Molecular Mechanisms Regulating the Vascular Prostacyclin Pathways and Their Adaptation during Pregnancy and in the Newborn

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Abstract——Prostacyclin (PGI_2) is a member of the prostanoid group of eicosanoids that regulate homeostasis, hemostasis, smooth muscle function and inflammation. Prostanoids are derived from arachidonic acid by the sequential actions of phospholipase A_2, cyclooxygenase (COX), and specific prostaglandin (PG) synthases. There are two major COX enzymes, COX1 and COX2, that differ in structure, tissue distribution, subcellular localization, and function. COX1 is largely constitutively expressed, whereas COX2 is induced at sites of inflammation and vascular injury. PGI_2 is produced by endothelial cells and influences many cardiovascular processes. PGI_2 acts mainly on the prostacyclin (IP) receptor, but because of receptor homology, PGI_2 analogs such as iloprost may act on other prostanoid receptors with variable affinities. PGI_2/IP interaction stimulates G protein-coupled increase in cAMP and protein kinase A, resulting in decreased $[Ca^{2+}]_i$, and could also cause inhibition of Rho kinase, leading to vascular smooth muscle relaxation. In addition, PGI_2 intracrine signaling may target nuclear peroxisome proliferator-activated receptors and regulate gene transcription. PGI_2 counteracts the vasoconstrictor and platelet aggregation effects of thromboxane A_2 (TXA_2), and both prostanoids create an important balance in cardiovascular homeostasis. The PGI_2/TXA_2 balance is particularly critical in the regulation of maternal and fetal vascular function during pregnancy and in the newborn. A decrease in PGI_2/TXA_2 ratio in the maternal, fetal, and neonatal circulation may contribute to preeclampsia, intrauterine growth restriction, and persistent pulmonary hypertension of the newborn (PPHN), respectively. On the other hand, increased PGI_2 activity may contribute to patent ductus arteriosus (PDA) and intraventricular hemorrhage in premature newborns. These observations have raised interest in the use of COX inhibitors and PGI_2 analogs in the management of pregnancy-associated and neonatal vascular disorders. The use of aspirin to decrease TXA_2 synthesis has shown little benefit in preeclampsia, whereas indomethacin and ibuprofen are used effectively to close PDA in the premature newborn. PGI_2 analogs have been used effectively in primary pulmonary hypertension in adults and have shown promise in PPHN. Careful examination of PGI_2 metabolism and the complex interplay with other prostanoids will help design specific modulators of the PGI_2-dependent pathways for the management of pregnancy-related and neonatal vascular disorders.

I. Introduction

Eicosanoids are lipid mediators derived from the hydrolysis of membrane phospholipids by phospholipase A_2 (PLA_2[1]) into arachidonic acid (AA), the key molecule in

[1]Abbreviations: 15R-TIC, (15*R*)-16-*m*-tolyl-17,18,19,20-tetranorisocarbacyclin; aa, amino acid; AA, arachidonic acid; ACh, acetylcholine; AFP-07, 18,19-didehydro-7,7-difluoro-16*S*,20-dimethyl-PGI_2; AH6809, 6-isopropoxy-9-oxoxanthene-2-carboxylic acid; BP, blood pressure; BW755C, 4,5-dihydro-1-(3-(trifluoromethyl)phenyl)-1*H*-pyrazol-3-amine; CAY10441, 4,5-dihydro-*N*-[4-[[4-(1-methylethoxy)phenyl]methyl]phenyl]-1*H*-imadazol-2-amine; CBF, cerebral blood flow; CNS, central nervous system; COX, cyclooxygenase; $cPLA_{2\alpha}$, cytosolic$cPLA_2$; CREB, cAMP-responsive element-binding protein; CVD, cardiovascular disease; DAG, diacylglycerol; DP, PGD_2 receptor; EC, endothelial cell; ECM, extracellular matrix; EDHF, endothelium-derived hyperpolarizing factor; EET, epoxyeicosatrienoic acid; eNOS, endothelial NO synthase; EP, PGE_2 receptor; EPC, endothelial progenitor cell; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; FDA, United States Food and Drug Administration; FP, $PGF_{2\alpha}$ receptor; HEK, human embryonic kidney; HETE, hydroxyeicosatetraenoic acid; HIE, hypoxic ischemic encephalopathy; IGF-1, insulin-like growth factor-1; IP, prostacyclin receptor; $iPLA_2$, calcium-independent PLA_2; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; K_{ATP}, ATP-sensitive potassium channel;

LOX, lipoxygenase; LT, leukotriene; MAPK, mitogen-activated protein kinase; MaxiK, large-conductance Ca^{2+}-dependent K^+ channel; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NS-398, *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide; NSAID, nonsteroidal anti-inflammatory drug; OKY1581, 2-methyl-3-(4-(3-pyridinyl-methyl)phenyl)-2-propenoic acid; P450, cytochrome P450; PAH, pulmonary arterial hypertension; PAI, plasminogen activator inhibitor; PAR, protease activated receptor; PDA, patent ductus arteriosus; PE, preeclampsia; PG, prostaglandin; PGDS, PGD synthase; PGES, PGE synthase; PGFS, PGF synthase; PGI_2, prostacyclin; PGIS, prostacyclin synthase; PKA, protein kinase A; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PPAR, peroxisome proliferator-activated receptor; PPHN, persistent pulmonary hypertension of the newborn; PR, prostanoid receptor; PVR, pulmonary vascular resistance; RAS-PE, renin-angiotensin-system rat model of PE; RNS, reactive nitrogen species; RO1138452, 2-(4-(4-isopropoxybenzyl)-phenylamino) imidazoline; RO3244794, (*R*)-3-(4-fluoro-phenyl)-2-(5-(4-fluoro-phenyl)-benzofuran-2-ylmethoxycarbonylamino)-propionic acid; ROP, retinopathy of prematurity; ROS, reactive oxygen species; RUPP, reduced uteroplacental perfusion pressure; S-18886, terutroban; sFlt-1, soluble fms-like tyrosine kinase-1; SMC, smooth muscle cell; $sPLA_2$, secretory PLA_2; SQ29548, 7-(3-((2-((phenylamino)carbonyl)hydrazino)methyl)-7-oxabicyclo(2.2.1)hept-2-yl)-5-heptenoic acid; TEI-9063, 17,20-dimethylisocarbacyclin; TMD, transmembrane domain; TP, thromboxane A_2 receptor; TXA_2, thromboxane A_2; TXAS, TXA syn-

eicosanoid biosynthesis. Eicosanoids include prostanoids, leukotrienes, epoxyeicosatrienoic acids (EETs), and hydroxyeicosatetraenoic acids (HETEs). Prostanoids are produced by the sequential actions of cyclooxygenase (COX) and specific prostanoid synthases to yield prostaglandin PGD_2, PGE_2, $PGF_{2\alpha}$, prostacyclin (PGI_2), and thromboxane A_2 (TXA_2) (Fig. 1). Leukotrienes are produced by the action of lipoxygenases (LOX) (Funk, 2001) and play a role in neutrophil chemotaxis and aggregation and in inflammation (Buczynski et al., 2009). EETs and HETEs are produced from AA by the actions of P450 monooxygenases, including epoxygenases and ω-hydroxylases, respectively. EETs are vasodilator and anti-inflammatory, whereas 20-HETE promotes vasoconstriction and natriuretic effects (Zordoky and El-Kadi, 2010). Free radical catalyzed nonenzymatic peroxidation of AA yields PG-like compounds known as isoprostanes (Fig. 1). In oxidative stress, isoprostane production exceeds that of COX-derived PGs (Hardy et al., 2000). Isoprostanes serve as biomarkers of oxidative stress (Buczynski et al., 2009) and are potent vasoconstrictors, especially during antioxidant deficiency such as in the premature infant (Wright et al., 2001).

Prostanoids are synthesized under basal conditions and in response to various stimuli, such as cytokines and growth factors, and regulate multiple functions including smooth muscle contraction/relaxation, platelet activity, and vascular homeostasis and hemostasis (Narumiya et al., 1999). Prostanoids act via cell surface G-protein-coupled receptors: DP, EP, FP, IP, and TP, which correlate with the prostanoid agonists PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2, respectively (Narumiya et al., 1999). Intracellular PGI_2 may also interact with nuclear peroxisome proliferator-activated receptors (PPARs) to activate intracrine nuclear pathways (Helliwell et al., 2004b).

The overall activity of the PGI_2 pathway is determined by the amount of biosynthetic enzymes, the subcellular localization of COX and PGI_2 synthase (PGIS), preferential IP binding versus interaction with other prostanoid receptors (PRs), and the post-PR intracellular signaling pathways. The PGI_2 autocrine/paracrine and intracrine signaling pathways could even be counter-regulatory. For instance, PGI_2/IP-mediated antiproliferative action on VSMCs is counterbalanced by PGI_2/PPARδ-mediated angiogenic response (Wise, 2003; Helliwell et al., 2004b). Such variability in the PGI_2 signaling pathways in different cells may explain the diverse biological and vascular effects of PGI_2 and other prostanoids, particularly during pregnancy and in the newborn (Helliwell et al., 2004b).

PGI_2 exerts protective cardiovascular effects that counterbalance the harmful effects of TXA_2. PGI_2 is a vasodilator and inhibitor of platelet aggregation, whereas TXA_2 promotes vasoconstriction and platelet aggregation

thase; U46619, 15-hydroxy-11α,9α-(epoxymethano)prosta-5,13-dienoic acid; VEGF, vascular endothelial growth factor.; VSMC, vascular smooth muscle cell; WAY-196025, 4-(3-(5-chloro-2-(2-(((2, 6-dimethylbenzyl)sulfonyl)amino)ethyl)-1-(diphenylmethyl)-1H-indol-3-yl)propyl)benzoic acid; Wy14643, pirixinic acid.

(Miller, 2006). Disturbance of the balance between PGI_2 and TXA_2 has been associated with vascular disorders such as pulmonary arterial hypertension (PAH). PGI_2 production is increased during normal pregnancy, as evidenced by elevated maternal urinary and plasma levels of its stable metabolite 6-keto-$PGF_{1\alpha}$. PGI_2 deficiency and PGI_2/TXA_2 imbalance during pregnancy may be associated with preeclampsia and may partly explain the hypertension, increased vascular reactivity, and platelet aggregation associated with the disease (Walsh, 2004). Decreased PGI_2/TXA_2 ratio has also been implicated in neonatal disorders, such as persistent pulmonary hypertension of the newborn (PPHN), whereas increased PGI_2 activity in premature infants may be involved in the pathogenesis of patent ductus arteriosus and cerebral intraventricular hemorrhage.

The observation that PGI_2/TXA_2 imbalance could play a role in vascular disorders, particularly those associated with pregnancy and in the newborn, has prompted the search for modulators of the PGI_2 pathway. One of the challenges in the design of modulators of the PGI_2 system is striking the correct prostanoid balance and targeting the specific prostanoid-related pathogenic mechanisms without altering other prostanoid-mediated physiological and protective mechanisms. This is particularly important for the well being of the mother and the newborn. For example, using low-dose aspirin to reduce the synthesis of TXA_2 in preeclampsia may decrease the synthesis of vasoprotective PGI_2 and minimize the net beneficial effects. Thus, it is imperative to carefully examine the different AA metabolic pathways, their various products, and their multiple target receptors and diverse signaling pathways. In this review, we first provide an overview of the AA metabolic pathways, the COX-mediated cascades, the various prostanoids and their target PRs, and the vasodilator effects of PGI_2 and its recently described vasoconstrictor actions. We will describe the changes in PGI_2/TXA_2 in vascular disease and some of the genetic polymorphisms in the PGI_2 system and their vascular consequences. We will then highlight the conditions associated with deficiency of vascular PGI_2 during pregnancy and in the neonatal period, or with excessive PGI_2 production in the premature newborn. Finally, we will provide some insight on recent reports regarding efficient use of PGI_2 modulators in pregnancy-related and neonatal vascular disorders.

II. Arachidonic Acid Metabolism and Prostacyclin Synthesis

Plasma membrane phospholipids such as phosphatidylethanolamine and phosphatidylserine are hydrolyzed by PLA_2 to produce AA and lysophospholipid, the precursors of eicosanoids and platelet-activating factor, respectively (Meyer et al., 2005). Free AA is metabolized by COXs to produce various prostanoids, including PGs and TXA_2 (Fig. 1). COX has two subtypes, COX1 and COX2, that metabolize AA to PGG_2 and PGH_2 (Zordoky and El-Kadi,
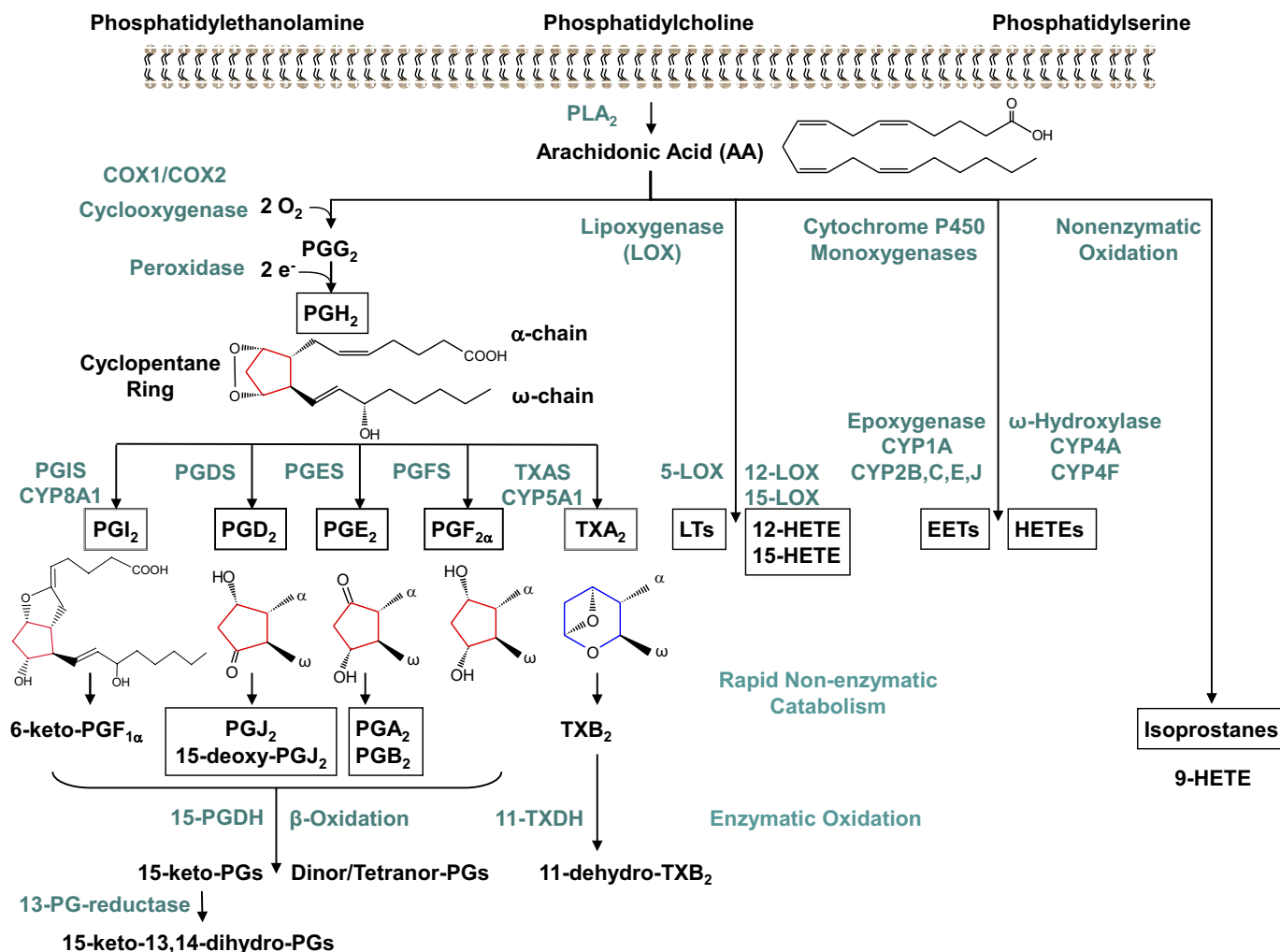
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FIG. 1. Eicosanoid and prostanoid biosynthesis and metabolism. Membrane phospholipids such as phosphatidylethanolamine are hydrolyzed by PLA_2 to produce AA. AA is metabolized by COX1 and COX2 to produce various prostanoids, 5-LOX to yield LTs and 12- or 15-LOX to yield 12- or 15-HETE, cytochrome P450 monoxygenases, including epoxygenases to produce EETs and ω-hydroxylases to produce HETEs, or undergo nonenzymatic lipid peroxidation to isoprostanes and 9-HETE. AA metabolism by COX yields PGG_2 then PGH_2. PGH_2 is acted upon by specific PG synthases (PGIS, PGDS, PGES, PGFS, and TXAS) to produce PGI_2, PGD_2, PGE_2, $PGF_{2\alpha}$, and TXA_2, respectively. PGI_2 and TXA_2 undergo rapid nonenzymatic hydrolysis to the stable and biologically inactive 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively. TXB_2 undergoes further and relatively slower enzymatic oxidation by 11-hydroxy-TXB_2 dehydrogenase (11-TXDH) to 11-dehydro-TXB_2. Nonenzymatic dehydration of PGD_2 and PGE_2 leads to the formation of the cyclopentenones PGJ_2 and 15-deoxy-PGJ_2, and PGA_2 and PGB_2, respectively. 6-Keto-$PGF_{1\alpha}$, PGD_2, PGE_2, and $PGF_{2\alpha}$ undergo either oxidation by 15-PG-dehydrogenase (15-PGDH) into the respective 15-keto-PGs, which are then reduced by 13-PG reductase to 15-keto-13,14-dihydro-PGs, or β-oxidation with subsequent loss of two or four carbons to form dinor- or tetranor-PGs. Boxed compounds are biologically active.

2010). PGH_2 is then metabolized by specific PG synthases to form PGI_2, PGD_2, PGE_2, $PGF_{2\alpha}$, and TXA_2. PGs contain 20 carbon atoms arranged in a five-carbon cyclopentane ring and two side chains, α and ω. Substitutions in the cyclopentane ring lead to different PGs. PGI_2 contains dual pentanoic acid moiety (Arehart et al., 2007), whereas TXA_2 has a six-membered oxane ring (Miller, 2006) (Fig. 1). PGI_2 synthesis is regulated by different intracellular processes, including phosphorylation by protein kinases and modification of nuclear transcriptional factors. For example, thrombin binds protease activated receptor-1 (PAR-1) to activate mitogen-activated protein kinase (MAPK), which causes phosphorylation and activation of a cytosolic PLA_2, leading to increased AA production and COX1-mediated rapid synthesis of PGI_2. In addition, activation of PAR-1 and PAR-2 increases the transcription factor NF-κB,

which in turn promotes the expression of COX2, leading to sustained PGI_2 synthesis (Wheeler-Jones, 2008).

The biologically active concentrations of prostanoids depend on their rates of synthesis and catabolism. Prostanoids are very labile compounds; therefore, quantitation of their inactive metabolites, rather than the active compounds, is used to assess their rate of synthesis. Prostanoids are catabolized via an initial rapid nonenzymatic process through which they lose most of their biological actions, followed by a relatively slower enzymatic oxidation (Miller, 2006) (Fig. 1). PGI_2 and TXA_2 have a very short half-life and are transformed spontaneously in the circulation into their inactive metabolites 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively (Miller, 2006). Catabolism of PGD_2, PGE_2, and $PGF_{2\alpha}$ occurs through oxidation by 15-PG dehydrogenase, which is highly expressed in the lung, liver,

kidney, pregnant uterus, and placenta (Kankofer, 1999). Most PGs could also undergo enzymatic β-oxidation in the liver, which increases the hydrophilicity of the molecules in preparation for urinary excretion (Diczfalusy, 1994; Buczynski et al., 2009).

Prostanoid biosynthesis requires coupling of COXs with upstream PLA$_2$ and downstream PG synthases, and compartmentalization of these enzymes is an important process in the regulation of prostanoid metabolism in different cell types (Funk, 2001). In the next sections, we will further discuss PLA$_2$, its different forms, its cellular and subcellular distribution, as well as COX1 and COX2, their structure, cellular expression, and preferential colocalization with specific PG synthases (Helliwell et al., 2004b) and describe how the differential expression of the specific PG synthases could ultimately determine the cell-specific prostanoid production (Wheeler-Jones, 2008).

A. Phospholipase A$_2$

1. Subtypes, Distribution, and Function. PLA$_2$ enzymes carry out the first step in eicosanoid synthesis. Depending on their primary structure, cellular localization, and Ca^{2+} requirement, PLA$_2$ enzymes are classified into secretory sPLA$_2$, cytosolic cPLA$_2$, and Ca^{2+}-independent iPLA$_2$ (Buczynski et al., 2009; Sharma et al., 2010). The PLA$_2$ enzymes are further divided into groups (I, II, III, IV, …) and subgroups (A, B, C, …) on the basis of their amino acid sequence. sPLA$_2$ acts mainly extracellularly and requires millimolar concentrations of Ca^{2+} for enzyme activity (Meyer et al., 2005). cPLA$_2$ functions in the cytoplasm and perinuclear region and requires micromolar concentrations of Ca^{2+} for translocation to the surface membrane (Helliwell et al., 2004b). Group IV-A cPLA$_2$ (cPLA$_{2\alpha}$) is a key player in eicosanoid production and is constitutively expressed in most cells especially ECs. In addition to cPLA$_2$, eicosanoid production also involves sPLA$_2$ (Wheeler-Jones, 2008; Sharma et al., 2010). Ca^{2+}-independent iPLA$_2$ is widely distributed in many tissues and cells (Meyer et al., 2005) and may contribute to overall PLA$_2$ activity and PGI$_2$ production in lung ECs (Sharma et al., 2010). Studies in knockout and transgenic mice support a role of PLA$_2$ in physiological processes, such as host defense and pathological processes such as inflammation and atherosclerosis (Murakami et al., 2010). Mice lacking cPLA$_{2\alpha}$ are deficient in PG and/or LT-mediated pathways and therefore demonstrate resistance to airway and joint inflammation and smaller brain infarct volume (Tai et al., 2010). Mice lacking group V and X sPLA$_2$ show reduced atherosclerosis and ischemia/reperfusion injury, respectively; although mice overexpressing group II-A sPLA$_2$ show increased atherosclerosis compared with nontransgenic littermates (Murakami et al., 2010). In addition, group II-A sPLA$_2$ has been localized in human atherosclerotic lesions, and its plasma levels are increased in humans at high risk for cardiovascular events (Garcia-Garcia and Serruys, 2009).

