan and Epac-mediated intimal thickening and that the EP4-PLC pathway attenuates elastogenesis in the DA. These cascades of events via different signaling pathways are thought to orchestrate the subsequent luminal DA reorganization (Fig. 35.1),

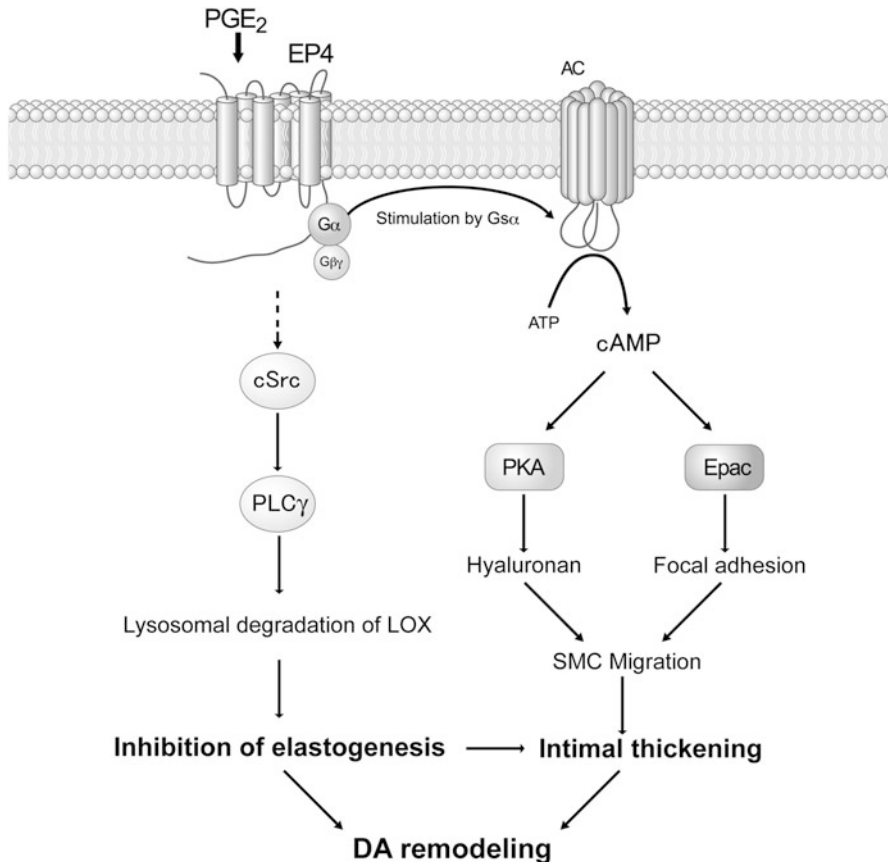


Fig. 35.1 The diverse EP4 signaling pathways. Both PKA and Epac synergistically promoted intimal cushion formation in the DA, but they work in two distinct ways. The cSrc-PLC pathway inhibited elastogenesis via degrading LOX proteins

leading to complete obliteration of the DA. In addition to its role in controlling vascular tone in the functional closure of the DA, the vascular remodeling of the DA is now attracting considerable attention as a target for novel therapeutic strategies for patients with persistently patent DA and DA-dependent cardiac anomalies.

Acknowledgments This work was supported by grants from the Ministry of Health, Labor and Welfare of Japan (U.Y.), the Ministry of Education, Culture, Sports, Science and Technology of Japan (U.Y., S.M.), the Yokohama Foundation for Advanced Medical Science (U.Y., S.M.), the “High-Tech Research Center” Project for Private Universities: MEXT (S.M.), MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S.M.), the Vehicle Racing Commemorative Foundation (U.Y., S.M.), Miyata Cardiology Research Promotion Funds (U.Y., S.M.), the Takeda Science Foundation (U.Y., S.M.), the Japan Heart Foundation Research Grant (U.Y.), the Kowa Life Science Foundation (U.Y.), the Sumitomo Foundation (U.Y.), and the Shimabara Science Promotion Foundation (S.M.).

Open Access This chapter is distributed under the terms of the Creative Commons Attribution-Noncommercial 2.5 License (<http://creativecommons.org/licenses/by-nc/2.5/>) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

The images or other third party material in this chapter are included in the work’s Creative Commons license, unless indicated otherwise in the credit line; if such material is not included in the work’s Creative Commons license and the respective action is not permitted by statutory regulation, users will need to obtain permission from the license holder to duplicate, adapt or reproduce the material.