2. Modulators of Phospholipase A2. The identification of the role of PLA$_2$ in inflammation has prompted investigation of the potential usefulness of PLA$_2$ inhibitors in inflammatory and vascular disease. Inhibitors of PLA$_2$ inhibit the hydrolysis of membrane phospholipids and decrease the synthesis of eicosanoids and platelet-activating factor. Glucocorticoids such as cortisol have wide anti-inflammatory properties partly as a result of inhibition of PLA$_2$ expression (Goppelt-Struebe, 1997). Other pharmacologic PLA$_2$ inhibitors have been developed. Pyrrolidine-based inhibitors such as pyrrophenone and indole derivatives such as ecopladib, efipladib, and 4-(3-(5-chloro-2-(2-(((2,6-dimethylbenzyl)sulfonyl)amino)ethyl)-1-(diphenylmethyl)-1H-indol-3-yl)propyl)benzoic acid (WAY-196025) are among the most potent and selective cPLA$_{2\alpha}$ inhibitors (Ramarao et al., 2008). Pyrroxyphene, a pyrrolidine-based cPLA$_{2\alpha}$ inhibitor that decreases PGE$_2$ and LTB$_4$ levels (and reduces COX2 mRNA expression, probably by inhibiting NF-κB), decreases arthritis, and bone damage in a rat model of collagen-induced arthritis (Tai et al., 2010). Target inhibition of cPLA$_{2\alpha}$ may also be beneficial in ischemia-reperfusion injury. In a mouse model of ischemia-reperfusion injury induced by middle cerebral artery occlusion, cPLA$_{2\alpha}$(+/+) mice have increased COX2 expression, reactive oxygen species (ROS), and neuronal swelling after 2 h of ischemia and 2 h of reperfusion. In comparison, cPLA$_{2\alpha}$(−/−) mice show no change in COX2 expression and decreased neuronal and non-neuronal cell injury after 2 h of ischemia (Kishimoto et al., 2010). Bromoenol lactone is a selective inhibitor of iPLA$_2$ (Meyer et al., 2005), and methyl arachidonyl fluorophosphonate irreversibly inhibits both cPLA$_2$ and iPLA$_2$ (Garcia-Garcia and Serruys, 2009). sPLA$_2$ inhibitors include indolizines such as indoxam and substituted indoles such as varespladib sodium (A-001) and the more potent varespladib methyl (A-002). Experimental animal studies and some clinical trials have examined the potential benefits of sPLA$_2$ inhibitors such as darapladib and varespladib in coronary artery disease and atherosclerosis (Garcia-Garcia and Serruys, 2009; Arsenault et al., 2011), but large phase III clinical trials are needed to evaluate their benefits in CVD.

B. Cyclooxygenases

1. Structure, Activity, and Distribution. COXs, the major enzymes in prostanoid synthesis, are homodimers. Each COX monomer has four structural domains: a signal peptide that disappears in the mature enzyme, a dimerization domain, a membrane-binding domain, and a large C-terminal catalytic domain (Fig. 2). COX activity involves two reactions, a cyclooxygenase reaction in which AA binds two O$_2$ molecules to form PGG$_2$, and a peroxidase reaction in which PGG$_2$ is reduced by two electrons to form PGH$_2$ (Smith et al., 2000; Arehart et al., 2007) (Fig. 1). COX-mediated metabolism of AA into PGH$_2$ is often associated with formation of superoxide anion (O$_2^-$) as a byproduct of the peroxidase activity of COX (McCullough et al., 2004). During oxidative stress, a feed-forward loop is created in which O$_2^-$ causes membrane lipid peroxidation, and the
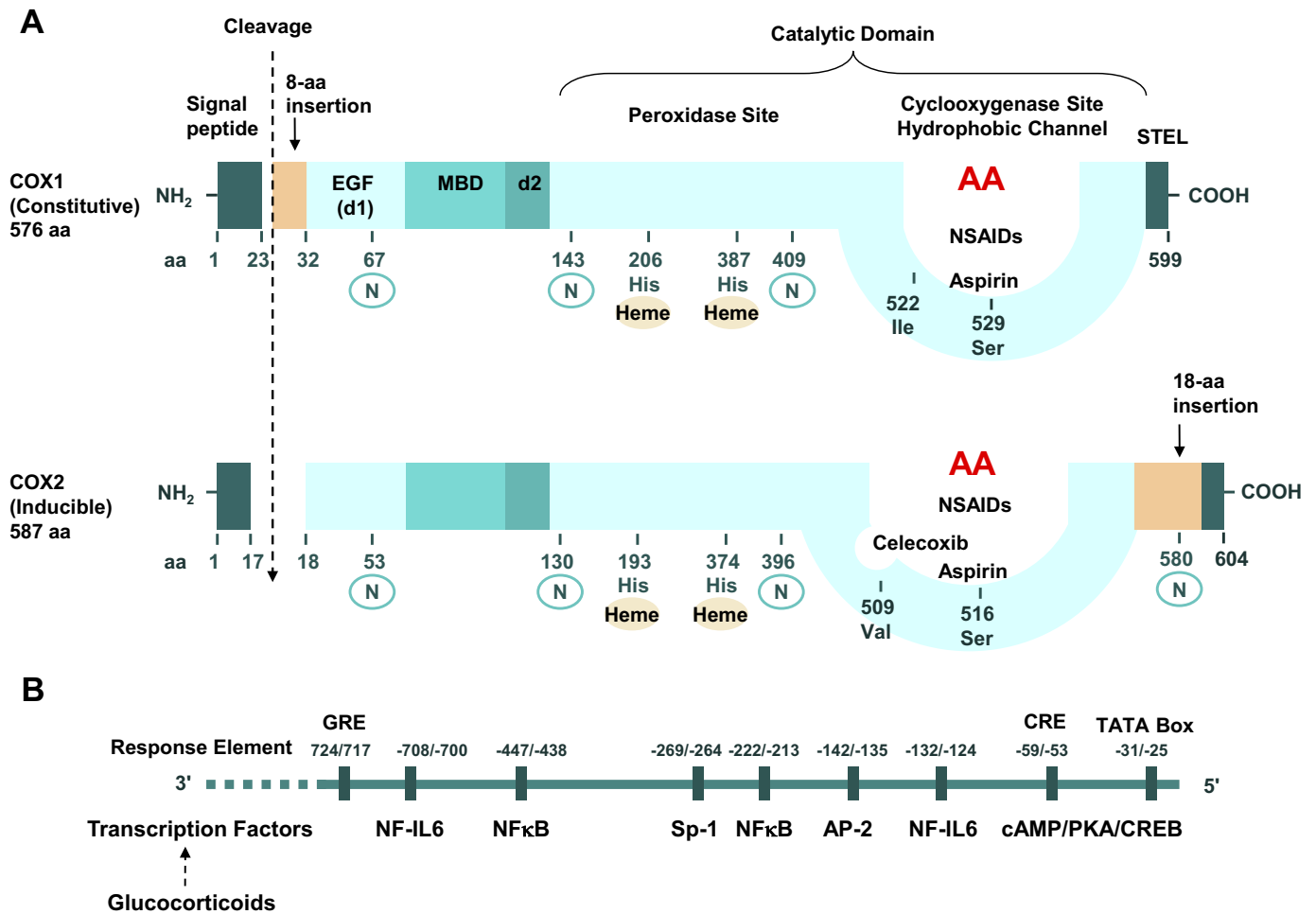
A

B

Fig. 2. Human COX1 and COX2. A, human COX1 and COX2 polypeptides share 61% primary sequence identity. The aa sequence of the COX protein starts with an N terminus with a signal peptide of 23 aa in COX1 and 17 aa in COX2 that is absent in the mature protein. Each COX monomer consists of three structural domains: dimerization domain composed of d1 (or the epidermal growth factor, EGF domain) and d2, membrane binding domain (MBD), and a large C-terminal globular catalytic domain. The catalytic domain contains a long narrow channel of hydrophobic character comprising the cyclooxygenase active site that binds AA, other fatty acids, and COX inhibitors such as aspirin and NSAIDs, and a peroxidase active site that binds heme. Both COX1 and COX2 contain C-terminal STEL-type sequence of 4 aa (Ser-Thr-Glu-Leu), which may constitute the signal for COX attachment to the ER. The mature COX1 has an 8-aa insertion at the N terminus, whereas COX2 has an 18-aa insertion at the C terminus. COX-1 and COX-2 share several N-glycosylation sites at homologous aa, and COX2 has an additional N-glycosylation site at the carboxyl terminal. The size of the cyclooxygenase active site is approximately 20% larger in COX2 than COX1, primarily due to substitution of Ile522 in human COX1 with Val509 in human COX2 that results in a small "side pocket" in the cyclooxygenase active site channel of COX2. Aspirin and other NSAIDs compete with AA at the cyclooxygenase active site. Ser529 in human COX1 (Ser530 in sheep COX1) and the corresponding Ser516 in COX2 represent the aspirin acetylation sites. The side pocket in the cyclooxygenase active site of COX2 can be accessed by selective COX2 inhibitors such as celecoxib. B, critical transcription factors and corresponding regulatory response elements on inducible COX2 promoter. In the transcription of inducible COX2, the COX2 5′-promoter contains regulatory response elements for various transcription factors starting from the TATA box, CRE (cAMP/PKA/CREB response element), NF-IL6, AP-2, NFκB, SP-1, and glucocorticoid receptor response element. Glucocorticoids inhibit transcription of COX2, but the intermediate transcription factor and response element involved are not clear.

lipid peroxides in turn increase COX activity, PGH_2 production, and formation of O_2^- (Davidge, 2001).

The *COX1* gene is constitutive, whereas *COX2* gene expression is controlled by numerous regulatory elements such as MAPK and inflammatory mediators such as the transcription factor NF-κB (Fig. 2). COX1 protein is constitutively expressed by most cell types, whereas COX2 protein expression is induced in ECs lining vascular lesions and in macrophages and monocytes during acute and chronic inflammatory states (Cipollone et al., 2008; Kawabe et al., 2010) (Table 1). In ECs, COX1 may be inducible in response to shear stress, vascular endothelial growth factor (VEGF), and thrombin (Morita, 2002), whereas

COX2 is constitutively expressed in some tissues such as kidney and neurons (Smith, 2006). In addition, immunohistochemistry studies on rat lung have shown that both COX1 and COX2 are constitutively expressed in pulmonary VSMCs and suggested that they may contribute to the regulation of pulmonary vascular tone (Ermert et al., 1998). In platelets, COX1 is the predominant constitutive isoform that catalyzes the production of TXA_2 (Morita, 2002). At the subcellular level, both COX1 and COX2 are expressed in the endoplasmic reticulum (ER) and the nucleus, but COX2 is more concentrated in the nuclear envelope (Table 1). The subcellular localizations of COX1 and COX2 could determine their distinct functions, and those

TABLE 1
Structure, distribution, activity and common inhibitors of COX1 and COX2

	COX1	COX2	Reference
Gene size	22 kb	8.3 kb	Vane et al., 1998
aa Sequence length	576	587	Smith et al., 2000
Active site size	Smaller	Larger	Morita, 2002
aa Substitutions causing differences in active site size	Ile523 Ile434 His513	Val523 Val434 Arg513	Smith et al., 2000
Enzyme activity	Largely constitutive	Largely inducible	Morita, 2002
Enzyme regulation and activation	High concentration of hydroperoxide required; negative allosteric regulation and decreased activity at low AA concentrations	10-fold lower hydroperoxide concentration required; greater activity and prostanoid synthesis than COX1 at low AA concentrations	Davidge, 2001
Substrate selectivity	AA, dihomo-γ-linolenic acid	AA, dihomo-γ-linolenic acid, other fatty acids (e.g., eicosapentoic acid, linolenic acid)	Vane et al., 1998
Tissue distribution	Constitutively expressed in most tissues; can be induced in ECs in response to shear stress, VEGF, and thrombin	Inducible expression especially in ECs by many stimulants such as inflammatory cytokines, LPS, hypoxia, and LDL; can be constitutively expressed in certain tissues (e.g. lung, kidney, brain)	Smith et al., 2000; Davidge, 2001; Morita, 2002
Subcellular distribution	ER and nuclear membrane	Mainly nuclear membrane	Morita, 2002
COX-deficient mice phenotypes	COX1(−/−) mice survive, but display platelet disaggregation, increased cerebral infarct volume, delayed labor with decreased offspring survival	COX2(−/−) mice develop nephropathy and may die early; display failure of ductus arteriosus closure; decreased cerebral infarct volume; defective ovulation, fertilization, implantation, and decidualization	Davidge, 2001; Loftin et al., 2002a,b; Morita, 2002
COX inhibitors			
Glucocorticoids	No effect	Inhibition	Vane et al., 1998; Morita, 2002
Aspirin	Complete inhibition	Incomplete inhibition of AA metabolism (transformed to active metabolite 15R-HETE)	
	Higher selectivity (IC$_{50}$, 1.67 μM)	Lower selectivity (IC$_{50}$, 278 μM)	
NSAIDS (IC$_{50}$)			
Indomethacin	0.028 μM	1.68 μM	
Diclofenac	1.57 μM	1.1 μM	
Selective COX2 inhibitors (IC$_{50}$)			
Celecoxib	15 μM	0.04 μM	
Meloxicam	4.8 μM	0.43 μM	

LDL, low-density lipoprotein; LPS, lipopolysaccharide.

localized at the nuclear envelope are more involved in gene transcription (Morita, 2002).

COXs are important regulators of vascular function. Gene deletion and overexpression of COX1 or COX2 in mice have delineated distinct biologic functions. COX1(−/−) mice survive but have reduced platelet aggregation. COX2(−/−) mice develop nephropathy and die early. COX2(−/−) mice are less prone to ischemic brain injury, but more prone to failure of closure of ductus arteriosus, resulting in 35% neonatal death within 48 h after birth (Davidge, 2001) (Table 1). In addition, 100% of mice deficient in both COX1 and COX2 have a patent ductus arteriosus and die within 12 h of birth (Davidge, 2001). Although COX2 is induced by inflammatory mediators and represent the major source of inflammatory PG production, studies in knockout mice suggest that both COX isoforms contribute to the acute inflammatory reaction, depending on the type of inflammatory stimulus, and the relative amount of each isoform in the target tissue. For example, in a mouse model of carrageenan-induced inflammation in subdermal air pouch, COX2 appears to be the major PGE$_2$-producing enzyme, because PGE$_2$ production in the subdermal air pouch exudate is decreased by 75% in COX2(−/−) mice and only by 25% in COX1(−/−) mice (Loftin et al., 2002a).

COXs are important during pregnancy, parturition, and in the newborn. COX1 deficiency in female mice does not impede conception or fetal development but causes a delay in the initiation of labor and thereby reduces offspring survival (Loftin et al., 2002b). COX2-deficient mice are infertile because of defects in ovulation, fertilization, and implantation (Loftin et al., 2002a). During pregnancy, COX1 may be more responsible for the increased capacity of uterine artery to produce PGI$_2$ (Magness et al., 2000). In human term placentas, both COX1 and COX2 mRNA expression are detected, and immunohistochemical staining shows both isoforms in the syncytiotrophoblast layer and in the capillary endothelium within the villi, suggesting a

role in the regulation of placental vascular function. However, the expression of COX1 and COX2 may differ in mid and late gestation. For example, in rat placentas, COX1 mRNA and protein expression are detected consistently from midgestation to term, with no noticeable change except for a slight increase at term, suggesting that COX1 may function as a "housekeeping" regulator during pregnancy and fetal development. On the other hand, in late pregnancy and toward labor, COX2 expression in the placenta increases substantially, leading to increased production of PGE_2 and $PGF_{2\alpha}$, which play a role in uterine contraction during the birth process (Vane et al., 1998; Simmons et al., 2004; Xu et al., 2005). In fetal tissues, COX1 expression is much greater than COX2 in the heart, kidney, and lung (Vane et al., 1998; Simmons et al., 2004), and both COX1 and COX2 are expressed in ECs and VSMCs of the ductus arteriosus (Østensen et al., 2006). The expression of COX1 and COX2 changes with maturation. In the brain, PGs are derived from COX2 in the fetus but catalyzed mainly by COX1 in adult life (Wright et al., 2001).

2. Cyclooxygenase Inhibitors. COX inhibitors include aspirin, indomethacin, other nonsteroidal anti-inflammatory drugs (NSAIDs), and recently developed selective COX2 inhibitors. It is noteworthy that the anti-inflammatory glucocorticoids not only inhibit PLA_2 activity but can also reduce COX2 mRNA, probably through a glucocorticoid-sensitive transcription factor that may interfere with transcriptional activation of the COX2 gene (Goppelt-Struebe, 1997).

COX inhibition by aspirin and other NSAIDs can take one of three forms: rapid reversible binding (e.g., ibuprofen), rapid lower affinity reversible binding followed by higher affinity slowly reversible binding (e.g., flurbiprofen), or rapid reversible binding followed by covalent irreversible modification (acetylation) of Ser530 (e.g., aspirin) (Smith et al., 2000). Aspirin displays marked selectivity for COX (Vane et al., 1998). Aspirin diffuses into the cyclooxygenase active site through the mouth of the channel and traverses up the channel to a constriction point formed by Arg120, Tyr355, and Glu524, where the carboxyl moiety of aspirin forms a weak ionic bond with the side chain of Arg120, positioning aspirin in the correct orientation for *trans*-acetylation at Ser530 (Fig. 2). Because the catalytic cyclooxygenase active site is larger in COX2 than COX1, aspirin does not fit snugly to Ser530 in COX2, and its *trans*-acetylation efficiency at COX2 is reduced; therefore, COX2 could still convert AA to 15-*R*-HETE in the presence of aspirin. This explains the 10- to 100-fold lower sensitivity of COX2 to aspirin compared with COX1 and why low-dose aspirin preferentially binds to COX1 (Morita, 2002; Simmons et al., 2004) (Table 1). Classic NSAIDs inhibit COXs by competing with AA for binding in the active site (Simmons et al., 2004) (Fig. 2) but bind more tightly to COX1 than COX2. NSAIDs bind in the upper part of the cyclooxygenase channel between Arg120 and Tyr385. Hydrogen bonding or electrostatic interactions at

Arg120 provide a major part of the binding energy and selectivity of classic acidic NSAIDs such as profens and fenamates. The remaining NSAID-COX drug-protein interactions are hydrophobic (Garavito et al., 2002).

The ability of NSAIDs to inhibit COX2, the principal isoenzyme responsible for production of inflammatory PGs, may explain their wide use as anti-inflammatory drugs, but their inhibition of COX1 causes adverse effects such as gastrointestinal and renal toxicity (Kawabe et al., 2010). To overcome the side effects of COX1 inhibition, the size difference between COX1 and COX2 active sites has been used in developing a new generation of selective and potent COX2 inhibitors. Some NSAIDs such as meloxicam (Mobic), nimesulide, and etodolac are preferential inhibitors of COX2. The first-generation selective COX2 inhibitors, including celecoxib (Celebrex) and rofecoxib (Vioxx), were first marketed in 1999. Second-generation highly selective COX2 inhibitors include valdecoxib (Bextra) and etoricoxib. Selective COX2 inhibitors have the same anti-inflammatory, antipyretic, and analgesic activities as NSAIDs but have little or none of the gastrointestinal side effects commonly observed with nonselective NSAIDs (Smith et al., 2000; Simmons et al., 2004).

III. Specific Prostanoid Synthases

1. Activity, Distribution, and Function. Specific prostanoids are synthesized by downstream isomerases and oxidoreductases that use PGH_2 as a common substrate. Prostanoid synthases include PGIS, PGDS, PGES, PGFS, and TXAS. PGDS has two forms, lipocalin L-PGDS and hematopoietic H-PGDS. Three distinct PGE synthases, responsible for the synthesis of PGE_2, have been identified and characterized, one cytosolic and two membrane-bound (Smith et al., 2000; Simmons et al., 2004; Smith, 2006). PGIS and TXAS are membrane-bound hemoproteins that belong to the P450 family but lack monooxygenase activity (Zordoky and El-Kadi, 2010). As an isomerase, PGIS rearranges PGH_2 to form PGI_2 using the peroxidase action of P450. The substrate channel of PGIS is lined by hydrophobic residues such as Cys441 and Tyr430, which are important for enzyme activity. Cys441 represents the binding site for heme leading to PGIS activation, whereas Tyr430 may be the site of PGIS nitration and enzyme inactivation. PGIS nitration by peroxynitrite ($ONOO^-$), a reaction product of O_2^- and NO, at Tyr430 near the active site reduces its catalytic activity. In contrast, $ONOO^-$ activates COX and in turn increases PGH_2 availability, and because TXAS is not affected by $ONOO^-$, this shifts prostanoid production toward TXA_2 (Wu and Liou, 2005; Zou, 2007). Therefore, under conditions of oxidative stress, PGIS inactivation together with increased COX expression favor the production of TXA_2 over PGI_2 (Hardy et al., 2005).

PGIS is distributed in a wide range of tissues including ECs, VSMCs (Nakayama, 2005), and nonvascular SMCs (Magness et al., 2000). Because of the consecutive actions of COX and PGIS, PGI_2 is the main product of AA in vascular tissues. PGI_2 synthesis is greatest in the intima

and decreases progressively toward the adventitia. In addition, among cultured vascular cells, ECs are the most active PGI_2 producers (Zou, 2007). PGI_2 synthesis takes place in highly vascularized organs such as the lung, kidney, uterus, testis, stomach, and spleen (Nakayama, 2005). PGI_2 production in ECs of the lung is critical in maintaining vasodilation in the pulmonary microcirculation (Miller, 2006). In ECs, PGIS is expressed under basal conditions and upon stimulation by thrombin, cytokines, growth factors, and mechanical stress (Wheeler-Jones, 2008). Sex hormones also affect PGIS expression/activity. Combined estradiol-17β and progesterone treatment of ovariectomized sheep increases the levels of $cPLA_2$ and COX1 proteins in uterine artery ECs, and each hormone increases PGIS in uterine artery VSMCs (Rupnow et al., 2002). During pregnancy, PGI_2 is produced by the placenta; umbilical, placental, and uterine vessels; amnion, chorion, and decidua; and fetal ductus arteriosus. ECs are the primary source of PGI_2 in these tissues, except for the amnion and chorion laeve, which are avascular (Walsh, 2004). PGI_2 is also synthesized by the myometrium during pregnancy (Fetalvero et al., 2008), and the protein levels of myometrial COX1 and PGIS increases before labor, possibly because of the cyclic mechanical stretch that occurs before and during labor (Korita et al., 2002).

TXAS is predominant in platelets but is also expressed in monocytes and VSMCs. In human placenta, immunohistochemical studies have shown that PGIS is localized in ECs within the placental villi, whereas TXAS is primarily localized in the trophoblasts (Helliwell et al., 2004a). At the subcellular level, PGIS and TXAS, like other P450s, are localized in the ER but are also found in the perinuclear region and nuclear envelope (Cipollone et al., 2008). In general, prostanoid synthases near the perinuclear membrane such as PGIS and membrane-bound PGES-1 are coupled with COX2, which is more localized at the nuclear envelope. On the other hand, prostanoid synthases such as cytosolic PGES, which is complexed with the cytosolic 90-kDa heat shock protein and casein kinase 2, couple more with COX1, because it is abundantly expressed in the ER (Smith, 2006). Colocalization of prostanoid synthases with COX1 or COX2 may also depend on whether the cells are in the resting state, when COX1 is constitutive, or in the stimulated state, when COX2 is induced. For example, in rat, dog, and human aortic ECs under basal conditions, constitutive COX1 couples with PGIS and TXAS to generate a balanced amount of PGI_2 and TXA_2 necessary for normal hemostasis and regulation of vascular function (Ruan and Dogné, 2006; Kawka et al., 2007). In addition, in bovine aortic ECs under resting conditions, constitutively expressed PGIS and COX1 colocalize to the nuclear envelope and ER, whereas in serum-treated ECs, PGIS colocalizes with inducible COX2 primarily in the ER (Liou et al., 2000). COX2 could also be constitutive and thereby colocalizes with PGIS to produce PGI_2 under physiological conditions. Studies in healthy human subjects have shown that the selective COX2 inhibitor celecoxib is associated

with a marked decrease in urinary PGI_2 (McAdam et al., 1999), suggesting that COX2 could be constitutive under normal conditions and possibly colocalizes with PGIS to produce PGI_2 in cells other than ECs, as has been shown in the lung bronchiolar epithelium (Kawka et al., 2007).

PGIS and TXAS yield PGI_2 and TXA_2, respectively, with opposite vascular effects. PGIS knockout mice have hypertension, increased fibrosis, vascular injury, and kidney infarction (Nakayama, 2005), and transgenic mice overexpressing PGIS are protected against hypoxic pulmonary hypertension (Nana-Sinkam et al., 2007). TXAS-deficient mice still produce PGH_2, which shifts prostanoid synthesis toward other PGs, as evidenced by increased levels of 6-keto-$PGF_{1\alpha}$. In addition, PGH_2 itself may act as a thromboxane receptor TP agonist. Therefore, the TXAS deletion phenotype may not reflect the full range of TXA_2 effects. TXAS($-/-$) mice have a mild hemostatic defect without overt spontaneous bleeding or defective AA-induced but not ADP- or collagen-induced platelet aggregation, and they are protected against AA-induced shock and death (Yu et al., 2004).

2. Modulators of Prostacyclin Synthase and Thromboxane Synthase.   Despite the attractive possibility of developing PGIS activators to enhance PGI_2 production, no specific activators of PGIS are currently available. Some drugs may increase PGI_2 production by increasing PGIS activity. Cicletanine, an antihypertensive drug acting by increasing NO synthesis and blocking Ca^{2+} channels, increases PGI_2 production possibly through activation of PGIS, stimulation of AA release, or increased cholesteryl ester hydrolase activity (Kalinowski et al., 1999). In addition, honokiol, a biphenyl neolignan in the cortex of *Magnolia officinalis*, has antithrombotic effects as a result of increased PGIS expression and inhibition of TXA_2 production (Zhang et al., 2007).

PGIS inhibitors such as tranylcypromine have been used experimentally but have not been tested clinically. Peroxynitrite ($ONOO^-$), but not H_2O_2 or O_2^-, is a major inhibitor of PGIS under conditions of oxidative stress. Hypochlorite inhibits PGIS but is less effective than $ONOO^-$. Other reagents such as tetranitromethane promote tyrosine nitration and inactivation of PGIS. Because nitration occurs in the active site of PGIS, it is prevented by competing substrates. For example, direct binding of the TXA_2/PGH_2 analog 15-hydroxy-11α,9α-(epoxymethano) prosta-5,13-dienoic acid (U46619) to the PGIS active site blocks its action but, in the meantime, protects PGIS from $ONOO^-$ binding, as evidenced by decreased tetranitromethane-induced PGIS staining with 3-nitrotyrosine antibody in the presence of U46619 (Zou et al., 1999). $ONOO^-$, as an oxidant, reacts with antioxidants such as SH groups (GSH) and vitamins C and E. However, none of these compounds can compete with PGIS for $ONOO^-$, probably because of a catalytic reaction of the iron-thiolate center of PGIS with $ONOO^-$ (Zou, 2007). On the other hand, heme-thiolate P450s that are similar in nature to PGIS, including the bacterial fatty acid monoxygenase $P450_{BM-3}$ and

the NADH-NO reductase $P450_{NOR}$, compete with PGIS for nitration by $ONOO^-$ and therefore rescue PGIS from $ONOO^-$ inhibition (Zou et al., 2000).

Gene therapy is an evolving strategy to increase PGIS. In rats, intravenous infusion of adenovirus Adv-COX1/PGIS gene reduces brain infarct size (Lin et al., 2002), decreases neointima formation in carotid artery after balloon injury, and restores 6-keto-$PGF_{1\alpha}$ to levels greater than those in control vessels (Imai et al., 2007). Gene transfer of both COX and PGIS may be preferred over PGIS alone because it increases both COX and PGIS, and the majority of PGH_2 produced by COX is used by PGIS to produce PGI_2. PGIS gene transfer alone would still have a limited amount of PGH_2 available for PGIS to produce PGI_2 (Imai et al., 2007). TriCat, a novel antithrombotic hybrid enzyme engineered by linking human COX2 with PGIS through a transmembrane domain, is 3-fold faster in converting AA to PGI_2 than the combination of COX2 and PGIS, and competes with endogenous COX1/TXAS to reduce TXA_2 production (Ruan et al., 2008).

Because of the physiological balance between PGI_2 and its counter-regulatory prostanoid TXA_2, the effects of PGI_2 could be modulated indirectly by altering TXA_2 production. TXAS inhibitors could exert antithrombotic effects not only by inhibiting TXA_2 production but also by increasing transcellular PGI_2 production. Inhibition of TXA_2 production in platelets leads to accumulation of PGH_2, which is taken-up by ECs and VSMCs and metabolized into PGI_2. Among the TXAS inhibitors are the imidazole ring group, including dazoxiben, pirmagrel, and ozagrel, and the pyridine ring group, including 2-methyl-3-(4-(3-pyridinylmethyl)phenyl)-2-propenoic acid (OKY1581), isobogrel, and furegrelate (Dogné et al., 2006). Although TXAS inhibitors were initially promising clinically, they have not been effective, partly because of their incomplete blockade of TXAS at the dosage used and the accumulation of PGH_2, which is chemically more stable than TXA_2 and exerts similar effects on TP receptor. PGH_2 has a half-life of 3 to 5 min compared with a half-life of 30 s for TXA_2 (Bunting et al., 1976). To overcome this disadvantage, dual-acting TXAS inhibitor/TP antagonists were developed. Ridogrel is a potent TXAS inhibitor but has very weak TP receptor affinity. Terbogrel has a balanced dual TXAS inhibitor/TP antagonist activity that effectively causes dose-dependent inhibition of platelet aggregation and enhances PGI_2 production. However, the clinical efficacy of TXAS/TP inhibitors is not conclusive despite promising results in animal models (Yu et al., 2004; Dogné et al., 2006).

IV. Prostacyclin Receptor

Several PRs have been identified in different tissues and cells. The PR family comprises eight classic members: DP1, EP1–4, FP, IP, and TP, which correlate with their primary endogenous ligands PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2, respectively (Funk, 2001) (Table 2). Although cloning studies have not provided data indicative of IP receptor sub-

types (Wilson et al., 2011), binding studies using radiolabeled PGI_2 analogs have suggested the presence of two IP receptor subtypes: the classic IP1 receptor, mainly identified in peripheral tissues, and IP2 receptor, largely expressed in the central nervous system (CNS), mainly in the thalamus, cerebral cortex, and striatum (Takechi et al., 1996). However, recent binding and functional studies have suggested coexpression of functionally distinct IP1 and IP2 receptors in the human airway epithelial cell line BEAS-2B (Wilson et al., 2011). Splice variants of EP3, FP, and TP receptors have also been described in human tissues (Norel, 2007). In addition to classic PRs, the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), also referred to as DP2, binds PGD_2, and the PPAR nuclear receptors may be a target for PGI_2 and some derivatives of PGD_2 (Norel, 2007).

A. Prostacyclin Receptor Structure

Classic PRs comprise seven transmembrane domains (TMD), an extracellular amino terminus, intracellular carboxyl terminus, three extracellular loops, and three intracellular loops (Fig. 3). PRs share some sequence homology, and most of the conserved amino acid sequences occur along TMD (Narumiya et al., 1999). In 1994, the cDNA of human IP receptor was cloned from a lung cDNA library. IP receptor shares 30 to 40% homology with other PRs (Tanaka et al., 2004) and has 386 amino acid residues with a calculated molecular mass of 40.96 kDa (Ruan and Dogné, 2006). However, the IP molecular mass ranges from 37 to 41 kDa, depending on its state of glycosylation (Arehart et al., 2007). Glycosylation of IP receptor at the amino terminus and first extracellular loop may affect ligand binding, receptor activation, and membrane localization. For example, PGI_2-induced activation of adenylyl cyclase may be reduced depending on the extent of IP receptor glycosylation (Stitham et al., 2007). TMD-I, along with a portion of the first extracellular loop, confer broader binding functions for recognition and interaction with the cyclopentane ring of PGI_2. In addition, protein segments within TMD-VI and TMD-VII may be involved in distinct interactions with PGI_2 side chains. A highly conserved Arg residue (Arg279) and a DPW (Asp-Pro-Trp) motif are located in the middle of TMD-VII and are 100% conserved among all PRs (Fig. 3). Arg279 is crucial for securing ligand within the binding domain and may represent the binding site of the carboxyl group in the α-chain of the prostanoid molecule, whereas DPW is important for receptor activity (Narumiya et al., 1999; Stitham et al., 2007). The coupling of IP receptor and downstream signaling pathways is modulated by C-terminal modification of the receptor. A highly conserved palmitoylation-isoprenylation (CAAX) sequence, CCLC (Cys-Cys-Leu-Cys), tethers part of the C-terminal tail to the cytoplasmic membrane, forming a fourth cytoloop (Fig. 3). Differential mutagenesis of this motif has shown that either Cys308 or Cys311 is sufficient for human IP coupling to G_s, but Cys308 is specifically needed for G_q coupling. In addition, C-terminal

TABLE 2

Biochemical characteristics, endogenous ligands, signaling pathways, and representative agonists and antagonists of prostanoid receptors

Data from Jones et al., 1995; Tsuboi et al., 2002; Nagata and Hirai, 2003; Hata and Breyer, 2004; Norel, 2007; Jones et al., 2009.

PR	MM	Endogenous Ligands	G-Protein	Second Messenger	Agonists	Antagonists
DP1	40.012 (M) 40.276 (H)	PGD_2 PGJ_2	G_s	Mainly ↑ cAMP Other: ↑ Ca^{2+}	BW245C[a]	AH6809 BWA868C[a,b,c] MK-0524 (laropiprant)[a,b] ONO-AE3-237[a]
DP2 (CRTH2)	43 (H)	PGD_2 15-Deoxy-PGJ_2	G_i G_q?	Mainly ↓ cAMP Other: ↑Ca^{2+}, PLC, PI3K, MAPK	13,14-Dihydro-15-keto-PGD_2[a] 15-(R)-methyl-PGD_2[a]	BAY-u3405 (ramatroban) TM-30642[a,b] TM-30089[a,b]
EP1	42.966 (M) 41.858 (H)	PGE_2 8-iso-PGE_2	G_q?	↑ Ca^{2+}	Iloprost,[b] ONO-DI-004[a] 17-Phenyl-PGE_2[b] Sulprostone[b]	AH6809 ONO-8713[a,b] SC51322[b]
EP2	40.478 (M) 39.38 (H)	PGE_2	G_s	Mainly ↑ cAMP Other: EGFR transactivation, GSK-3/β-catenin	AH13205 Butaprost[a] 16,16-Dimethyl-PGE_2[b] ONO-AE1-259,[a] PGE_1[b]	AH6809
EP3	40.077 (M) 40.5–43.315 (H)	PGE_2 8-iso-PGE_2	G_i G_q G_s	Mainly ↓ cAMP Other: ↑ IP_3/ DAG, cAMP	11-Deoxy-PGE_1[b] GR-63799X[a,b] Iloprost, M&B-28767[b] ONO-AE248,[a] PGE_1[b] SC-46275[a] Sulprostone[b]	DG041[b] L826266 ONO-AE3-240[b]
EP4	56.157 (M) 53.115 (H)	PGE_2	G_s	Mainly ↑ cAMP Other: PI3K, ERK1/ 2, GSK-3/β-catenin	11-Deoxy-PGE1[b] L-902688 Misoprostol ONO-AE-329[a,b]	AH23848 EP4A[a] L161982[b] ONO-AE3-227[b]
FP	40.077 (M)	$PGF_{2\alpha}$ PGD_2 PGE_2	G_q	Mainly ↑ IP_3/DAG Other: ERK, EGFR *trans*-activation, β-catenin	Cloprostenol Fluprostenol[a,b] Latanoprost[a] 17-Phenyl-PGE_2	AS604872
IP	44.722 (M) 40.06 (H)	PGI_2 PGE_2	G_s G_q G_i	Mainly ↑ cAMP, Rac/PAK1, ↓ Rho-A Other: ↑ IP_3/DAG, ↓ cAMP ↓Akt-1, ↓ ↑MAPK	Beraprost[b] Carbacyclin Cicaprost[b] Iloprost[b] Isocarbacyclin[b] PGE_1	RO1138452[a,b] RO3244794[a,b]
TP	37.114 (M) 37.429 (H)	TXA_2 PGH_2 8-iso-$PGF_{2\alpha}$	G_q $G_{12/13}$ G_i G_s	Mainly ↑ IP_3/DAG, ↑ Rho-A Other: ↓ cAMP ↑ cAMP ↑ MAPK	GR-32191 (vapiprost)[b] I-BOP[b] STA2[a,b] U-46619[a]	AH23848[b] BAYu3405 (ramatroban)[b] GR32191 (vapiprost)[b]

AH13205, *trans*-2-(4-(1-hydroxyhexyl)phenyl)-5-oxocyclopentaneheptanoic acid; AH23848, *(4Z)*-7-[(rel-1*S*,2*S*,5*R*)-5-((1,1′-biphenyl-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid; AH23848, 7-(5-(((1,1-biphenyl)-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl)-4-heptanoic acid; AS604872, (2*S*)-3-((1,1′-biphenyl)-4-ylsulfonyl)-*N*-((*R*)-phenyl(2-pyridinyl)methyl)-1,3-thiazolidine-2-carboxamide; BW245C, 3-((2-cyclohexyl-2-hydroxyethylidene) amino)-2,5-dioxo-4-imidazolidineheptanoic acid; BWA868C, 3-((2-cyclohexyl-2-hydroxyethyl)amino)-2,5-dioxo-1-(phenylmethyl)-4-imidazolidineheptanoic acid; DG041, 2,3-dichlorothiophene-5-sulfonic acid, 3-[1-(2,4-dichlorobenzyl)-5-fluoro-3-methyl-1*H*-indol-7-yl]acryloylamide; GR-63799X, (4-benzamidophenyl)-(*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(2*R*)-2-hydroxy-3-phenoxypropoxy]-5-oxocyclopentyl]hept-5-enoate; GSK-3, glycogen synthase kinase-3; (H), human; I-BOP, [1*S*-[1α,2α(*Z*),3β(1E,3*S**),4α]]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabi-cyclo[2.2.1]hept-2-yl]5-heptenoic acid; IP_3, inositol trisphosphate; L161982, *N*-[[4′-((3-butyl-5-oxo-1-[2-(trifluoromethyl)phenyl]-1,5-dihydro-4*H*-1,2,4-triazol-4-yl]methyl)biphenyl-2-yl] sulfonyl]-3-methylthiophene-2-carboxamide; L826266, [(2*E*)-*N*-[(5-bromo-2-methoxyphenyl)-sulfonyl]-3-[5-chloro-2-(2-naphthylmethyl)phenyl]acrylamide; L-902688, 5*R*-[(1*E*)-4,4-difluoro-3*R*-hydroxy-4-phenylbut-1-en-1-yl]-1-[6-(1*H*-tertrazol-5-yl)hexyl]pyrrolidin-2-one; (M), mouse; M&B-28767, 7-[(1*R*,2*R*)-2-[(*E*,3*R*)-3-hydroxy-4-(phenoxy)but-1-enyl]-5-oxocyclopentyl]heptanoic acid; MM, molecular mass of cloned receptor; ONO-8713, (*E*)-3-[4-[[2-(furan-2-ylsulfonyl-(2-methylpropyl)amino)-5-(trifluoromethyl)phenoxy]methyl]phenyl]prop-2-enoic acid; ONO-AE1-259, 9-deoxy-9-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-19,20-didehydroprostaglandin E$_2$; ONO-AE248, 16*S*-9-deoxy-9β-chloro-15-deoxy-16-hydroxy-17,17-trimethylene-19, 20-didehydro-prostaglandin F$_2$; ONO-AE3-227, Hata and Breyer (2004); ONO-AE3-237, Jones et al. (2009); ONO-AE3-240, 2-[2-[[4-methyl-2-(1-naphthyl)pentanoyl]amino]-4-(1*H*-pyrazol-1-ylmethyl)benzyl]benzoic acid; ONO-AE-329, 2-[3-[(1*R*,2*S*,3*R*)-3-hydroxy-2-[(*E*,3*S*)-3-hydroxy-5-[2-(methoxymethyl)phenyl]pent-1-enyl]-5-oxocyclopentyl]sulfanylpropylsulfanyl]acetic acid; ONO-DI-004, 17*S*-2,5-ethano-6-oxo-17,20-dimethyl prostaglandin E$_1$; PI3K, phosphatidylinositol 3-kinase; SC-46275, methyl 7-(2β-(6-(1-cyclo-pentyl-yl)-4*R*-hydroxy-4-methyl-1*E*,5*E*-hexadienyl)-3α-hydroxy-5-oxo-1*R*,1α-cyclopentyl)-4*Z*-heptenoate; SC51322, 8-chlorodibenz[*b*,*f*][1,4]oxazepine-10(11H)-carboxylic acid, 2-[3-2-(furanylmethyl)thio]-1-oxopropyl]hydrazide; STA2, 9,11-epithio-11,12-methanothromboxane A$_2$; TM-30089, (+)-3-[[(4-fluorophenyl)sulfonyl]methylamino]-1,2,3,4-tetrahydro-9*H*-carbazole-9-acetic acid; TM-30642, Jones et al. (2009).

[a] High receptor selectivity.
[b] High affinity (agonist binding $K_i \leq 25$ in mouse tissue or $pA_2 \geq 8$ in different species).
[c] Partial agonist activity.

serine residues in the IP receptor are phosphorylated by G-protein-coupled receptor kinases or second-messenger-activated kinases such as protein kinase C (PKC) and protein kinase A (PKA) and therefore play a role in IP receptor intracellular phosphorylation and kinase-mediated desensitization (Hata and Breyer, 2004; Stitham et al., 2007).

Similar to other GPCRs, the IP receptor has been shown to dimerize/oligomerize, at least in COS-7 cells. The cysteine residues of the IP receptor extracellular domains are important determinants of receptor expression, because individual substitution with Ser at any of the four (Cys5, Cys92, Cys165, and Cys170) results in substantial decrease in IP receptor protein levels in COS-7 cells. Coim-

FIG. 3. Prostanoid receptor structure. PRs have three domains: an extracellular domain consisting of a short N-terminal tail and three extracellular loops, a transmembrane domain (TMD) composed of seven transmembrane-spanning α-helices, and a cytoplasmic domain made of three intracellular loops (in most PRs), and a C terminus. There are 34 amino acid residues conserved in all PRs in different species (white circles), mainly located within the TMDs. A highly conserved Arg residue (Arg279) and a DPW (Asp-Pro-Trp) motif (100% conserved among all PRs) are located in the middle of TMD-VII. In all PRs, a conserved disulfide bond is formed in the extracellular domain between Cys92 at the top of TMD-III and Cys170 in the second extracellular loop of human IP receptor. Another disulfide bond specific for human IP receptor is formed between Cys5 at the top of TMD-I and Cys165 in the second extracellular loop. One or more consensus sequences for N-glycosylation sites, demonstrated as CHO ($C_6H_{12}O_6$; glucose), are present in the amino terminus extracellular portion (asparagine residues Asn7 and Asn78 in human IP). In the cytoplasmic domain of IP receptor, lipid isoprenylation-palmitoylation anchoring sites starting at Cys308 (Cys-Cys-Leu-Cys) create a fourth intracellular loop.

munoprecipitation studies of differentially tagged IP receptors expressed in COS-7 cells demonstrated that IP receptor can form homodimers/oligomers, and the cysteine residues of the IP extracellular domains are essential for receptor dimerization, possibly by forming disulfide links between Cys5 and Cys165 and between Cys92 and Cys170. Treatment with carbacyclin does not alter the ratios of IP oligomeric/dimeric/monomeric forms, suggesting that IP receptor dimerization/oligomerization is an agonist-independent process. It is noteworthy that in COS-7 cells lacking cell surface expression of the IP receptor because of individual mutation in Cys5, Cys92, Cys165, or Cys170, IP receptors can still form dimers/oligomers, suggesting that dimerization may occur intracellularly (Giguère et al., 2004).

B. Prostanoid Receptors Cellular and Subcellular Distribution

Among PRs, DP1 is the least abundant, with low expression in human small intestine and mouse brain leptomeninges (Narumiya et al., 1999). Among EP receptors, EP3 and EP4 mRNA expression is detected in almost all mouse tissues tested. EP3 mRNA expression is particularly abundant in the brain, kidney, and SMCs of ileum, colon, and myometrium. EP1 and EP2 have more limited distribution, but EP2 mRNA expression can be induced by various stimuli. FP mRNA is mainly expressed in the corpus luteum, kidney, heart, lung, and stomach (Narumiya et al., 1999), and FP receptor has been identified in human uri-

nary bladder and myometrium (Walch et al., 2001; Wright et al., 2001). IP and TP receptor mRNAs are abundantly expressed in the lung, kidney, heart, and other highly vascularized organs (Narumiya et al., 1999; Tsuboi et al., 2002). At the cellular level, IP and TP receptors are expressed in human VSM and platelets. The vascular distribution of PRs differs between blood vessels and between arteries and veins within the same vascular bed. Contraction/relaxation studies have suggested that IP, TP, and EP3 receptors are the main PRs in human pulmonary artery, whereas IP, DP, TP, EP1, and probably EP2 receptors are present in human pulmonary vein (Walch et al., 1999, 2001). Vascular PRs are mainly localized in the VSM layer, as determined by immunohistochemistry, contraction/relaxation studies of isolated vascular preparations without endothelium, and physiological responses of isolated VSMCs. Most human VSMCs express both IP and TP receptors. Some contraction/relaxation studies have suggested the presence of PRs in ECs of certain blood vessels such as human hand veins. In these vessels, the vascular response to prostanoid agonists may change with endothelium removal, probably because of the release of an endothelial-derived relaxing factor in response to activation of an endothelial PR (Norel, 2007).