References

1. Smith GC. The pharmacology of the ductus arteriosus. *Pharmacol Rev.* 1998;50:35–58.
2. Clyman RI. Mechanisms regulating the ductus arteriosus. *Biol Neonate.* 2006;89:330–5.
3. Yokoyama U, Minamisawa S, Quan H, Ghatak S, Akaie T, Segi-Nishida E, Iwasaki S, Iwamoto M, Misra S, Tamura K, Hori H, Yokota S, Toole BP, Sugimoto Y, Ishikawa Y. Chronic activation of the prostaglandin receptor EP4 promotes hyaluronan-mediated neointimal formation in the ductus arteriosus. *J Clin Invest.* 2006;116:3026–34.
4. Gittenberger-de Groot AC, Strengers JL, Mentink M, Poelmann RE, Patterson DF. Histologic studies on normal and persistent ductus arteriosus in the dog. *J Am Coll Cardiol.* 1985;6:394–404.
5. De Reeder EG, Girard N, Poelmann RE, Van Munsteren JC, Patterson DF, Gittenberger-De Groot AC. Hyaluronic acid accumulation and endothelial cell detachment in intimal thickening of the vessel wall. The normal and genetically defective ductus arteriosus. *Am J Pathol.* 1988;132:574–85.
6. Slomp J, Gittenberger-de Groot AC, Glukhova MA, Conny van Munsteren J, Kockx MM, Schwartz SM, Kotliansky VE. Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. *Arterioscler Thromb Vasc Biol.* 1997;17:1003–9.
7. Segi E, Sugimoto Y, Yamasaki A, Aze Y, Oida H, Nishimura T, Murata T, Matsuoka T, Ushikubi F, Hirose M, Tanaka T, Yoshida N, Narumiya S, Ichikawa A. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem Biophys Res Commun.* 1998;246:7–12.

8. Nguyen M, Camenisch T, Snouwaert JN, Hicks E, Coffman TM, Anderson PA, Malouf NN, Koller BH. The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. *Nature*. 1997;390:78–81.
9. Loftin CD, Trivedi DB, Tiano HF, Clark JA, Lee CA, Epstein JA, Morham SG, Breyer MD, Nguyen M, Hawkins BM, Goulet JL, Smithies O, Koller BH, Langenbach R. Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. *Proc Natl Acad Sci U S A*. 2001;98:1059–64.
10. Yokoyama U, Minamisawa S, Katayama A, Tang T, Suzuki S, Iwatsubo K, Iwasaki S, Kurotani R, Okumura S, Sato M, Yokota S, Hammond HK, Ishikawa Y. Differential regulation of vascular tone and remodeling via stimulation of type 2 and type 6 adenylyl cyclases in the ductus arteriosus. *Circ Res*. 2010;106:1882–92.
11. de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, Bos JL. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature*. 1998;396:474–7.
12. Bos JL. Epac: a new cAMP target and new avenues in cAMP research. *Nat Rev Mol Cell Biol*. 2003;4:733–8.
13. Yokoyama U, Minamisawa S, Quan H, Akaike T, Suzuki S, Jin M, Jiao Q, Watanabe M, Otsu K, Iwasaki S, Nishimaki S, Sato M, Ishikawa Y. Prostaglandin E2-activated Epac promotes neointimal formation of the rat ductus arteriosus by a process distinct from that of cAMP-dependent protein kinase A. *J Biol Chem*. 2008;283:28702–9.
14. Yokoyama U, Minamisawa S, Shioda A, Ishiwata R, Jin MH, Masuda M, Asou T, Sugimoto Y, Aoki H, Nakamura T, Ishikawa Y. Prostaglandin E2 inhibits elastogenesis in the ductus arteriosus via EP4 signaling. *Circulation*. 2014;129:487–96.
15. Yokoyama U, Iwatsubo K, Umemura M, Fujita T, Ishikawa Y. The prostanoid EP4 receptor and its signaling pathway. *Pharmacol Rev*. 2013;65:1010–52.
16. Hermes-DeSantis ER, Clyman RI. Patent ductus arteriosus: pathophysiology and management. *J Perinatol*. 2006;26 Suppl 1:S14–8; discussion S22–13.
17. Mitchell SC, Korones SB, Berendes HW. Congenital heart disease in 56,109 births. Incidence and natural history. *Circulation*. 1971;43:323–32.
18. Van Overmeire B, Allegaert K, Casaer A, Debauche C, Decaluwe W, Jaspers A, Weyler J, Harrewijn I, Langhendries JP. Prophylactic ibuprofen in premature infants: a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet*. 2004;364:1945–9.