Although most PRs are localized at the plasma membrane, some are located at the nuclear envelope (Funk, 2001). Plasma membrane PRs elicit rapid physiological actions, whereas nuclear PRs convey gene regulation. EP1, EP4, and EP3 receptors have been localized in the perinu-

clear region of ECs of porcine cerebral microvessels. IP receptor may also be present at the nucleus, because PGIS has been colocalized with COX1 at the perinuclear area of human ECs (Norel, 2007). Other nuclear receptors for PGI_2 include PPARδ and PPARα, which are expressed in a wide range of tissues, and PPARγ, which has more restricted expression, especially in adipose tissue (Helliwell et al., 2004b). PPARα, -γ, and -δ may use PGI_2 as an endogenous ligand (Norel, 2007; Kurtz et al., 2010), and PPARγ is activated by natural derivatives of PGD_2 (e.g., $\Delta12$-PGJ_2 and 15-deoxy-PGJ_2).

PRs are widely distributed in maternal and fetal tissues during pregnancy, and their expression varies in the different stages of gestation. Human uterine myocytes express mRNA of all eight PRs, but the expression levels differ among nonpregnant, pregnant, and postpartum myometrium. The mRNA expression of procontraction PRs such as EP3, FP, and TP in the human myometrium decreases during pregnancy, whereas mRNA expression of DP and IP does not change in the different reproductive states (Sooranna et al., 2005). PRs undergo developmental changes in their expression in fetal and neonatal vascular tissues of the ductus arteriosus, brain, and retina. When measured by radiolabeled ligand binding, EP and FP receptors have 2- to 3-fold lower receptor density in brain microvessels of newborn pig compared with adult animals (Li et al., 1994). In the pig ductus arteriosus, EP receptor density decreases by 3-fold in the immediate newborn compared with the fetus. Although EP2, EP3, and EP4 receptors are expressed in equivalent densities in pig fetal ductus arteriosus, only EP2 is found in the imme-

diate postnatal ductus in newborn pig (Bhattacharya et al., 1999). This is different from the rabbit ductus, where developmental changes in mRNA expression of the EP receptor subtypes occur toward term, with an increase in EP3, a decrease in EP4, and no change in the expression of EP1 and EP2 receptors (Fan et al., 2010). Nuclear PRs, including PPARα, -γ, and -δ, are expressed in placental tissues, and PPARδ seems to be essential for embryo implantation, early embryogenesis, and placental development (Wise, 2003; Higa et al., 2007; Kurtz et al., 2010).

C. Prostacyclin Receptor Agonists and Antagonists

Because PGI_2 is very labile and transforms to 6-keto-$PGF_{1\alpha}$ within minutes, stable PGI_2 analogs, including prostanoid and nonprostanoid IP agonists, have been developed (Lim and Dey, 2002). Similar to PGI_2, prostanoid IP agonists have a carboxyl group at C1 and two OH groups at C11 and C15 (Fig. 4). Nonprostanoid IP agonists maintain the obligatory C1-carboxyl group, the general hydrophobic chains, and the critical orientation of the functional groups capable of forming hydrogen bond at a distance from the C1-carboxyl group comparable with the C11-OH group in PGI_2. Although the OH groups at C11 and C15 in prostanoid IP agonists facilitate hydrogen bonding to PRs, heterocyclic nitrogen, oxime nitrogen, or ester groups serve this function in nonprostanoid agonists. Prostanoid IP agonists have higher affinity over nonprostanoid agonists, largely because of their ability to form an additional hydrogen bond to IP receptor (Stoll et al., 2002). Nonprostanoid compounds could have partial or dual IP agonist,
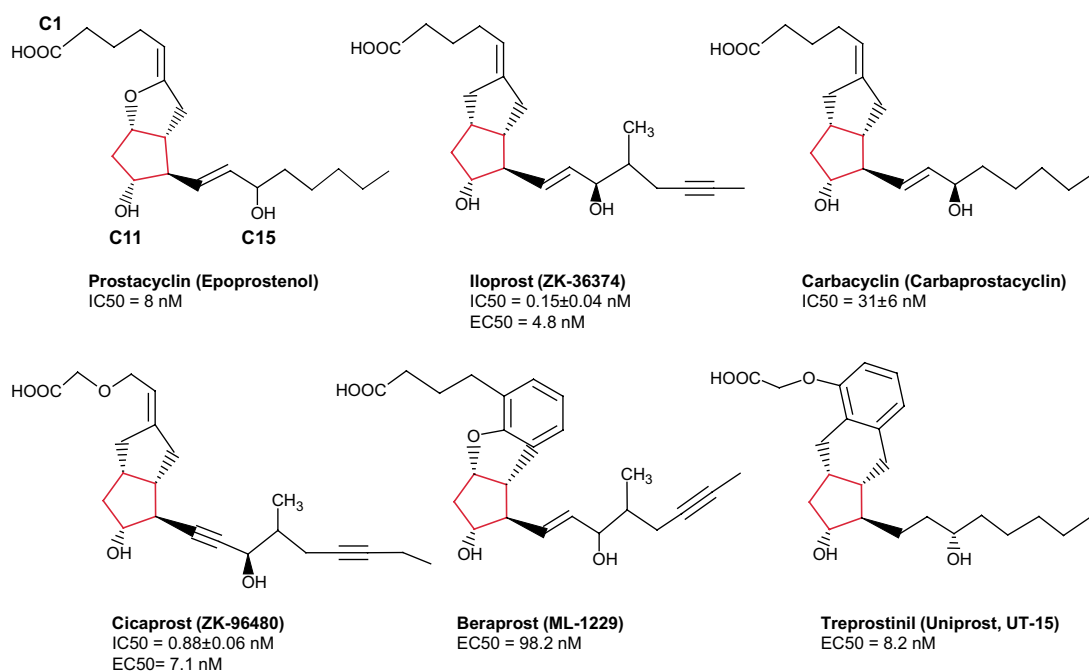

FIG. 4. Commonly used prostanoid-type prostacyclin analogs. Prostanoid analogs share a primary structure that determines their binding to the IP receptor, including the essential carboxyl group at C1, and hydrogen-bonding OH groups at C11 and C15. Functional IC_{50} is the half-maximal inhibitory concentration of platelet aggregation. Functional EC_{50} is the half-maximal effective concentration of inducing a cAMP response in cultured human pulmonary artery SMCs.

TXA$_2$/TP antagonist, or TXAS inhibitor activity (Stoll et al., 2002; Arehart et al., 2007) (Table 3). Commonly used IP analogs in clinical trials include epoprostenol, iloprost, carbacyclin, cicaprost, beraprost, and treprostinil (Tanaka et al., 2004) (Fig. 4).

PGI$_2$ analogs bind with different affinities to IP receptor subtypes. Binding studies have shown that, compared with the peripheral IP1, the CNS-type IP2 receptor has almost similar affinity for isocarbacyclin (K_d 7.8 nM for IP2 versus 3.9 nM for IP1), but very low affinity for iloprost (K_d, 159 nM for IP2 versus 6.8 nM for IP1) (Takechi et al., 1996). (15R)-16-m-Tolyl-17,18,19,20-tetranorisocarbacyclin (15R-

TIC) is a specific ligand for CNS-type IP2 with low affinity for peripheral IP1 and therefore exerts few effects on circulatory parameters such as blood pressure (BP), heart rate, and platelet aggregation (Cui et al., 2006). In the human airway epithelial cell line BEAS-2B, PGI$_2$ analogs act with different potencies for IP1 and IP2 receptors to independently regulate two distinct functions. For example, iloprost activates CRE and glucocorticoid receptor response element reporter through IP1 but not IP2 receptor, whereas 15-deoxy-TIC inhibits chemokine CXCL9 and CXCL10 release through IP2 with minimal activity at IP1 receptor (Wilson et al., 2011).

TABLE 3
Nonprostanoid IP receptor agonists

Binding IC$_{50}$ refers to competitive binding with radiolabeled iloprost in human platelets. Functional IC$_{50}$ refers to inhibition of ADP-induced platelet aggregation in human platelet-rich plasma.

Groups and Subgroups	Representative Compound	Pharmacokinetics and Pharmacodynamics			Reference
		K_i	IC$_{50}$		
			Binding	Functional	
			μM	μM	
IP agonists					
Tetrahydro-naphthalene oxyacetic acid derivatives					
4-Benzhydryl-oxyimines	ONO-AP-227		0.15	0.15	Kondo and Hamanaka, 1995;
Oximes/amides/ethers	Compound 12		0.01	0.057	Tsubaki et al., 2000
4-Benzhydryl pyrazoles	ONO-AP-437		0.008	0.026	
Pyridazinones	FR181877	0.094		0.081	
Diphenyloxazole derivatives					Meanwell et al., 1992a;
Cyclohexenes	FR181157	~0.054		≈0.060	Hattori et al., 2005a,b
Tetrahydro-naphthalenes and pyrrolidines	FR193262	~0.012		0.019 ± 0.0038	
Diphenylcarbamate derivatives with a tetrahydro-naphthalene skeleton	FK788	0.02		0.01	Hattori et al., 2005c
2 Amino-5,6 diphenylpyrazine derivatives	NS-304	0.02		0.2	Asaki et al., 2007
IP partial agonists					
Phenylated pyrazol alkanoic acid derivatives (Octimibate-like)					
Triphenylated pyrazol alkanoic acids	BMY 42239		0.16	0.4	Meanwell et al., 1992a,b; Seiler et al., 1997
Diphenyloxazole derivatives demonstrating partial agonist activity	BMY42393		0.245	1.2	
	BMY45778	~0.0068		0.035 ± 0.012	
IP agonist/TXAS inhibitor					
Tetrahydro-naphthalene 5-oxyacetic acid derivatives with a 3-pyridyl instead of phenyl group	ONO-AP-500-02 (ONO-1301)			0.24[a] 0.04[b]	Kondo et al., 1995
IP agonist/TXA2 antagonist					
Benzofuran sulfides	DHMB-acetic acid (Compound 9b)			2.2 ± 0.4[c] 0.17 ± 0.01[d]	Ohno et al., 2005
3,4-Dihydro-2H benzo oxazine derivatives	TRA-418			1.8[c] 0.55[d]	Ohno et al., 2006
PG endoperoxide analogs with diphenylmethyl oxime or azine residues in the ω-chain	EP157			0.5[c] 0.3[d]	Armstrong et al., 1986

BMY 42239, Meanwell et al. (1992b); BMY 42393, 2-[3-[2-(4,5-diphenyl-2-oxazolyl)ethyl]phenoxy]acetic acid; BMY 45778, [3-[4-(4,5-diphenyl-2-oxazolyl)-5-oxazolyl]phenoxy]acetic acid; Compound 12, Kondo and Hamanaka (1995); DHMB-acetic acid, (3-(2-(1,1-diphenylethylsulfanyl)ethyl)-2-hydroxymethylbenzofuran-7-yloxy)acetic acid; EP157, *rac*-5-endo-(6′-carboxyhex-2′-Z-enyl)-6-exo-diphenylmethoxyiminomethyl-bicyclo[2.2.2]oct-2-ene; FK788, 2-[[(6R)-6-(diphenylcarbamoyloxymethyl)-6-hydroxy-7,8-dihydro-5H-naphthalen-1-yl]oxy]acetic acid; FR181157, sodium (3-{[(1S)-2-(4,5-diphenyl-1,3-oxazol-2-yl)-2-cyclohexen-1-yl]methyl}phenoxy)acetate; FR181877, [[(2S)-2β-(3-oxo-6-benzhydryl-2,3-dihydropyridazine-2-ylmethyl)-5-tetralinyl]oxy]acetic acid; FR193262, sodium ({(5R)-5-[(2R)-2-(4,5-diphenyl-1,3-oxazol-2-yl)pyrrolidin-1-yl]-5,6,7,8-tetrahydronaphthalen-1-yl}oxy)acetate; NS-304, 2-(4-((5,6-diphenylpyrazin-2-yl)(isopropyl)amino)butoxy)-N-(methylsulfonyl)acetamide; ONO-AP-227, Kondo and Hamanaka (1995); ONO-AP-437, Kondo and Hamanaka (1995); ONO-AP-500-02, 7,8-dihydro-5-(2-((α-(3-pyridyl)benzylideneaminooxy)ethyl)-1-naphthyloxy)acetic acid; TRA-418, (4-(2-(1,1-diphenylethylsulfanyl)ethyl)-3,4-dihydro-2H-benzo(1,4)oxazin-8-yloxy)acetic acid N methyl-D-glucamine salt.

[a] IP agonist.
[b] TXAS inhibition.
[c] ADP-induced.
[d] U46619-induced.

Because of the partial homology between IP receptor and other PRs, and the structural similarities between PGI_2 and other prostanoids, cross-activation of IP receptor by other prostanoids and of other PRs by PGI_2 analogs could occur (Tanaka et al., 2004; Arehart et al., 2007) (Table 2). Compared with PGI_2 analogs, PGD_2 and PGE_2 bind to human IP receptor in the following affinity order: iloprost $\gg$ carbacyclin $>$ PGE_2 $\gg$ PGD_2 (Tsuboi et al., 2002). In addition, none of the PGI_2 analogs is absolutely specific for the IP receptor. Iloprost has equivalent binding affinity for IP and EP1, appreciable affinity for EP4, and only slightly lower affinity for EP3 receptor. In comparison, carbacyclin has comparable binding affinity for IP, DP, EP, and FP receptors, whereas isocarbacyclin has affinity for EP2 and EP3 receptors (Wise, 2003; Hata and Breyer, 2004; Wilson et al., 2011). Cicaprost and beraprost have higher affinity and are considered better pharmacological tools than iloprost to investigate IP receptor-mediated biological responses (Tanaka et al., 2004).

PGI_2 analogs can also bind to nuclear PPARs with variable affinities. For example, the PGI_2 analogs carbacyclin, iloprost, cicaprost, and treprostinil inhibit cell proliferation in cultured human lung fibroblasts at concentrations greater than those expected to activate IP receptors ($>10^{-7}$ M) but within the range for PGI_2 mimetics acting on PPARδ receptors ($>10^{-6}$ M) (Ali et al., 2006). Carbacyclin, iloprost, and cicaprost bind to PPARα with relative affinity $\approx$120, 100, and 10%, respectively, of the selective PPARα activator pirixinic acid (Wy14643), and bind to PPARδ with relatively strong affinity for carbacyclin (100%) compared with lower affinity for iloprost (80%) and very little affinity for cicaprost (8%) (Forman et al., 1997). In IP transfected HEK-293 cells, carbacyclin, cicaprost, and treprostinil increase PPARγ activity, whereas in HEK-293 cells expressing an empty vector, neither carbacyclin nor treprostinil activates PPARγ (Falcetti et al., 2007). In addition, reporter gene assays in IP nontransfected HEK-293 cells have shown that treprostinil activates PPARδ, but not PPARγ, suggesting a role of IP receptor in treprostinil-induced activation of PPARγ (Ali et al., 2006). Thus, PGI_2 analogs bind to nuclear PPARs with variable affinities, and this may explain some of the genomic effects of PGI_2.

Although several PGI_2 analogs are available, few IP receptor antagonists have been developed, possibly because of the lack of clinical utility (Arehart et al., 2007). IP receptor antagonists include the 2-(phenylamino)-imidazoline series such as 2-(4-(4-isopropoxybenzyl)-phenylamino) imidazoline (RO1138452), and the N-substituted phenylalanine series such as RO3244019 (Fitch et al., 2004) and its difluoro analog (R)-3-(4-fluoro-phenyl)-2-(5-(4-fluoro-phenyl)-benzofuran-2-ylmethoxycarbonylamino)-propionic acid (RO3244794) (Jones et al., 2006). RO1138452 and RO3244794 inhibit carbacyclin-induced adenylate cyclase activity in cultured Chinese hamster ovary-K1 cells overexpressing human IP receptor. RO1138452 is more potent than RO3244794 as an IP receptor antagonist and exhibits

no or weak affinity for EP1, EP3, FP, and TP receptors (Bley et al., 2006). IP antagonists can also have different affinities for IP receptor subtypes. For instance, in the human respiratory epithelial cell line BEAS-2B the affinity of RO3244794 is ~10-fold higher for IP2 than for IP1 receptor (Wilson et al., 2011).

D. Prostacyclin Receptor Signaling Pathways

PRs belong to GPCRs and are coupled to different GTP-binding proteins, including G_s, G_q, and G_i, to activate downstream signaling pathways. Phylogenetic analyses show that PRs sharing a common signaling pathway have greater sequence homology than receptors sharing a common prostanoid as their preferential ligand (Wright et al., 2001). On the basis of their signal transduction and activated G-protein, PRs can be either relaxant receptors (IP, DP, EP2, and EP4), which couple to G_s to mediate increases in cAMP and SMC relaxation; contractile receptors (EP1, FP, and TP), which couple to G_q and mediate Ca^{2+} mobilization and SMC contraction; or inhibitory receptors such as EP3, which couples to G_i, mediates decreases in cAMP, and inhibits SMC relaxation (Narumiya et al., 1999; Funk, 2001) (Table 2). DP2 also signals through a G_i-mediated pathway (Nagata and Hirai, 2003). In general, activation of IP, DP, EP2, and EP4 receptors in VSMCs induces vasodilation, whereas activation of EP1, EP3, FP, and TP receptors causes vasoconstriction (Norel, 2007).

Although PGI_2/IP couples primarily to G_s to activate adenylyl cyclase and increase cAMP, and in turn activates PKA (Lim and Dey, 2002) (Fig. 5), PGI_2/IP may also couple to G_q, leading to increased phosphoinositide turnover and $[Ca^{2+}]_i$ in a cell-specific manner (Wright et al., 2001). IP receptor subtypes may have distinct downstream signaling pathways and activate different functions in different tissues. Unlike IP1 receptor, activation of the CNS-type IP2 receptor does not couple to increased cAMP production or phosphoinositide turnover but enhances neuronal excitatory transmission by an unidentified mechanism (Takechi et al., 1996). On the other hand, in the human respiratory epithelial cell line BEAS-2B, IP1 and IP2 receptors couple to activation of canonical cAMP/PKA cascades, although they mediate two distinct functions in these cells (Wilson et al., 2011).

PGI_2 may act via other pathways to control various cellular processes (Wise, 2003; Helliwell et al., 2004b). PGI_2/IP signaling may modulate the MAPK pathway and consequently gene regulation, cell growth, and differentiation. PGI_2/IP activation of PKA could impair ERK signaling, and thus interfere with TXA_2 signaling. In addition to PGI_2-activated cell surface receptors, the presence of COX2/PGIS at the nuclear and ER membranes suggests PGI_2 intracrine pathways involving nuclear receptors (Lim and Dey, 2002). Intracrine PGI_2 binding to perinuclear PPARs causes translocation of PPAR to the nucleus and the formation of heterodimers with retinoic acid receptors, which in turn bind to per-
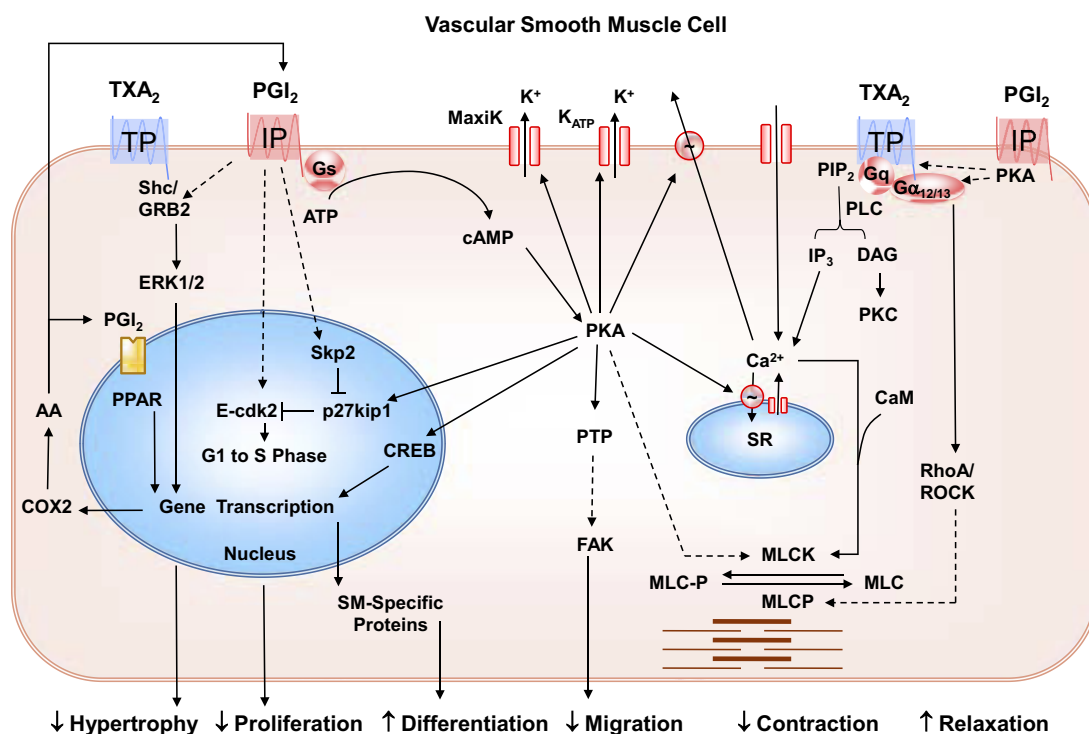
Vascular Smooth Muscle Cell

FIG. 5. PGI$_2$ signaling pathways in VSMCs. PGI2/IP cell surface interaction is coupled primarily to G$_s$ to activate cAMP/PKA leading to Ca^{2+} extrusion via cell surface and sarcoplasmic reticulum (SR) Ca^{2+} pumps, and activation of different K$^+$ channels (including ATP-sensitive K$^+$ channels and MaxiK channels), which in turn cause VSMC hyperpolarization and relaxation. In contrast, TXA$_2$/TP interaction causes stimulation of G$_q$, activation of phospholipase C (PLC), and increased production of inositol-1,4,5-trisphosphate (IP$_3$), which stimulates Ca^{2+} release from SR, and DAG, which activates PKC. Increased [Ca^{2+}]$_i$ causes activation of Ca^{2+}/calmodulin/myosin light chain kinase (MLCK) pathway and stimulation of VSM contraction. TP-mediated stimulation of G$\alpha_{12/13}$ activates Rho signaling and the RhoA/Rho-associated protein kinase (ROCK) pathway, which inhibits myosin-light chain phosphatase (MLCP) and causes Ca^{2+} sensitization and enhancement of VSMC contraction. PGI$_2$/IP signaling via cAMP/PKA can inhibit TXA$_2$-induced VSMC contraction by PKA-mediated TPα phosphorylation and thereby inhibition of both G$_q$/PLCβ/Ca^{2+}-dependent and G$_{12/13}$/RhoA Ca^{2+}-independent signaling pathways. PGI$_2$ also activate genomic pathways and cellular processes including, as shown from left to right, PGI$_2$-induced PGI$_2$ release, PPAR, VSMC hypertrophy, proliferation, differentiation, and migration. PGI$_2$/IP signaling can induce COX2 expression in VSMCs to metabolize AA and produce PGI$_2$, which in turn may act in an intracrine fashion on the same VSMC or paracrine on nearby VSMCs (feedback loop). PGI$_2$ intracrine signaling may involve direct binding to PPAR nuclear receptors and gene transcription. PGI$_2$/IP signaling can inhibit Shc/GRB2 complex formation and subsequent ERK1/2 activation by TXA$_2$/TP signaling, thus inhibiting VSMC hypertrophy. PGI$_2$ also inhibits VSMC proliferation by inhibiting G$_1$-to-S phase progression through inhibition of cyclin E-cyclin-dependent kinase (cdk2) as well as activation of p27^{kip1}, which keeps cyclin E-cdk2 in an inactive state, either directly or via inhibition of the gene for S-phase kinase-associated protein (Skp2), which causes p27^{kip1} degradation. PGI$_2$ can also act through IP/cAMP/PKA-mediated activation of CREB or other SM-specific transcription factors to increase the expression of SM-specific differentiation markers and cause VSM differentiation. PGI$_2$ via cAMP-dependent activation of protein tyrosine phosphatase (PTP) causes inhibition of focal adhesion kinase (FAK) and disruption of focal adhesion formation, leading to inhibition of cell migration.

oxisome proliferator response element and lead to genomic effects (Fig. 5).

V. Effects of Prostacyclin on Blood Vessels

PGI$_2$ is particularly known for its prominent effects on cardiovascular homeostasis and hemostasis. PGI$_2$ exerts major effects on various cell types of the vessel wall (Fig. 6). PGI$_2$ plays an essential role in endothelial cell integrity and regulation of vasomotor function, particularly in larger arteries. PGI$_2$ can also affect VSM differentiation, proliferation, and migration as well as extracellular matrix metabolism and vascular tissue remodeling. The effects of PGI$_2$ in vascular cells are mediated by different signaling pathways, including the classic PGI$_2$/IP/G$_s$/cAMP pathway and the intracrine pathways involving nuclear receptors. Although several reports suggest that the effects of PGI$_2$ and its analogs involve an increase in cAMP production, whether these effects are mediated by IP receptor or other cAMP-producing PRs may not be evident in many cases

because the studies were conducted before specific IP receptor agonists and antagonists became available.

A. Effects of Prostacyclin on Endothelial Cells

The endothelium plays a major role in maintaining vascular integrity, and endothelium-derived NO is a major relaxing factor and regulator of vascular tone. Endothelium-derived PGI$_2$ also plays a role in maintaining a healthy endothelium and regulating EC function (Zardi et al., 2005). Beraprost increases EC barrier integrity by promoting cortical F-actin ring formation and inhibiting central stress fibers at areas covered by adherens junctions, a process mediated via Rac1 and its effector p21-activated kinase, and effectively counteracts the stimulatory effects of RhoA/Rho-associated protein kinase on stress fiber formation (Birukova et al., 2007). PGI$_2$ also maintains EC integrity by inhibiting cell apoptosis. PGI$_2$/PPARδ signaling inhibits H$_2$O$_2$-induced apoptosis by decreasing the activity of the proapoptotic factor Bad (Kawabe et al., 2010)

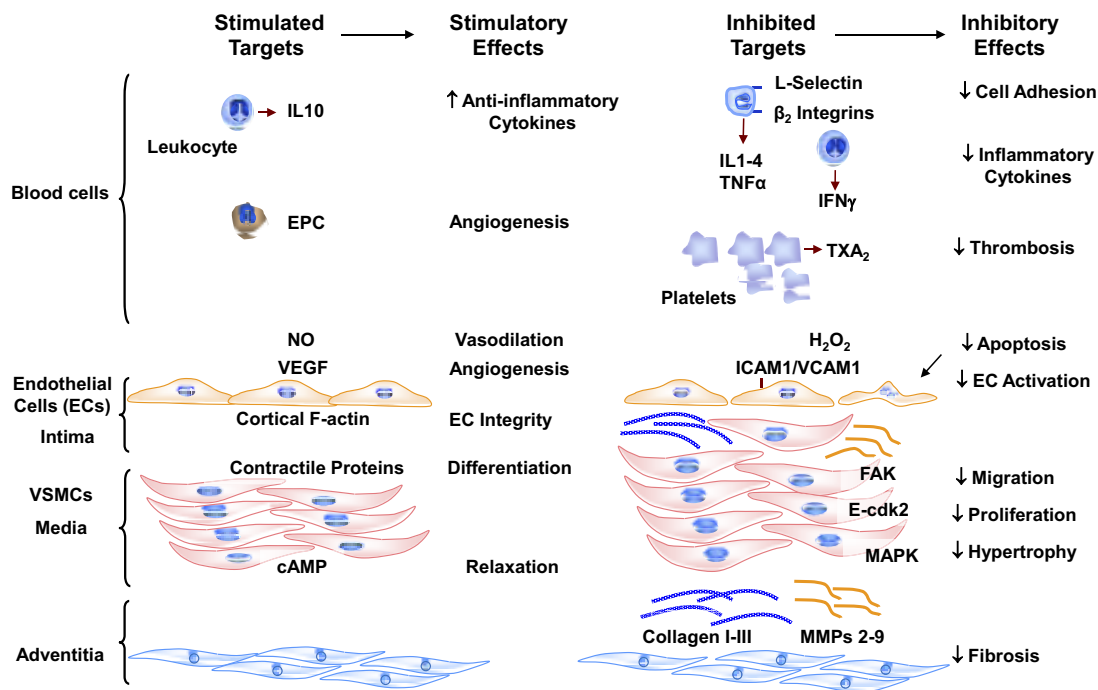## Vascular Effects of PGI$_2$

FIG. 6. Vascular effects of PGI$_2$. PGI$_2$ interact with multiple stimulatory and inhibitory target molecules in circulating platelets, leukocytes, EPCs, and cells of the vascular wall, including ECs, VSMCs, and adventitial fibroblasts. The interaction of PGI$_2$ with the stimulatory and inhibitory target molecules lead to corresponding stimulation or inhibition of cell signaling or function. cdk, cyclin-dependent kinase; FAK, focal adhesion kinase; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

(Fig. 6). In addition, overexpression of PGIS in a mouse model of cigarette smoke-induced emphysema decreases caspase-3 expression and apoptosis in lung ECs of the PGIS transgenic mice compared with control littermates (Nana-Sinkam et al., 2007). Iloprost decreases expression of adhesion molecules on ECs and inhibits the interaction between circulating platelets and leukocytes on the EC surface (Mazzone et al., 2002). In addition, iloprost treatment in patients with Raynaud's disease inhibits the increase in the soluble endothelium-dependent adhesion molecules E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 (Mittag et al., 2001).

PGI$_2$ could also affect EC function, and some of these effects could involve cross-talk with the NO pathway. For example, beraprost causes a cAMP/PKA-mediated transcriptional increase of endothelial NO synthase (eNOS) mRNA expression and enhancement of eNOS mRNA stability in cultured human aortic ECs (Niwano et al., 2003).

B. Effects of Prostacyclin on Smooth Muscle Cells

1. Prostacyclin and Smooth Muscle Relaxation. Vascular ECs generate several vasoactive substances that cause relaxation of the underlying VSMCs. The primary endothelium-derived vasorelaxant mediators are NO, PGI$_2$, and hyperpolarizing factors (EDHFs) (Tanaka et al., 2004). PGI$_2$'s contribution to vascular relaxation compared with other endothelium-derived vasodilators generally depends on the size of the blood vessel, the specific vascular

bed, and the animal species studied. This is supported by the variable effects of COX inhibitors in various blood vessels from different species (Table 4).

Compared with NO, PGI$_2$ contributes very little to endothelium-dependent relaxation of rat aorta and large proximal branches of mesenteric arteries (Shimokawa et al., 1996) and human proximal gastroepiploic arteries (Urakami-Harasawa et al., 1997). In contrast, EDHF is the major endothelium-derived relaxing factor in small-resistance rat mesenteric arteries and human gastroepiploic arteries that are known to control vascular tone and BP. In addition, in vivo data have shown that endogenous PGI$_2$ may have little role in the regulation of BP compared with NO because pharmacological blockade of COX with aspirin or indomethacin does not substantially affect resting BP in humans or rats, whereas pharmacological blockade or genetic ablation of NO synthase has a profound effect on BP, increasing it by 30 to 50 mm Hg. Although some studies have shown that mice lacking PGIS have greater BP compared with wild-type, this has been attributed not to changes in PGI$_2$-mediated relaxation but to the associated gross abnormalities in the kidney or reduction in the lumen-to-wall ratio in the aorta and pulmonary and renal vessels caused by PGI$_2$ deficiency (Parkington et al., 2004).

Cross-talk between the PGI$_2$-dependent and NO-dependent pathways may also play a role in the control of vascular relaxation. In some blood vessels, relaxation of

TABLE 4
Effects of cyclooxygenase inhibition on vascular response to various vasoactive substances

Species	Vessel	Vasoactive Agent	Effect	COX Inhibitor	Effect	Reference
Human	Uterine artery rings partially contracted with phenylephrine	AngII (4×10^{-8} M)	Transient contraction ($3.1 \pm 0.6\%$) followed by relaxation ($40.8 \pm 6.5\%$)	Indomethacin (10^{-6} M)	↑ Contraction and abolished relaxation	Kimura et al., 2001
	Pulmonary artery rings precontracted with U46619	ACh (1 nM–3 μM)	Relaxation (E_{max} $45.1 \pm 12.1\%$)	Flurbiprofen (1 μM)	Partly attenuated relaxation ($33.4 \pm 13.5\%$)	Lawrence et al., 1998
	Middle cerebral artery blood flow by ultrasonography	Hypercapnia (8% CO_2)	Vasodilation (87.7% of baseline velocity)	Indomethacin	Insignificant reduction in vasodilation (61%)	Markus et al., 1994
		Hypocapnia Hyperventilation	Vasoconstriction (37.5%)		↓ Vasoconstrictor response (20.7%*)	
	Placentofetal resistance arteries from the placental villus stem	Bradykinin ($10^{-6} - 10^{-9}$ M)	Vasoconstriction through release of vasoconstrictor PGs	Meclofenamate (10^{-7} M)	↓ Vasoconstriction at bradykinin concentrations $>3 \times 10^{-7}$ M	Tulenko, 1981
Dog	Coronary artery (in vivo measurement of coronary blood flow)	ACh (10–300 ng)	Increased coronary blood flow by ~10 ml/min	Naproxen (10 mg/kg)	No effect	Gross and Moore, 2004
		AA (30–1000 μg)	Increased coronary blood flow by ~7 ml/min		↓ Blood flow by 10–20 ml/min	
	Pulmonary arterial and venous vascular resistance in perfused lung lobe	Serotonin (100 μg)	Increased lobar vascular resistance (both lobar arterial and venous resistance)	Meclofenamate (45 μM)	↑ Lobar arterial resistance by ~26 and venous resistance by ~6 cm H_2O/l/min	Hofman et al., 1991
	Lobar intrapulmonary artery rings	Serotonin (10^{-7} M)	Contraction (1 g active tension)	Indomethacin (10 μM)	No change	
	Mesenteric Artery (in vitro isolated rings)	AngII (2×10^{-8} M)	~60% contraction compared with KCl	Indomethacin (10^{-6} M)	~60% increase in contraction	Yamazaki and Toda, 1991
	Retinal artery rings precontracted with U46619	Bradykinin ($10^{-10} - 10^{-6}$ M)	Relaxation (max $94.2 \pm 2.5\%$ of maximum relaxation diameter to papaverine)	Indomethacin (10 μM)	No significant effect ($92.5 \pm 3.4\%$)	Jeppesen et al., 2002
	Middle cerebral artery rings	AA (10 μg/ml)	Contraction (maximum 1.8 g)	Indomethacin (10^{-5} M)	Insignificant ↑ in contraction by ~9%	Jancar et al., 1987
Rabbit	Celiac artery rings	NE (5×10^{-8} M)	Contraction (1.62 mN)	Indomethacin (0.8 μM)	$160 \pm 15\%$ Increase in contraction	Hadházy et al., 1984
	Pulmonary artery		Contraction (2.4 mN)		$26 \pm 6\%$ ↑ Contraction	
	Femoral artery		Contraction (9.3 mN)		$15 \pm 3\%$ ↓ Contraction	
	Aorta		Contraction (4.58 mN)		$7 \pm 3\%$ ↑ Contraction	
	Aortic rings precontracted with NE 10^{-7} M	AA ($10^{-8} - 10^{-5}$ M)	Relaxation (max $31 \pm 2\%$)	Indomethacin (10^{-5} M)	Increased relaxation ($42 \pm 2\%$)	Pfister and Campbell, 1992
		ACh ($10^{-8} - 10^{-5}$ M)	Relaxation (max $43 \pm 3\%$)		No effect ($41 \pm 3\%$)	
Rat	Cerebral pial arteriole (in vivo measurement of arteriolar diameter)	ACh (4×10^{-4} M)	Relaxation ($111 \pm 2\%$ of baseline diameter)	Indomethacin (6×10^{-5} M)	No significant effect ($107 \pm 1\%$ of baseline diameter)	Rosenblum et al., 1989
		A-23187, calcium ionophore (10^{-5} M)	Relaxation ($107 \pm 1\%$ of baseline diameter)	Indomethacin (6×10^{-5} M)	↓ Relaxation ($101 \pm 1\%$ of baseline diameter)	

TABLE 4—*Continued.*

Species	Vessel	Vasoactive Agent	Effect	COX Inhibitor	Effect	Reference
	Uterine arterioles (in vivo measurement of arteriolar diameter)	ACh ($10^{-8} – 10^{-4}$ M)	Dilation	Ibuprofen (10^{-4} M)	↓ Dilation at higher ACh concentrations (>1 μM)	Saha et al., 1998
		AngII ($10^{-11} – 10^{-7}$ M)	Constriction		Enhanced constriction	
		Phe ($10^{-8} – 10^{-4}$ M)	Constriction		No effect	
	Renal arcuate artery rings	ACh (0.01–100 μM)	Relaxation (maximum response $17.2 \pm 1.7\%$)	Indomethacin (14 μM)	↑ Relaxation (maximum response $36.5 \pm 3.3\%$)	Wu et al., 1994
		NE (0.01–10 μM)	Contraction (maximum 1.84 ± 0.27 mN/mm)		↓ Contraction (max 1.48 ± 0.21 mN/mm)	

VSM induced by PGI_2 can be amplified by endothelium-derived NO. In small fifth-order porcine pulmonary artery, basal release of NO and formation of cGMP could lead to inhibition of cAMP phosphodiesterase, thus decreasing the hydrolysis of cAMP and increasing its concentration in VSMCs and thereby enhancing the PGI_2-cAMP pathway and pulmonary artery relaxation (Zellers et al., 2000). In isolated canine pulmonary artery, the synergistic interaction between endothelium-derived NO and PGI_2 during bradykinin-induced relaxation seems to involve activation of ATP-sensitive K^+ channels (Gambone et al., 1997). Decreased NO production may also affect PGI_2 release/activity. When NO availability is impaired, a compensatory increase in the role of endothelium-derived PGI_2 may occur to overcome the increase in vascular resistance. For example, PGI_2 has a more prominent role in modulating basal vascular tone when NO production is impaired in gracilis muscle arterioles of eNOS knockout mice (Sun et al., 2006) and in rat proximal mesenteric arteries or dog coronary arteries subjected to long-term inhibition of NO synthesis (Puybasset et al., 1996; Henrion et al., 1997).

The contribution of endothelium-derived factors to vascular relaxation seems to be different in such specialized vessels as the coronary, cerebral, and pulmonary arteries. PGI_2, NO, and possibly an EDHF contribute to methacholine and bradykinin-induced endothelium-dependent vascular relaxation of ovine coronary artery precontracted with U46619 (Pratt et al., 1996). In addition, removal of the endothelium or pretreatment with inhibitors of both NO and PGI_2 synthesis reduces (R)-α-methyl-histamine-induced relaxation of rabbit middle cerebral artery rings precontracted with KCl (Ea Kim et al., 1992) and increases the vasoconstriction response of goat middle cerebral artery to 5-hydroxytryptamine (Miranda et al., 1993). In the pulmonary vasculature, the contribution of PGI_2 to endothelium-dependent relaxation seems to be different in pulmonary arteries and veins and between species. Both PGI_2 and NO contribute to ACh-induced relaxation of human pulmonary artery (3–6 mm internal diameter), but NO is the major endothelium-derived mediator of ACh-induced relaxation of human pulmonary vein. The observation that the COX inhibitor indomethacin reduces ACh relaxation in

isolated human pulmonary artery but not in the pulmonary vein supports little role of PGI_2 in relaxation of pulmonary vein. In contrast, NO seems to be the sole endothelium-derived vasorelaxant released by ACh in porcine pulmonary artery (Lawrence et al., 1998; Norel et al., 2004). In canine pulmonary vessels, PGI_2 does not contribute to endothelium-dependent relaxation in the large arterial rings but has an important vasodilator effect in the small microvessels. In isolated blood-perfused canine left lower lung lobe, treatment with indomethacin increases the resting postcapillary venous pressure and blocks the serotonin-induced decrease in precapillary arterial pressure, suggesting that PGI_2 release is a major endothelium-derived relaxing factor in the postcapillary venous circulation under basal conditions as well as in precapillary resistance arteries stimulated with serotonin (Hofman et al., 1991). Collectively, endothelium-derived PGI_2 has an important role in enhancing vasodilation in arteries supplying vital organs such as pulmonary and cerebral arteries, but its overall contribution to basal systemic vascular tone, especially as a function of vessel size, as well as its role in the control of BP, may depend on whether the release of other vasodilators such as NO is intact or compromised.

The role of PGI_2 in mediating vasodilation depends not only on the amount of endogenous endothelium-derived PGI_2 but also on the amount of IP and other PRs expressed in VSMCs, the relative potency and selectivity of the PGI_2 analog, and the postreceptor signaling mechanisms. Therefore, although endogenous PGI_2 production may not have significant effect in the control of vascular tone compared with NO in certain vascular beds, exogenous PGI_2 and its analogs could have marked effects on VSM relaxation (Table 5). For example, the PGI_2 analog isocarbacyclin causes dose-dependent relaxation of isolated monkey cerebral and peripheral arteries precontracted with 5-hydroxytryptamine, with potency as follows: mesenteric > renal > cerebral > coronary > popliteal arteries. Isocarbacyclin causes almost the same relaxation in endothelium-denuded as endothelium-intact cerebral and mesenteric arteries, suggesting that the regional difference in the relaxation response of the cerebral and peripheral arteries is

TABLE 5
Effects of prostacyclin analogs on representative vascular preparations

Species	Blood Vessel	Agonist and Dose	Receptor Involved	Effect	Reference
Human	Pulmonary arteries and veins precontracted with norepinephrine	PGI_2 (0.1 nM–10 μM)	IP (Arteries)	Dose-dependent relaxation	Norel et al., 2004
			EP1, IP (Veins)	Reduced relaxation	
Monkey	Cerebral arteries precontracted with 5-hydroxytryptamine	Isocarbacyclin 0.1 nM–10 μM	IP	Dose-dependent relaxation	Kawai and Ohhashi, 1994
		$\geq$ 1 μM	TP, IP	Transient contraction followed by sustained relaxation	
Piglets	Saphenous vein precontracted with phenylephrine	AFP-07 (0.3–14 nM)	EP4, IP	Relaxation	Jones and Chan, 2001
Guinea pig	Aorta precontracted with phenylephrine	Iloprost (1–50 nM)	IP	Relaxation	Jones and Chan, 2001
		Carbacyclin (3 to 43 nM)	EP3, IP	Contraction followed by relaxation at higher doses	
Rabbit	Mesenteric artery precontracted with phenylephrine	Carbacyclin Initial 1–14 nM then higher	EP3, IP	Contraction followed by relaxation at higher doses	Jones and Chan, 2001
Rat	Aorta precontracted with norepinephrine	PGI_2, Carbacyclin			Williams et al., 1994
		$\leq$1 μM	IP	Relaxation	
		>1 μM	TP	↓ PGI_2-induced relaxation	

probably due to a difference in IP receptor expression or the post-IP receptor signaling mechanisms in VSMCs rather than the ability of the endothelium to produce NO (Kawai and Ohhashi, 1994). The pig carotid artery, rabbit mesenteric artery, and guinea pig aorta are considered IP receptor preparations with relative potencies of PGI_2 analogs in the following order: 18,19-didehydro-7,7-difluro-16S,20-dimethyl-PGI_2 (AFP-07) > 17,20-dimethylisocarbacyclin (TEI-9063) $\geq$ cicaprost > iloprost (Jones and Chan, 2001). In human uterine artery preconstricted with phenylephrine, both PGI_2 and PGE_2 are potent relaxant agonists, mainly through IP and possibly EP4 receptors. In addition, the selective IP receptor agonists cicaprost and iloprost cause dilation of human uterine artery and are ~10-fold more potent than PGI_2 (Baxter et al., 1995). AFP-07 and cicaprost are highly potent IP receptor agonists but moderately potent EP4 receptor agonists. In isolated rabbit saphenous vein, an EP4 preparation in which PGE_2 is the most potent agonist, cicaprost and AFP-07 cause relaxation by acting on EP4 and not IP receptor (Jones and Chan, 2001). Thus, PGI_2-mediated vascular relaxation may depend on the relative expression of IP and other PRs and the potency of the PGI_2 analog used to stimulate these receptors in a specific blood vessel.

PGI_2 causes vasodilation and VSM relaxation via several signaling mechanisms (Fig. 5). PGI_2/IP couples primarily to G_s to activate cAMP/PKA, causing activation of plasmalemmal and sarcoplasmic reticulum Ca-ATPases, increased Ca^{2+} extrusion and reuptake, and a decrease in $[Ca^{2+}]_i$. PGI_2/IP-induced increase in cAMP/PKA can also cause SMC relaxation by inhibiting TXA_2/$TP\alpha$ receptor-mediated signaling pathways (Fig. 5). PGI_2 could inhibit $TP\alpha$ G_q/PLCβ Ca^{2+}-dependent or $TP\alpha$ G_{12}/RhoA Ca^{2+}-independent signaling through cAMP/PKA-mediated phosphorylation of $TP\alpha$ at Ser329 within its unique C-tail domain (Wikström et al., 2008).

PGI_2 through IP receptor and in a cAMP-dependent and/or -independent manner could also cause activation of K^+ channels, including large-conductance Ca^{2+}-dependent K^+ channel (BK_{ca}, MaxiK) (Tanaka et al., 2004), small conductance SK_{Ca} (Dong et al., 1998), ATP-sensitive (K_{ATP}) (Dumas et al., 1997), voltage-gated (K_V) (Dong et al., 1998), inward-rectifier (K_{IR}) (Orie et al., 2006), and two-pore-domain K^+ channel (K2P) (Olschewski et al., 2006), leading to VSMC membrane hyperpolarization, decreased Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels, and VSMC relaxation. In guinea pig aorta, beraprost causes activation of MaxiK partly by a direct G_s protein-mediated cAMP-independent mechanism, although a cAMP-dependent/PKA-induced MaxiK phosphorylation could contribute to VSMC relaxation in this vascular bed (Tanaka et al., 2004). In isolated rat perfused lung, iloprost decreases the hypoxia-induced increase in pulmonary perfusion pressure and causes pulmonary vasorelaxation through K_{ATP} channels, K_{Ca} channels, and NO release. In the same preparation, forskolin, a cAMP activator, causes pulmonary vasorelaxation, and glibenclamide, a K_{ATP} channel blocker, decreases forskolin-induced relaxation, suggesting a link between cAMP and activation of K_{ATP} channels. In addition, glibenclamide inhibits the relaxation induced by iloprost to a greater extent than that induced by forskolin, raising the possibility of a role of cAMP in iloprost-induced K_{ATP}-mediated vascular relaxation (Dumas et al., 1997). In rabbit coronary artery, PGI_2 and iloprost activate K_{ATP} and cause vasorelaxation, but the role of cAMP in this response is not conclusive (Jackson et al., 1993). In rabbit middle cerebral artery, endogenous PGI_2 contributes to endothelium-dependent ACh relaxation through cAMP/PKA-mediated phosphorylation of BK_{Ca}, SK_{Ca}, and K_V channels, but not K_{ATP} (Dong et al., 1998). In addition, treprostinil treatment of isolated human pulmonary artery VSMCs results

in membrane hyperpolarization through a cAMP/PKA-mediated activation of two-pore domain acid-sensitive K^+ channel-1 (TASK-1), a member of K2P channels (Olschewski et al., 2006). In contrast, in rat tail artery, cicaprost induces VSMC relaxation through cAMP-independent activation of K_{IR} channel (Orie et al., 2006). Thus, the contribution of a specific K^+ channel to PGI_2-induced vascular relaxation depends on the vascular tissue and species studied and the amount and colocalization of K^+ channels with different components of the IP receptor signaling pathways in the specific vascular bed (Tanaka et al., 2004).

2. Prostacyclin Signaling Causing Smooth Muscle Contraction. Although PGI_2 is largely known as a vaso-dilator (Miller, 2006), some ex vivo studies suggest that, depending on the vessel type and/or concentration tested, PGI_2 may induce VSM contraction (Williams et al., 1994) (Table 5). In rat aorta precontracted with norepinephrine, PGI_2 and carbacyclin elicit a biphasic concentration-response curve such that lower concentrations elicit relaxation, whereas at higher concentrations, the relaxation decreases (Williams et al., 1994). High PGI_2 concentrations also cause contraction in cat (Uski et al., 1983), dog (Chapleau and White, 1979), monkey (Kawai and Ohhashi, 1994), and human cerebral arteries (Uski et al., 1983). In isolated myometrial strips from pregnant human, PGI_2 and its analogs cause either an inhibitory or a biphasic effect on contraction (Fetalvero et al., 2008). The contractile effects of PGI_2 have been attributed in part to elevation in $[Ca^{2+}]_i$ as a result of IP receptor-mediated inositol phosphate formation (Tanaka et al., 2004). In the mouse IP receptor, initial G_s-mediated PKA activation leads to phosphorylation of Ser357 in the C-terminal of the receptor, which in turn couples IP with G_i, leading to inhibition of adenylyl cyclase and G_q-mediated activation of PLC. Although the mouse IP requires PKA-induced phosphorylation for the G_s to G_q switching, human IP does not couple to G_i and can couple to G_q independent of PKA-induced phosphorylation (Stitham et al., 2007). The biphasic effect of different PGI_2 concentrations on VSMCs has also been related to difference in the dissociation constant of various PRs, the level of expression of PRs, and the specificity of the PGI_2 analog toward IP receptor. PGI_2 may directly activate EP1 or TP receptors, which would counterbalance the PGI_2/IP relaxant effect (Tanaka et al., 2004). For example, in rat aorta precontracted with nor-epinephrine, the relaxation induced by lower concentrations of PGI_2 is likely to involve IP receptors, whereas the decreased relaxation in response to higher PGI_2 concentrations is abolished by the TP receptor antagonist 7-(3-((2-((phenylamino)carbonyl)hydrazino)methyl)-7-oxabicyclo(2.2.1)hept-2-yl)-5-heptenoic acid (SQ29548), suggesting that at higher concentrations, PGI_2 binds to TP receptor (Williams et al., 1994). In addition, exogenous PGI_2 causes less relaxation in isolated human pulmonary veins than arteries precontracted with noradrenaline, probably because of activation of EP1 receptor; in the pres-

ence of the EP1 receptor antagonist 6-isopropoxy-9-oxoxanthene-2-carboxylic acid (AH6809), PGI_2-induced relaxation in the veins is similar to that in the arteries (Norel et al., 2004). In addition to EP1 and TP receptors, some PGI_2 analogs may target EP3 receptor and cause VSM contraction. Carbacyclin, but not cicaprost, acting via EP3 receptors, produces a biphasic response with initial contraction followed by relaxation in guinea pig aorta and rabbit mesenteric artery (Jones and Chan, 2001) (Table 5). The endothelium may play a role in mediating the vaso-constrictor effect of PGI_2 whereby PGI_2-induced endothelial NO release through IP receptor is counterbalanced by PGI_2-induced activation of endothelial EP1 and TP receptors, which could cause IP receptor desensitization and internalization, leading to reduced NO release and decreased vascular relaxation (Xavier et al., 2009). In aortic rings of spontaneously hypertensive rat and Wistar Kyoto rat, PGI_2 unexpectedly acts as an endothelial-derived contracting factor, probably as a result of decreased IP receptor expression and PGI_2-mediated activation of TP receptor (Gluais et al., 2005). The paradoxical contractile effects of PGI_2 could also be related to the amount of PGIS expressed in vascular tissues. PGIS gene expression in ECs seems to be augmented with aging and in hypertension. It has been shown that during endothelium-dependent contraction of spontaneously hypertensive rat aorta in response to ACh, PGI_2 production is larger than that of other prostanoids and reaches levels compatible with activation of TP receptors in VSMCs (Vanhoutte, 2011). In these rat models, inhibition of PGIS by tranylcypromine further enhances ACh-induced endothelium-dependent contraction, probably because of enhanced PGH_2 spillover, a more potent TP receptor agonist than PGI_2 (Gluais et al., 2005). The vasoconstrictor effects of PGI_2 may increase the cardiovascular risk, and further investigation of the mechanisms involved may identify new therapeutic targets in vascular disease.

3. Effects of Prostacyclin on Smooth Muscle Differentiation, Proliferation, and Migration. During vascular injury, VSMCs re-enter the cell cycle and undergo proliferation, migration toward attractants, and phenotypic change to synthetic VSMCs with decreased expression of contractile proteins and increased extracellular matrix proteins (Arehart et al., 2007). PGI_2 through IP/cAMP/PKA signaling, PKA-regulated CREB, and SMC-specific transcription factors may play a role in maintaining myometrial SMCs in a differentiated contractile phenotype by inducing the transcription of the thick filament protein myosin heavy chain, the thin filament-associated proteins α-SMA and calponin, and the gap junction protein connexin 43 (Figs. 5 and 6), an effect that is blocked by both the IP antagonist RO3244794 and PKA knockdown (Fetalvero et al., 2008). It is noteworthy that the PGI_2 signal that mediates VSMC differentiation may propagate through the blood vessel tunica media by a PGI_2-induced PGI_2 release mechanism (Fig. 5). This is supported by the observation that iloprost induces COX2 up-regulation and

VSMC differentiation directly in a single layer of cultured human aortic VSMCs as well as in an adjacent layer of cells that has not been directly exposed to iloprost. These effects are probably mediated by IP receptors, because the selective IP antagonist 4,5-dihydro-N-[4-[[4-(1-methylethoxy)phenyl]methyl]phenyl]-1H-imadazol-2-amine (CAY10441) abolishes iloprost-induced COX2 protein up-regulation. PGI_2-induced induction of COX2 and subsequent stimulation of PGI_2 synthesis and release to adjacent VSMCs may involve downstream signaling by cAMP/PKA and two other PKA-independent pathways involving stimulation of ERK1/2 MAPKs or inhibition of Akt1 (Kasza et al., 2009).

PGI_2 also exerts protective effects in the vasculature by inhibiting VSMC hypertrophy, migration, proliferation, and neointima formation (Miller, 2006) (Fig. 6). Compared with wild-type mice, IP-deficient mice show marked increase in BP and thickening of the aortic media and adventitia (Jin et al., 2005). In addition, PGIS gene transfer modulates COX2-derived PGI_2 synthesis and inhibits neointima formation in animal models of arterial injury (Kawabe et al., 2010). The PGI_2 analog ciprostene may inhibit the hypertrophic effects of TXA_2 in VSMCs by attenuating TXA_2-induced ShC/GRB2 complex formation and subsequent activation of MAPK through a cAMP-independent mechanism that could stem from TP receptor desensitization (Jones et al., 1995). In vitro, iloprost, and cicaprost inhibit migration of human aortic SMCs through cAMP-dependent activation of protein tyrosine phosphatases that counteract the actions of focal adhesion kinase and thereby cause disruption of focal adhesions (Bulin et al., 2005). PGI_2 analogs can inhibit proliferation of VSMCs by inhibiting G_1 phase to S phase cell cycle progression (Fetalvero et al., 2007) (Fig. 5). Cicaprost inhibits proliferation of cultured mouse aortic VSMCs by inhibiting the G_1-phase cyclin-dependent kinase cyclin E-cdk2, probably through an IP-receptor mediated mechanism, because the effects of cicaprost on G_1-phase progression are lost in primary aortic VSMCs from IP($-/-$) mice (Kothapalli et al., 2003). Beraprost also inhibits VSMC proliferation and causes cell cycle arrest in the G_1 phase in SMCs from canine coronary artery after balloon-induced injury by preventing the down-regulation of the cell cycle inhibitor p27^{kip1} in a cAMP-dependent manner (Ii et al., 2001). Iloprost has also been shown to modify the expression of 83 genes implicated in SMC growth and migration of human VSMCs, including 51 up-regulated genes such as VEGF and 32 down-regulated genes such as plasminogen activator inhibitor (PAI-1) (Norel, 2007).

PGI_2 signaling through PPAR receptors may play a role in modulating VSMC proliferation and/or differentiation. PGI_2/PPARδ signaling stimulates expression of inducible NO synthase and consequently inhibits VSMC proliferation (Lin et al., 2008). PGI_2/PPARδ signaling can also mediate long-term effects on VSMCs similar to those independently mediated by PGI_2/cAMP. For example, beraprost not only induces cAMP-mediated prevention of p27^{kip1}

down-regulation (Fig. 5) (Ii et al., 2001) but can also inhibit VSMC proliferation through PPARδ-mediated nuclear translocation of CREB-binding protein, which increases CRE/PPAR response element enhancer activity and downstream transactivation of p21/p27^{kip1} (Sue et al., 2009). In addition, endogenously released PGI_2 in response to laminar shear stress may act through activation of PPARα/δ to induce synthetic-to-contractile phenotypic modulation in VSMCs by increasing contractile protein marker expression and decreasing proinflammatory gene expression and the percentage of cells in S phase (Tsai et al., 2009). PGI_2, through PPARα, in addition to its effects via the IP/cAMP pathway, can also inhibit TNFα production during renal ischemia/reperfusion injury (Chen et al., 2009a). Although the effects of PGI_2-induced activation of PPARα and -δ do not require IP receptors in most cells, some studies suggest that IP receptor through a cAMP-independent mechanism may contribute to the regulation of PPARs. For example, PPARγ may partly mediate the antiproliferative effects of treprostinil in HEK-293 cells expressing IP receptor through a signaling mechanism that is dependent on IP receptor (Falcetti et al., 2007).

C. Effects of Prostacyclin on Vascular Extracellular Matrix

Extracellular matrix (ECM) is a major component of the vascular wall synthesized by vascular cells such as fibroblasts, SMCs, and inflammatory cells. ECM contains collagen and elastin fibers embedded in a viscoelastic gel of a variety of ECM proteins. ECM metabolism plays a role in the vascular remodeling associated with vascular tissue injury. In fibroblasts, PGI_2 decreases ECM accumulation and fibrosis by different mechanisms. PGI_2 suppresses connective tissue growth factor gene transcription and inhibits type I collagen synthesis (Zardi et al., 2005). In rat cardiac fibroblasts, bradykinin-stimulated PGI_2 and the PGI_2 analog beraprost both decrease cell proliferation and expression of collagen types I and III (Yu et al., 1997) (Fig. 6). Some of the effects of PGI_2 on ECM proteins are mediated through its action on matrix metalloproteinases (MMPs), which contribute to cell migration and proliferation and the pathogenesis of vascular disease. Beraprost suppresses MMP-2 and MMP-9 activities in the lung of a rat model of cigarette smoke-induced emphysema and phorbol ester-induced MMP-9 activity in cultured human mesangial cells (Chen et al., 2009b).

V. Alterations in the Prostacyclin Pathways in the Pathophysiology of Vascular Disease

Vascular tissue injury in response to various insults disturbs EC function, alters vascular homeostasis and hemostasis, and eventually leads to vascular disease. Endothelial dysfunction involves increased vascular permeability, decreased relaxation, platelet aggregation, increased coagulation, leukocyte adhesion, and VSMC proliferation.

As part of endothelial dysfunction, exaggerated endothelial activation in response to injury stimuli may occur, and the activated endothelium acquires a procoagulant, proadhesive, and proinflammatory phenotype. Some of the injury stimuli causing endothelial dysfunction/activation include ROS and reactive nitrogen species (RNS), which reduce the bioavailability of NO and PGI_2, increase TXA_2 and endothelin-1 (ET-1), and enhance the expression of adhesion molecules and leukocyte adhesion to ECs (Hata and Breyer, 2004). O_2^--induced endothelial dysfunction and altered PGI_2/TXA_2 balance have been demonstrated in atherosclerosis, hypertension, ischemia, endotoxic shock, and diabetes (Zou, 2007). The role of PGI_2 in maintaining the PGI_2/TXA_2 balance, regulation of EC angiogenesis, and control of blood coagulation and vascular inflammation supports its use in the management of certain vascular inflammatory and ischemic diseases. On the other hand, perturbation in PGI_2 metabolism as a result of naturally occurring and environmentally induced mutations may explain why PGI_2 and its analogs have shown benefits against vascular disease in some studies but not in others.

A. Prostacyclin/Thromboxane A_2 Balance

The vasodilator and antithrombotic effects of PGI_2 are opposed by the vasoconstrictor and prothrombotic effects of TXA_2, which also promotes EC apoptosis and VSMC proliferation (Wheeler-Jones, 2008). IP(−/−) mice show aggravated atherogenesis and enhanced platelet activation in response to endothelial damage, whereas TP(−/−) mice show bleeding tendency, decreased platelet aggregation, and delayed atherogenesis. Mice with deletion of both IP and TP show no difference in vascular remodeling compared with wild-type control mice supporting a cross-talk between the two opposing PGI_2 and TXA_2 signaling pathways (Kawabe et al., 2010). PGI_2 modulates platelet-vascular interactions in vivo and limits the response to TXA_2 (de Leval et al., 2004). In addition, platelet-derived endoperoxide precursors of TXA_2, such as PGH_2, can be used by endothelial PGIS to generate PGI_2. Therefore, pharmacological inhibition of TXAS in vivo allows diversion of endoperoxides to produce PGI_2 (Cheng et al., 2002).

TP agonists such as TXA_2 and its analogs can modulate PGI_2 release and cAMP production from ECs, but this mechanism is dependent on the TP agonist concentration and the exposure time. In cultured ECs, low concentrations of U46619 stimulate PLA_2 activity with dose-dependent increase in PGI_2 production, but repeated exposure to higher U46619 concentrations inhibits endothelial phosphodiesterase, leading to accumulation of cAMP and feedback inhibition of PLA_2 activity and PGI_2 production. These findings may have in vivo implications whereby physiological concentrations of endogenous TP receptor activators could increase PGI_2 production by ECs and promote vasodilation, whereas in pathological conditions associated with higher concentrations and repeated exposure to endogenous TP activators, feedback inhibition of PGI_2 production by ECs would occur and leads to further increase in vasoconstriction and vascular pathology (Nicholson et al., 1984).

Cross-talk between PGI_2 and TXA_2 may also occur at the receptor level. Similar to other GPCRs, IP receptors may associate with TP receptor to form a heterodimer. IP/TP heterodimerization and cross desensitization in a given cell or tissue may lead to functional changes that depend on the activated dimeric receptor partner (Xavier et al., 2009), the milieu of activating ligands, the cellular complement of accessory proteins, the expression levels of the individual receptors, and their propensity to heterodimerize under physiological or pathological conditions (Gluais et al., 2005). IP/TP heterodimerization may also influence the ligand binding affinity and/or potency, signal transduction, and biological response (Giguère et al., 2004). The formation of IP/TP heterodimer may limit the cellular functions of TXA_2 and promote a "PGI_2-like" cellular response, and vice versa. Activation of IP receptor in the IP/TP heterodimer may cause $TP\alpha$-mediated cAMP production. Conversely, activation of TP receptor in the TP/IP heterodimer causes loss of 25 to 30% of surface IP in cultured human aortic VSMCs and reduction in IP signaling efficiency and cAMP production. Depending on the activated dimeric partner, IP/TP heterodimerization can also modify the receptor internalization, endocytosis, and trafficking pattern of the other dimeric partner. When homologous IP receptors are stimulated by an agonist, they are internalized and undergo either rapid recycling and restoration of IP receptor at the cell surface, such as in IP receptor-transfected HEK-293 cells, or receptor sequestration, as in aortic VSMCs (Wilson et al., 2004, 2007). In contrast, homologous TP receptor internalization leads to receptor degradation in both TP receptor-transfected HEK-293 cells and aortic VSMCs. During IP-induced internalization of IP/$TP\alpha$ heterodimer, $TP\alpha$ is rapidly recycled to the cell surface in coexpressing HEK-293 cells but undergoes sequestration in aortic VSMCs, in accordance with the postendocytotic pathways of IP receptor (Wilson et al., 2007). Therefore, when IP and $TP\alpha$ are present in the same cell, a common occurrence in vascular cells, IP may limit the cellular effects of $TP\alpha$ by evoking a PGI_2-like response that counterbalances the effects of TP, by promoting sequestration of $TP\alpha$ from the cell surface and limiting $TP\alpha$-PLC signaling as in VSMCs, and/or by restoring $TP\alpha$ recycling in cells where IP recycling occurs, and thereby further promoting the formation and responsiveness of the IP/$TP\alpha$ heterodimer and its PGI_2-like signaling (Wilson et al., 2007).

Because of the opposing actions of PGI_2 and TXA_2, many aspects of cardiovascular disease have been explained by alterations in the PGI_2/TXA_2 ratio. PGI_2/TXA_2 imbalance may also explain the observed clinical association between the use of selective COX2 inhibitors and increase in cardiovascular risk. Selective inhibition of COX2 leads to decreased production of the antiatherogenic PGI_2, whereas the production of the atherogenic TXA_2, mostly COX1-dependent, remains unaffected (Kawabe et al., 2010). Se-

cretion of PGI_2, but not TXA_2 metabolite, is reduced by 75 to 80% in volunteers given anti-inflammatory doses of the selective COX2 inhibitors celecoxib (Celebrex) and rofecoxib (Vioxx) (Smith et al., 2000). The VIGOR and APPROVE clinical trials, and other retrospective and prospective studies and meta-analyses, related the use of COX2 inhibitors to increased cardiovascular risk, primarily myocardial infarction and stroke (Miller, 2006). Therefore, rofecoxib was removed from the market voluntarily by the manufacturer in 2004. In addition, the FDA has warned against marketing of valdecoxib (Bextra) because of insufficient data on cardiovascular safety. However, because the benefits of celecoxib outweigh the potential risks in properly selected and informed patients, FDA allowed the use of celecoxib and advised manufacturers to include a warning in the label highlighting the potential for increased risk of cardiovascular events.

Modulators of PGI_2 and TXA_2 production such as PGI_2 analogs and TXAS inhibitors may be useful therapeutic agents to improve the PGI_2/TXA_2 imbalance in CVD. In addition, dysregulation of IP/TP receptor heterodimerization at the receptor level may contribute to the development of CVD and understanding the mechanisms involved may lead to the development of new therapeutic targets.

B. The Prostacyclin Pathway and Blood Coagulation

In addition to its role in vascular homeostasis, PGI_2 is an essential factor in hemostasis. Studies in IP receptor knockout mice have suggested antithrombotic effects of PGI_2. IP$(-/-)$ mice are more susceptible to thrombosis than wild-type mice (Nakae et al., 2005). Although IP-deficient mice have normal bleeding time under basal conditions, a 4-h ferric chloride-induced endothelial damage in carotid arteries leads to the development of obstructive thrombi in two thirds of IP$(-/-)$ mice, whereas wild-type mice show substantially less thrombus formation. In addition, 30% of the IP-deficient mice die within 1 day as a result of bilateral occlusions of the carotid arteries and/or embolic stroke, whereas wild-type mice survive this period (Murata et al., 1997). These findings suggest that PGI_2 plays an antithrombotic role in conditions associated with platelet activation such as vessel wall injury. Studies in animal models of vascular injury and in humans have also supported antithrombotic effects of PGI_2. Intravenous infusion of PGI_2 and its analogs decreases thrombosis in dogs undergoing stent implantation in the common iliac vein (Seiji et al., 2009), in extracorporeal circuit of human patients undergoing hemofiltration (Kozek-Langenecker et al., 2003), and in patients with critical limb ischemia (Blardi et al., 2006). The mechanisms of PGI_2 effects may involve decreased expression of platelet activation markers such as platelet fibrinogen receptor and P-selectin, decreased platelet-leukocyte aggregation (Kozek-Langenecker et al., 2003), and decreased platelet granular release of serotonin (Blardi et al., 2006).

PGI_2 also exerts its antithrombotic effects by disrupting some of the complex platelet aggregation reactions that occur during injury of vascular tissues. During vascular injury, ADP and thrombin are released from adherent platelets, and platelet membrane disruption results in activation of PLA_2 and generation of TXA_2, the most potent platelet aggregator known. TXA_2 mobilizes Ca^{2+} from intracellular storage sites to trigger platelet granule release of active substances such as β-thromboglobulin, coagulation factor VIII, von Willebrand factor, fibrinogen, and ADP. In the final phase of thrombus formation, thrombin-generated fibrin stabilizes the platelet mass. Thrombin also stimulates ECs to produce PGI_2, thus creating a critical balance with TXA_2 to modulate platelet-vessel wall interaction (Jin et al., 2005; Miller, 2006). PGI_2 inhibits platelet aggregation in vivo, disperses existing circulating aggregates, and counteracts platelet activation by TXA_2 (Jin et al., 2005; Miller, 2006). PGI_2-induced inhibition of platelet aggregation involves cAMP-mediated inhibition of Ca^{2+} mobilization and granule release of various mediators such as ADP and fibrinogen, as well as inhibition of PLA_2 activity and TXA_2 production (Miller, 2006). In addition, PGI_2-induced phosphorylation of vasodilator-stimulated phosphoprotein (VASP) modulates filamentous membrane structure reorganization and decreases fibrinogen-dependent platelet cross-linking (Jin et al., 2005). Platelet-platelet aggregation is inhibited at much lower PGI_2 concentrations than platelet-collagen adhesion, enabling platelets to repair vascular wall injury while minimizing thrombus generation (Miller, 2006). Epoprostenol at nanomolar concentrations inhibits in vitro platelet aggregation and induces intraplatelet VASP phosphorylation in whole blood activated by the combination of collagen and ADP (Tamburrelli et al., 2011). It is noteworthy that earlier studies showed that low-dose aspirin failed to inhibit platelet aggregation induced by two aggregating stimuli such as epinephrine and plasma-activating factor, although it effectively inhibited platelet aggregation in response to either agent alone. The platelet aggregating effects of combined epinephrine and plasma activating factor was inhibited by the LOX inhibitor 4,5-dihydro-1-(3-(trifluoromethyl)phenyl)-1H-pyrazol-3-amine (BW755C), suggesting a cyclooxygenase-independent platelet aggregation mechanism that is likely to involve LOX (Cerletti et al., 1986).

In addition to inhibiting platelet aggregation, PGI_2 may inhibit thrombosis by inhibiting coagulation factors Va, VIIIa (Rabausch et al., 2005), tissue factor (Crutchley et al., 1992), and other proteins involved in coagulation, such as PAI-1 (Atsuta et al., 2009), von Willebrand factor, and D-dimer (Kim et al., 2009). Iloprost and cicaprost increase mRNA expression and protein amount of thrombomodulin, which binds to thrombin with high affinity, resulting in activation of protein C, which in turn proteolytically degrades factors Va and VIIIa, thus inhibiting further thrombin generation and blood coagulation (Rabausch et al., 2005).

In association with the role of IP receptor agonists in preventing thrombosis, TP receptor antagonists such as

ifetroban and sulotroban may have additive antithrombotic effects by blocking the action of TXA_2 and other endogenous TP receptor agonists such as PGH_2, PGD_2, and isoprostanes. Terutroban (S-18886) is a TP antagonist that has shown clinical benefit over aspirin as antithrombotic agent and as an effective agent in endothelial dysfunction, making it an interesting candidate drug for further clinical trials to evaluate its potential benefits in atherosclerosis (Dogné et al., 2006). Dual agents with TP antagonist/IP agonist properties may have additive or synergistic antithrombotic action compared with TP antagonist or IP agonist alone. (4-(2-(1,1-Diphenylethylsulfanyl) ethyl)-3,4-dihydro-2H-benzo(1,4)oxazin-8-yloxy)actic acid N methyl-D-glucamime salt TRA-418 is a dual-acting TP antagonist/IP agonist with K_i for binding to human platelet membranes of 0.05 and 0.43 μM for TP receptor and IP receptor, respectively. TRA-418 inhibits human platelet aggregation, fibrinogen receptor GPIIb/IIIa activation and P-selectin expression on the membrane of activated platelets. An additional advantage of TRA-418 over individual TP antagonists and IP agonists is that it has a better effect on reducing platelet-leukocyte complex formation, increasing its potential as an antithrombotic agent (Miyamoto et al., 2010).

C. The Prostacyclin Pathway and Angiogenesis

PGI_2 plays a role in angiogenesis, an important process in various physiologic processes and in pathological conditions such as cancer, chronic inflammatory conditions, and ischemic disease. PGI_2 decreases hypoxic ischemic injury by inducing neovascularization in ischemic tissues and enhances the biological effects of proangiogenic factors such as heparin growth factor and VEGF in response to ischemia (Kawabe et al., 2010) (Fig. 6). PGI_2 induces VEGF production in a human monocytic cell line and increases VEGF gene expression in isolated rat lung and rat aortic VSMCs (Pola et al., 2004). Beraprost augments hypoxia-induced VEGF mRNA expression in cultured aortic VSMCs through PKA/CREB-dependent mechanisms (Atsuta et al., 2009). The angiogenic property of PGI_2 mediated by VEGF may involve PGI_2 binding to nuclear PPAR receptors because only PGI_2 analogs that act as PPARs ligands, such as iloprost and carbacyclin, but not cicaprost, are able to induce angiogenesis in murine cornea (Pola et al., 2004). The proangiogenic effects of PGI_2 may also involve bone marrow-derived mononuclear cells and endothelial progenitor cells (EPCs), which play a role in vascular wall repair after ischemic injury. EPCs express the IP receptor, and EPC adhesion to ECM and migration are tightly regulated by PGI_2/IP receptor. In addition, transfusion of wild-type EPCs, but not IP-deficient EPCs, into a mouse model of wire-induced vascular injury promotes reendothelialization and limits vascular remodeling (Kawabe et al., 2010). PGI_2/PPARδ signaling is also an important mechanism

underlying the proangiogenic effects of EPCs (He et al., 2008).

D. Prostacyclin and Vascular Inflammation

COX inhibitors are commonly used to reduce PGI_2 production and to alleviate pain and inflammation but could adversely affect vascular function, making it important to discuss the role of PGI_2 in the inflammatory process. PGI_2 is a mediator of edema and pain in both acute and chronic inflammation (Pulichino et al., 2006). Bradykinin induces PGI_2 formation and enhances microvascular permeability and edema (Hata and Breyer, 2004). PGI_2 contributes to nociceptive pain in acute inflammation and to hyperalgesia (Smith, 2006). IP($-/-$) mice display impaired acute inflammatory response in the carrageenan-induced paw edema and acetic acid-induced writhing models (Nakae et al., 2005). PGI_2 antagonists such as RO1138452 and RO3244794 reduce pain, hyperalgesia, edema formation, and chronic joint arthritis in rats (Bley et al., 2006).

Although PGI_2 exerts a proinflammatory effect in nonallergic acute and chronic inflammation, it could have a protective effect in vascular inflammation and an immunosuppressive role on Th2-mediated allergic response. IP knockout mice demonstrate increased allergic responses and capillary permeability in the airways (Matsuoka and Narumiya, 2007), increased antigen-induced leukocyte accumulation in the lungs, increased Th2 inflammatory cytokines IL4 and IL5, and decreased Th2 anti-inflammatory IL10 (Hata and Breyer, 2004). Iloprost inhibits TNFα synthesis by leukocytes and macrophages, down-regulates MAPK in macrophages (Lo et al., 1998), and decreases tissue factor expression in monocytes (Crutchley et al., 1992; Mittag et al., 2001). Iloprost also decreases expression of $\alpha_M\beta_2$ integrin of phagocytes that has a key role in leukocyte-endothelium interactions in inflammation and thrombosis, inhibits chemotaxis, and modulates the expression of adhesion molecules on ECs, neutrophils, and monocytes (Mazzone et al., 2002). In vivo studies with iloprost support the idea that PGI_2 could control vascular inflammation by reducing leukocyte activation and migration and adhesion to ECs (Lindemann et al., 2003). It is noteworthy that atherosclerosis is considered a chronic inflammatory vascular disease, and IP-deficient mice have increased atherosclerosis and injury-induced restenosis (Fetalvero et al., 2007).

Thus, although PGI_2 is a mediator of pain in acute and chronic inflammation, it may reduce vascular and allergic inflammation. This may explain some of the side effects of IP analogs and COX inhibitors. For example, in patients with atherosclerotic ischemic injuries, intravenous administration of the IP analog iloprost causes flushing, headaches, and pain at the injection site (de Leval et al., 2004). In addition, the common use of NSAIDs to treat acute and chronic inflammation may increase the risk for asthmatic attacks in certain people (Matsuoka and Narumiya, 2007). In addition, in a mouse model of antigen-induced airway inflammation, the use of the COX2 inhibitor N-(2-

Cyclohexyloxy-4-nitrophenyl)methanesulfonamide (NS-398) is associated with decreased PGI_2 and increased Th2-mediated lung inflammation (Hata and Breyer, 2004).

E. The Prostacyclin Pathway and Ischemia/Reperfusion Injury

The PGI_2/IP pathway may have protective effects against endothelial activation associated with ischemia/reperfusion injury (Hata and Breyer, 2004). Ischemia/reperfusion injury involves "no-reflow" or failure of nutritive capillary perfusion thought to be caused by EC swelling and intravascular hemoconcentration and is probably precipitated by the initial reoxygenation-associated "reflow." Reperfusion as a prerequisite of tissue reoxygenation and elimination of toxic mediators from the ischemic tissue aggravates tissue damage, leading to impaired endothelial barrier, increased capillary permeability, leukocyte-endothelium interaction, and release of proinflammatory cytokines and ROS (Tauber et al., 2004). An imbalance of the PGI_2/TXA_2 ratio in favor of increased TXA_2 production may be involved in the pathogenesis of ischemia/reperfusion injury (Zardi et al., 2007). Administration of PGI_2 in animal models of ischemia/reperfusion injury reduces postischemic injury in myocardium, liver, pancreas, and lung. The protective effects of PGI_2 in ischemia/reperfusion injury include anti-inflammatory inhibition of neutrophil-mediated endothelial injury and reduction of endothelial permeability and edema. Examination of the microcirculatory changes in pressure-induced ischemia/reperfusion injury of hamster striated skin muscle demonstrated that 6 h of PGI_2 infusion during ischemia and early reperfusion at doses that do not cause systemic vasodilation attenuated postischemic leukocyte adhesion but did not improve the postischemic failure of capillary perfusion or reverse the increased microvascular permeability (Tauber et al., 2004). On the other hand, in a buffer-perfused rabbit lung model of reperfusion injury, short-term aerosolized PGI_2 at the beginning of ischemia suppresses the increase of the capillary filtration coefficient and progressive edema formation. In addition, the increase in pulmonary vascular resistance observed after reperfusion, mostly attributable to enhanced precapillary resistance, reflects an increase in TXA_2 production because it is suppressed by a TXA_2 receptor antagonist (Schütte et al., 2001). Thus, PGI_2 has protective effects in ischemia/reperfusion injury that may not be related to its vasodilator effects but mainly involve its anti-inflammatory effects, maintaining endothelial integrity and decreasing capillary permeability and edema.

F. Genetic Polymorphisms in the Vascular Prostacyclin System

Genetic polymorphisms in PGI_2 synthesis/activity could predispose to or protect against certain cardiovascular conditions, depending on the population studied. Several genetic polymorphic variants in COX1, COX2, PGIS, and TXAS genes have been identified, and some of them are functionally defective (Lemaitre et al., 2009). For example, a COX2 single nucleotide polymorphism involving a G-to-C substitution in the promoter region 765 base pairs before the start of the protein coding sequence, is common in the white population of the United Kingdom ($>25\%$ of healthy people carry the C allele) and is associated with reduced COX2 gene expression and PG synthesis. The COX2 C allele is also associated with reduced levels of circulating C reactive protein (Papafili et al., 2002) and decreased incidence of myocardial infarction and stroke in an Italian population but increased risk of stroke in African Americans (Lee et al., 2008; Lemaitre et al., 2009). In addition, in a largely white population study, commonly observed single nucleotide polymorphism variations in TXAS and PGIS are associated with increased risk of incident myocardial infarction (Lemaitre et al., 2009). Overall, up to six genetic variants in the *PGIS* gene have been linked to increased risk of essential hypertension, stroke, and myocardial infarction (Nakayama, 2005). However, whether the variations in the TXAS and PGIS genes affect the production of TXA_2 and PGI_2 was not measured in these studies (Lemaitre et al., 2009).

IP receptor polymorphic variants could also affect IP cell-surface expression, ligand binding. and G protein activation. A gene-mapping study of 1761 human subjects has identified and characterized 18 rare ($<2\%$ prevalence) nonsynonymous mutations in the coding region of the human IP receptor. Of these gene variants, eight were functionally defective, namely R77C, L104R, M113T, R212C, R212H, R215C, R279C, and I293N (Stitham et al., 2011). The first identified IP receptor R212H variant [an arginine-to-histidine substitution at amino acid (aa) 212] demonstrated a marked decrease in binding affinity at low pH 6.8 and abnormal activation and cAMP generation at both normal pH 7.4 and low pH (Stitham et al., 2007). Therefore, R212H polymorphism could have predictive value of disease progression in conditions associated with acidosis, such as cardiac, renal, and respiratory failure. The IP R212C variant is associated with intimal hyperplasia, atherothrombosis, increased severity of coronary artery disease and cardiovascular events. The IP mutant R212C is also functionally defective in vitro. Iloprost generates lower levels of cAMP in COS-1 cells transfected with the IP mutant R212C than in wild-type cells (Patrignani et al., 2008). L104R, M113T, and R279C were identified at highly conserved positions (100%) in the TMD region across all PRs and exhibited severely decreased ligand binding. R77C, L104R, M113T, and R215C exhibited reduced IP receptor cell-surface expression. L104R, M113T, R212C, R212H, and R279C mutants exhibited reduced cAMP production at physiological IP agonist concentrations and abnormal EC_{50}. The R77C, R215C, and I293N IP receptor mutations show a clear decrease in cAMP production at the physiological range but have normal EC_{50}. A case-control study comparing patients with dysfunctional IP mutations and decreased cAMP production and either 1) patients with silent nonsynonymous mutations with no

biochemical defect or 2) age- and risk factor-matched control subjects with no IP mutation showed that only functionally active mutations correlated with the severity of CVD (Stitham et al., 2011).

Although all patients with IP R212C polymorphism displayed impaired cAMP signaling and accelerated CVD, all but one IP R212C carrier identified to date are heterozygous for the mutation, suggesting dominant action of IP R212C over wild-type IP. Receptor homodimerization and heterodimerization experiments in cultured cells have provided insight into the dominant role of IP R212C observed in heterozygous individuals. Mutant IP R212C/wild-type IP homodimerization in coexpressing HEK-293 cells enhanced ER retention, decreased expression of wild-type IP and reduced the cAMP signaling response to cicaprost. It is noteworthy that when TPα was heterodimerized with wild-type IP in coexpressing HEK-293 cells, the response to a TXA$_2$ analog shifted from IP$_3$ to cAMP production. On the other hand, when TPα and IP R212C were heterodimerized in coexpressing cells, cAMP production was nearly abolished to the levels detected in single TPα-transfected cells. Thus, a cellular mechanism through which IP can limit TPα function is minimized in IP R212C carriers and possibly contributes to accelerated CVD in these people (Ibrahim et al., 2010). Further characterization of polymorphisms involving the PGI$_2$ metabolic pathways and the IP receptor and signaling mechanisms could identify those at higher risk of CVD and allow early targeted therapeutic intervention in these patients.

G. Modulators of the Prostacyclin Pathway in the Management of Vascular Disease

Because of the wide range of protective effects of PGI$_2$ on the vessel wall, PGI$_2$ analogs have been used clinically in such CVDs as pulmonary arterial hypertension (PAH) (Table 6). Decreased PGI$_2$ production has been implicated in the pathogenesis of PAH, an often-fatal disease that may be attributed to an abnormally elevated TXA$_2$/PGI$_2$ ratio. PGI$_2$ treatment over the long term improves quality of life and survival in patients with PAH. Three IP analogs are currently approved by the FDA for treatment of PAH: epoprostenol and treprostinil, administered by subcutaneous infusion and inhalation, and iloprost, given by inhalation (Ivy, 2010). IP analogs are also used in peripheral vascular diseases such as acute lower limb ischemia (Ruffolo et al., 2010), diabetic macro- and microangiopathy, intermittent claudication, Buerger's disease, arteriosclerosis obliterans, and Raynaud's phenomenon (Melian and Goa, 2002; de Leval et al., 2004). On the other hand, the effectiveness of IP analogs in sepsis and adult respiratory distress syndrome is controversial (Zardi et al., 2007; Afshari et al., 2010). In addition to IP analogs, "pleiotropic" releasers of endogenous PGI$_2$ may have cardiovascular protective effects. These drugs include angiotensin-converting enzyme inhibitors, statins, some β-adrenergic receptor-blockers, antiplatelet thienopyridines, some antidiabetic drugs such as metformin (Gryglewski, 2008),

and N^1-methyl-nicotinamide (Bryniarski et al., 2008). IP antagonists are being examined as potential analgesics to replace nonselective COX inhibitors, although there remains a concern for the perturbation of the TXA$_2$/PGI$_2$ balance, which could be greater with IP antagonists than with COX2 inhibitors (Jones et al., 2006).

VI. Prostacyclin Metabolism in Pregnancy-Associated Vascular Disorders

Normal pregnancy is an estrogen- and progesterone-dominated state characterized by increased cardiac output and uteroplacental blood flow, decreased uterine and systemic vascular resistance, and neovascularization of the uteroplacental vascular bed that is critical for optimum fetal growth and development (Magness et al., 2000). Because the placenta lacks innervation, locally released molecules play a key role in maintaining proper mother-to-fetus transfer of nutrients, including oxygen, glucose, and amino acids (Sobrevia et al., 2011). Vasodilators such as NO, VEGF, the vasodilator component of the renin-angiotensin system Ang-(1-7), and the kallikrein-kinin system (bradykinin) (Valdes et al., 2009) are involved in the control of uteroplacental and fetoplacental hemodynamics. Eicosanoid production as well as prostanoids and PGI$_2$ could also play a role in regulating placental vascular tone and maintaining placental blood flow and nutrient transfer to the fetus. In addition, PGI$_2$ production by the uterine artery increases 2- to 3-fold in pregnant compared with nonpregnant ewes, probably because of up-regulation of COX1 and PGIS in response to shear stress forces and hormone-dependent mechanisms (Krishnamurthy et al., 1999; Magness et al., 2000). Normotensive pregnant women are refractory to infused vasoactive agents such as angiotensin II, partly as a result of increased synthesis of the vasodilators PGI$_2$ and PGE$_2$ (Keith et al., 1993; Krishnamurthy et al., 1999; Magness et al., 2000).

In late pregnancy and during labor, PGE$_2$ and PGF$_{2\alpha}$ increase and play a role in the physiological events associated with parturition, such as membrane rupture, cervical dilation, and myometrial contraction (Reese et al., 2000). Exogenous PGE$_2$ is commonly used to induce labor, whereas inhibitors of PG synthesis such as indomethacin may have tocolytic effects (Reese et al., 2000; Polydorides et al., 2007). PGI$_2$ paradoxically increases before labor, priming the uterus for contraction by enhancing oxytocin-induced contractions and increasing the expression of contractile proteins and connexin 43 to allow myocytes to act synchronously during labor (Fetalvero et al., 2008).

Alterations in placental eicosanoid production and imbalanced PGI$_2$/TXA$_2$ ratio have been implicated in complications of pregnancy and abnormal fetal growth and development (Kuhn et al., 1990). Altered PG metabolism during pregnancy may lead to hemodynamic disturbances, impaired fetoplacental circulation, and pregnancy-related vascular disorders (Fig. 7). Deficient PGI$_2$ and PGI$_2$/TXA$_2$ imbalance may be associated with maternal disorders such

TABLE 6
Prostacyclin analogs used in clinical practice

PGI$_2$ Analog	Indications	Route & Dosage	Side Effects	Reference
Epoprostenol	PAH NYHA class III or IV	Continuous i.v. infusion	Flushing, hypotension, palpitations, rebound pulmonary hypertension upon rapid decrease of infusion rate. Abdominal pain, nausea, vomiting, diarrhea. Headache, anxiety, agitation, dizziness, paresthesia. Jaw and leg pain	FDA
	Scleroderma-associated pulmonary hypertension	Initial: 2 ng/kg/min		
		Maintenance: increase by 2 ng/kg/min every 15 min as tolerated		
Iloprost	Severe forms of PAH class III or IV	Inhalation 2.5 or 5 μg/puff 6–9 times/day (~0.37 ng/kg/day). Median inhaled dose 30 μg/day	Flushing, hypotension, palpitations. Flu, cough, hemoptysis, pneumonia, bronchospasm. Nausea, vomiting. Trismus, insomnia	FDA
	Digital ischemia of systemic sclerosis (scleroderma)	0.5–3 ng/kg/min continuous 6-h infusion for 5 days, then once every 3 weeks	Common infusion-related side effects: headache, nausea, vomiting, flushing, hypotension	Bettoni et al., 2002; Blardi et al., 2006; de Donato et al., 2006; Beirne et al., 2008
	Critical limb ischemia unsuitable for revascularization surgery (Fontaine stage III or IV)	1.5 ng/kg/min 16-h i.v. infusion daily for 7 days	Reported long-term severe events: renal failure, intracranial hemorrhage, retinal vein thrombosis	
	As adjuvant to surgery for critical limb ischemia (peri-operative) (ILAILL study)	Intra-arterial bolus of 3000 ng immediately after revascularization in the affected artery then i.v. infusion 0.5–2 ng/kg/min 6 h/day for 4–7 days		
	Abdominal aortic aneurysm as postoperative adjuvant	i.v. infusion 1 ng/kg/min for 72 h postoperatively		
Treprostinil	PAH NYHA Class II-IV	Continuous s.c./i.v. infusion	Flushing, hypotension	FDA
	Transition from Epoprostenol to reduce rate of clinical deterioration	Initial: 1.25 ng/kg/min Maintenance: increase by 1.25 to 2.5 ng/kg/min per week as tolerated	Nausea, diarrhea Headache, dizziness Rash, pruritus, infusion site pain, bleeding/bruising	
	PAH NYHA Class III	Inhalation system Initial: 3 breaths (18 μg)	Flushing, hypotension Cough, hemoptysis, pneumonia, bronchospasm	FDA
		Maintenance: increase by 3 breaths every 1–2 weeks up to 9 breaths	Nausea, throat irritation, diarrhea. Dizziness	
Beraprost	PAH NYHA classes <IV Peripheral vaso-occlusive disease: Raynaud's phenomena, Buerger disease, arteriosclerosis obliterans, intermittent claudication	Oral 20–40 μg t.i.d., increase gradually up to 180 μg/day	Usually highly tolerated. Most common side effects reported: flushing, nausea, diarrhea, headache, jaw and leg pain	Melian and Goa, 2002

NYHA, New York Heart Association; for FDA approved drugs, search at http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.

as preeclampsia (PE). In addition, fetal disorders such as intrauterine growth restriction (IUGR) and the dysmorphogenesis associated with maternal diabetes partly involve placental vascular dysfunction and PGI$_2$ deficiency.

A. Prostacyclin Metabolism in Preeclampsia

PE is a maternal disorder unique to human pregnancy characterized by hypertension, proteinuria, and occasionally edema and increased platelet aggregation in the second half of pregnancy (Reslan and Khalil, 2010). PE is also associated with a 4-fold increase in IUGR as a result of inadequate fetal blood and nutrient supply from the damaged placenta (Ojeda et al., 2008; Bujold et al., 2010). The pathogenesis of PE is unclear but may be related to insufficient spiral artery remodeling by endovascular trophoblasts resulting in impaired placental blood flow and reduced uteroplacental perfusion pressure (RUPP) (Granger

et al., 2002). Because PE is a human-specific pregnancy-associated disorder, and because numerous genetic, immunological, and environmental factors have been implicated in the pathogenesis of PE, there is no perfect animal model of the disease. However, some animal models of pregnancy-induced hypertension, including the RUPP rat model (Granger et al., 2002) and the transgenic activated renin-angiotensin-system rat model of PE (RAS-PE) (Verlohren et al., 2008), have shown some features of human PE. RUPP leads to systemic release of such antiangiogenic factors as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin, inflammatory cytokines, ROS, and hypoxia-inducible factors that could ultimately lead to generalized endotheliosis, endothelial cell dysfunction, and disturbed balance between vasodilator and vasoconstrictor systems. Reduced NO production or decreased bioavailability secondary to oxidative stress and increased ROS

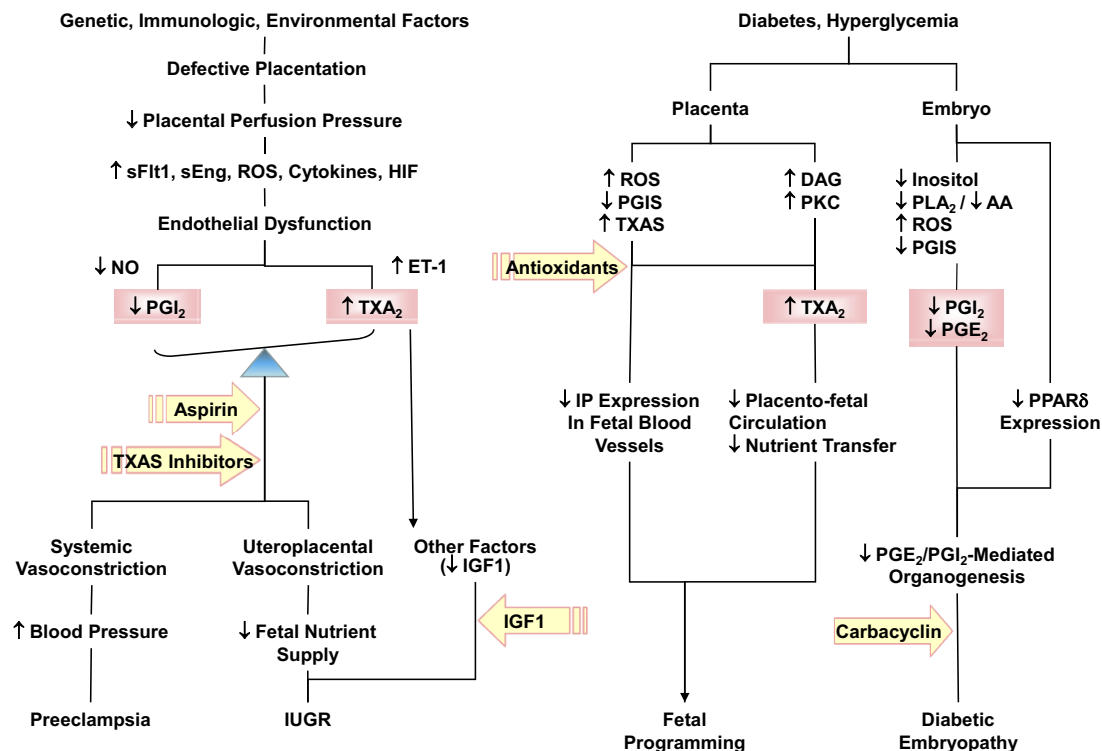## PGI$_2$ Metabolism and Pregnancy-Associated Vascular Disorders

FIG. 7. PGI$_2$ metabolism and pregnancy-associated vascular disorders. Maternal genetic, immunologic, and environmental factors cause defective placentation early in pregnancy, leading to placental hypoperfusion/hypoxia and the release of cytotoxic factors such as sFlt1, soluble endoglin, ROS, cytokines, and hypoxia-inducible factor (HIF). The circulating cytotoxic factors cause EC dysfunction, with decreased vasodilator NO and PGI$_2$ and increased vasoconstrictor ET-1 and TXA$_2$, leading to the development of preeclampsia and IUGR. Defective placentation and other maternal factors such as deficient IGF1 may also lead to IUGR independent of preeclampsia. Maternal diabetes and hyperglycemia lead to increased ROS and DAG in the placental circulation. ROS decreases PGIS activity and leads to increased TXA$_2$ synthesis over PGI$_2$, whereas increased DAG increases PKC and TXA$_2$ activity. This leads to decreased placental perfusion and nutrient transport to the developing fetus, which, together with decreased expression of IP receptors in fetal blood vessels, increases the risk for fetal programming and the development of later adult vascular disease. In addition, in maternal diabetes, hyperglycemia in the embryo leads to increased ROS that decreases PGIS activity, in addition to decreased inositol, PKC, and PLA$_2$ activity resulting in decreased AA availability and PG synthesis and net decrease in PGE$_2$ and PGI$_2$ that are necessary for organogenesis, thereby increasing the risk for diabetic embryopathy. Modulators of PGI$_2$ or TXA$_2$ production or activity could affect the course of maternal/fetal vascular disease. Low-dose aspirin and TXAS inhibitors could decrease TXA$_2$ in preeclampsia, antioxidants decrease ROS production and thereby PGIS inactivation in maternal diabetes, IGF-1 may counter TXA$_2$ overproduction in IUGR, and carbacyclin can overcome the deficient fetal PGI$_2$ activity in diabetic embryopathy.

could lead to increased vascular resistance and hypertension of pregnancy. This is supported by the observation that isolated vascular strips from RUPP rat model show reduced NO production compared with control pregnant rats (Granger et al., 2002). Pathological pregnancies could also be associated with increased vasoconstrictor mediators that reduce placental blood flow including angiotensin II, which stimulates AT1 receptor-mediated vasoconstriction; plasma asymmetric dimethylarginine, which is an endogenous inhibitor of NOS; and sFlt-1, which reduces binding of VEGF to its cell receptors (Valdes et al., 2009; Reslan and Khalil, 2010). ET-1 is also a major vasoconstrictor that has been suggested as a potential mediator of PE (George et al., 2012). It is noteworthy that ET-1 may induce the relase PGI$_2$ (Thiemermann et al., 1989), and PGI$_2$ may regulate ET-1 generation and action (Nakaki et al., 1991; Wort et al., 2000).

EC dysfunction and consequent decrease in PGI$_2$ and increase in TXA$_2$ may also play a role in the hypertension associated with PE (Walsh, 2004). Urinary excretion of

TXB$_2$ is higher in RUPP than in normal pregnant rats (Granger et al., 2002). In addition, uterine arteries from transgenic RAS-PE rats show increased contraction to phenylephrine and paradoxical contraction to high doses of ACh that is reversed by indomethacin and TXA$_2$ antagonists. The increased vasopressor response and TXA$_2$ production are associated with reduced 6-keto-PGF$_{1\alpha}$/TXB$_2$ ratio in plasma of RAS-PE compared with control pregnant rats (Verlohren et al., 2008).

Measurements of plasma and urine levels of PGI$_2$ and TXA$_2$ metabolites show decreased PGI$_2$/TXA$_2$ ratio as early as 9 to 12 weeks of gestation and throughout pregnancy in high-risk women, even before PE symptoms appear (Mills et al., 1999; Chavarría et al., 2003) (Table 7). The decreased PGI$_2$/TXA$_2$ ratio during the first trimester of pregnancies complicated later by PE may be due either to initial decrease in PGI$_2$ (Mills et al., 1999) or increase in TXA$_2$ that is followed by decreased PGI$_2$ in later trimesters (Chavarría et al., 2003). A 6-keto-PGF$_{1\alpha}$/TXB$_2$ ratio of ≤3.0 at 22 to 26 weeks of gestation may be used as a

TABLE 7
PGI$_2$ and TXA$_2$ metabolite levels in representative pregnancy and neonatal disorders vs. levels in normal pregnant or newborn subjects or experimental animals

Most studies showed consistently increased TXB$_2$, with decreased, increased, or unchanged 6-keto-PGF$_{1\alpha}$ and a net decrease in PGI$_2$/TXA$_2$ ratio.

Disorder	Subject/Animal	Sample	6-keto-PGF$_{1\alpha}$	TXB$_2$	PGI$_2$/TXA$_2$	Reference
Preeclampsia	Pregnant women	Blood (1st trimester)	545 ± 4 vs. 551 ± 28.4 pg/ml	188 ± 17 vs. 119 ± 4.8 pg/ml	3.1 ± 0.18 vs. 4.6 ± 0.12	Chavarria et al., 2003
		Blood (3rd trimester)	191 ± 9.8 vs. 288 ± 10.4 pg/ml	90 ± 4.6 vs. 72 ± 2 pg/ml	2.05 ± 0.06 vs. 3.9 ± 0.07	Chavarria et al., 2003
	Human placenta	Cytotrophoblast culture	0.38 ± 0.05 vs. 0.20 ± 0.05 pg/μg	4.33 ± 1.03 vs. 1.84 ± 0.29 pg/μg	≈ 0.087 vs. ≈ 0.108	Bowen et al., 2005
Maternal diabetes	Pregnant women with insulin-dependent diabetes	In vitro perfused placenta with labeled AA	18 vs. 23%	9.5 vs. 5%	1.9 vs. 4.5%	Kuhn et al., 1990
PPHN	Lamb, in utero induced PPHN (by partial DA constriction)	Lung tissue	≈ 7700 vs. ≈ 4200 pg/g	≈ 9000 vs. ≈ 1500 pg/g	≈ 0.8 vs. ≈ 3	Abman and Stenmark, 1992
	Piglet hypoxia-induced PPHN	Cannulated small pulmonary artery	45 ± 6 vs. 103 ± 27 pg/mg	2.3 ± 4.5 vs. 0.8 ± 0.2 pg/mg	≈ 25 vs. ≈ 200	Fike et al., 2003
ROP	Newborn piglet hypoxia-model of ROP	Retinal tissue 5 min after hypoxia	≈ 40 vs. ≈ 20 pmol/g protein	≈ 30 vs. ≈ 10 pmol/g protein	≈ 1.3 vs. ≈ 2.1	Chemtob et al., 1995
		60 min after hypoxia	≈ 30 vs. ≈ 20 pmol/g protein	≈ 50 vs. ≈ 10 pmol/g protein	≈ 0.6 vs. ≈ 2.1	Chemtob et al., 1995
HIE	Human newborn with moderate to severe HIE	CSF	168.47 vs. 86.23 ng/l	206.06 vs. 41.77 ng/l	0.82 vs. 2.06	Liu et al., 2003

marker of early diagnosis of PE in patients at high risk (Chavarría et al., 2003). It is noteworthy that cultured ECs incubated for 24 h in plasma from preeclamptic pregnancies have higher PGI$_2$ production than cells exposed to plasma from normal pregnancies. However, prolonged 72-h EC exposure to PE plasma is associated with decreased PGI$_2$ production, suggesting that chronic exposure to a circulating factor(s) is responsible for the EC dysfunction and PGI$_2$ deficiency associated with PE (Baker et al., 1996). At the placental level, the PGI$_2$/TXA$_2$ ratio is lower in villous cytotrophoblast from PE pregnancies, suggesting that the placenta may contribute to PGI$_2$/TXA$_2$ imbalance in PE (Valdes et al., 2009). In addition, cytotrophoblast cultures from PE placentas show decreased PGI$_2$/TXA$_2$ with increased TXA$_2$ (Ding et al., 2002) or both reduced PGI$_2$ and increased TXA$_2$ secretion (Ding et al., 1997).

Although COX inhibition by low dose aspirin (50–150 mg/day) could induce greater inhibitory effects on TXA$_2$ than on PGI$_2$, this approach did not prevent the development of PE, possibly because of three factors. First, in early preeclamptic pregnancy, the primary defect is probably decreased production of PGI$_2$ with secondary increase in TXA$_2$ (Mills et al., 1999). Second, aspirin may have nonselective inhibitory effects on the synthesis of both PGI$_2$ and TXA$_2$ (Ding et al., 2002). Third, factors other than PGI$_2$/TXA$_2$ imbalance contribute to the pathogenesis of PE (Reslan and Khalil, 2010). Other causes of the negative or partially positive results with aspirin in PE may be related to the timing and dosage of aspirin (Vainio et al., 2002; Walsh, 2004). Late initiation of treatment with aspirin may not prevent the development of PE, because placental implantation is already established by week 18 of gestation (Vainio et al., 2002). In addition, aspirin at 50 to 60 mg/day is effective in inhibiting TXA$_2$ production by platelets but

not that by placental trophoblasts or maternal leukocytes (Walsh, 2004). Early use of aspirin at 12 to 14 weeks of gestation in high-risk women may decrease the risk of PE to 4.7% compared with 23.3% in patients not receiving aspirin (Vainio et al., 2002). In addition, aspirin at the higher end of the dose range, 80 to 150 mg/day, could be more effective, although it may carry the risk of more side effects to both mother and fetus (Walsh, 2004; Duley et al., 2007). In the large CLASP trial (Collaborative Low-Dose Aspirin Study in Pregnancy, 1994), aspirin used at a dose of 60 mg starting at a mean gestational age of 18 weeks did not provide prophylaxis against PE. However, two recent meta-analysis showed that low-dose aspirin started at 12 to 16 weeks of gestation or earlier in high-risk women reduced the risk of development of PE (Duley et al., 2007; Bujold et al., 2010). In addition, there was a greater reduction in the risk of PE in trials using higher doses of aspirin (>75 mg) compared with trials using aspirin dose of 75 mg or less, although no trials made a direct comparison of different doses of aspirin (Duley et al., 2007).

In search of alternatives to the inhibitory effects of aspirin on the synthesis of both TXA$_2$ and PGI$_2$, studies have examined the potential use of selective TXAS inhibitors or specific IP analogs. Selective TXAS inhibitors such as pirmagrel and ozagrel have shown promising results in bovine animal models and in humans with PE; however, these findings were not followed by larger randomized clinical trials (Keith et al., 1993; Ding et al., 2002). The use of IP analogs in prophylaxis or treatment of PE has been less studied. In a hypertensive pregnant rat model induced by the NO synthase inhibitor N^ω-nitro-L-arginine methyl ester, iloprost partially reversed the hypertension and cicaprost decreased BP to control levels, but their effects on other elements of PE such as thrombocytopenia, protein-

uria, and tissue ischemia were unclear (Zlatnik et al., 1999). Thus, low-dose aspirin may have moderate benefits in preventing PE in patients at high risk, whereas TXAS inhibitors and PGI_2 analogs still need further study.

Although research into the potential use of TP antagonists in such CVDs as myocardial infarction and stroke has shown promising results, little information is available regarding the potential use of TP antagonists in PE and other pregnancy-associated vascular disorders in which TXA_2 is implicated. TP antagonists have the advantage of blocking the effects of other TP receptor activators such as isoprostanes, which are increased in PE, but they lack the additive effects of TXAS inhibitors, which increase PGI_2 production as a result of PGH_2 accumulation (Dogné et al., 2006). Dual-acting TXAS inhibitors/TP antagonists have entered into clinical trials for potential use in CVD and should be evaluated for their potential benefit in PE. Dual-acting agents having PGI_2 agonist/TXA_2 antagonist or TXAS inhibitor properties can also be more effective in PE than individual agents.

B. Prostacyclin Metabolism and Intrauterine Growth Restriction

IUGR is defined as a birth weight in less than the 10th percentile compared with the reference weight value for the current gestational age and is a major cause of premature delivery and fetal mortality (Karowicz-Bilinska et al., 2007). In the absence of fetal genetic abnormalities, IUGR is often ascribed to placental insufficiency (Wareing et al., 2006), because it is often associated with extensive atherosclerotic and thrombotic lesions in the placental arteries and with placental infarction (Stuart et al., 1981). Placental oxidative stress, defective trophoblast invasion of arterioles, and RUPP similar to that seen in PE may be the underlying pathogenic mechanisms of both idiopathic IUGR and PE/IUGR pregnancies (Figueroa and Maulik, 2006; Karowicz-Bilinska et al., 2007).

In addition to the potential role of decreased NO and increased ET-1, the role of PGI_2/TXA_2 metabolism in the pathologic processes leading to IUGR has been examined (Hophy et al., 1996). Studies in rat IUGR model induced by occlusion of uterine arteries during late pregnancy have shown increased maternal urinary levels of both 6-keto-$PGF_{1\alpha}$ and TXB_2, but the elevation of both prostanoids seemed to originate from the uterus and not the placenta, which would lead to increased maternal systemic PGI_2 levels but may not translate into similar levels or effects in the placental circulation (Hophy et al., 1996). In humans, PGI_2 production was decreased in umbilical cord arteries of a small cohort of IUGR newborns born to mothers with chronic placental insufficiency and was related to chronic hypertension and PE or to unidentified cause (Stuart et al., 1981). However, in vitro, human placentas of severe IUGR pregnancies without hypertension produced similar or lower quanti-

ties of both PGI_2 and TXA_2 compared with controls, with no reduction in PGI_2/TXA_2 ratio (Sorem and Siler-Khodr, 1995). Some studies suggest that placental insufficiency in IUGR may be related to increased TXA_2 production secondary to tissue hypoxia. TXA_2 limits biochemical and morphologic differentiation and promotes apoptosis in cultured human villous trophoblasts exposed to hypoxia (Yusuf et al., 2001). In addition, in chorionic vessels isolated from IUGR placentas and examined at low oxygen levels, the TXA_2 agonist U46619 causes greater venous contraction and no change in arterial contraction compared with vessels from normal placentas exposed to the same oxygen concentration. The venoconstriction in response to hypoxia and increased TXA_2 production may partly explain the increased fetoplacental vascular resistance associated with IUGR (Wareing et al., 2006). In contrast to the hypoxia-induced increase in TXA_2 activity in IUGR, insulin-like growth factor-1 (IGF-1), which regulates cell proliferation, differentiation, and apoptotic modulation, shows protective effects and dose-dependent inhibition of TXA_2 release in normal and IUGR placentas. Mutations of the IGF-1 molecule and receptor genes and decreased IGF-1 levels have been demonstrated in placentas, umbilical cord blood, and decidual explant cultures from human pregnancies complicated by IUGR. In a study of human IUGR placentas, IGF-1 caused dose-dependent inhibition of TXA_2 production, three of five placentas demonstrating favorable increase in PGI_2/TXA_2 ratio in response to lower doses of IGF-1. The two IUGR placentas that showed no TXA_2 response to IGF-1 had higher PGI_2/TXA_2 ratios before adding IGF-1, suggesting insensitivity that was probably due to prolonged in vivo exposure to IGF-1 as a compensatory mechanism to IUGR (Sorem and Siler-Khodr, 1997).

Maternal therapeutic interventions for IUGR include oxygen therapy, nutritional, and vitamin supplementation. Exogenous NO donors and low-dose aspirin have also been tested (Figueroa and Maulik, 2006). In a meta-analysis of 38 studies on the use of aspirin in pregnancies at high risk of fetal loss, PE, or IUGR, there was no significant change in the number of small-for-gestational-age infants, although offspring of women treated with aspirin were slightly heavier than those of women treated with placebo. In addition, there was little or no change in the risk of perinatal mortality but a decreased risk of preterm delivery (Kozer et al., 2003). Earlier initiation of therapy before 17 weeks of gestation and higher aspirin dose may provide more effective prophylaxis in pregnancies at high risk of IUGR (Figueroa and Maulik, 2006). Thus, whether PGI_2 deficiency contributes to IUGR is unclear, but excess TXA_2 in association with hypoxia seems to be a major contributor to placental insufficiency in IUGR. No major clinical trials studied the use of aspirin in IUGR other than that associated with PE, and the effects of low-dose aspirin in prophylaxis and prevention of idiopathic IUGR or IUGR

secondary to conditions other than PE needs to be further studied.

C. Prostacyclin Metabolism, Vasculopathic Diabetic Embryopathy, and Fetal Programming

Maternal diabetes is often associated with disrupted fetal development, teratogenesis, neural tube defects, and cardiac anomalies and could promote fetal programming and development of adulthood hypertension and metabolic syndrome. Placentas from diabetic pregnancies (i.e., pregnancy in women who have diabetes before pregnancy) show microangiopathy, increased fibrosis, and SMC proliferation (Jawerbaum et al., 1998). Therefore, in maternal diabetes, there is reduced placental blood flow, altered nutrient transfer, placental hypoxia, oxidative and nitrative stress, and hyperglycemia-induced changes in placental and fetal circulations, any of which could lead to congenital anomalies, and fetal programming of vascular disease (Kuhn et al., 1990; Jansson and Powell, 2007).

Several markers of endothelial dysfunction have been reported in gestational diabetes, including increased levels of ROS, RNS, and asymmetric dimethylarginine levels in maternal plasma. In addition, abnormal transport and metabolism of L-arginine and the NO pathway in the human placental endothelium have been implicated in the pathogenesis of placental dysfunction in gestational diabetes (Sobrevia et al., 2011). Disturbances in prostanoid metabolism have also been suggested to play a role in the placental dysfunction associated with diabetes during pregnancy. PGI_2/TXA_2 ratio is markedly decreased in both maternal and fetal effluents of in vitro perfused human placentas from insulin-dependent diabetic pregnancies (Kuhn et al., 1990). In addition, animal models of diabetes demonstrate reduced placental blood flow, decreased PGI_2, and increased TXA_2 production in both fetal and maternal circulations (Kuhn et al., 1990). Placental conversion of labeled AA into TXB_2 is higher and 6-keto-$PGF_{1\alpha}$ production is lower in placentas from late gestational diabetic rats compared with control rats (Jawerbaum et al., 1998) (Table 7). Hyperglycemia also causes disturbance in the PGI_2/TXA_2 ratio in blood vessels of diabetic mothers and in the maternofetal circulation, partly by increasing the levels of diacylglycerol (DAG), leading to increased PKC activity and TXA_2 production. Elevated glucose also increases ROS, which inactivates PGIS but not the ROS-resistant TXAS, resulting in further decrease in PGI_2/TXA_2 ratio in placental vessels (Jawerbaum et al., 1998) (Fig. 7).

In addition to the importance of sufficient placental blood flow for fetal growth, adequate transfer of AA and eicosanoid across the placenta is critical for normal fetal development, membrane biosynthesis, energy needs, and synthesis of precursors of signaling molecules (Kurtz et al., 2010). Alterations in AA and PGI_2 metabolism and transfer across the maternofetal circulation in placentas of diabetic pregnancies may contribute to abnormal embryo organogenesis (Kuhn et al., 1990). Hyperglycemia causes alteration in lipid signaling in the embryo, leading to decreased inositol, AA, PGI_2, PGE_2, and PPARδ expression and, consequently, abnormal embryo organogenesis in early pregnancy (Eriksson et al., 2003). In addition, IP receptor expression is reduced by 50% in the aorta of rat offspring that were exposed in utero to maternal diabetes and later developed adult hypertension (Duong Van Huyen et al., 2010) (Fig. 7). PGI_2 analogs improve both placental and embryonic function in animal models of maternal diabetes. Carbacyclin improves lipid catabolism and reduces lipid peroxidation in diabetic rat placentas by increasing PPARδ and acyl-CoA oxidase expression (Kurtz et al., 2010). In addition, carbacyclin acting through PPARδ decreases the incidence of neural tube defects by up-regulating phospholipids and PGE_2 in diabetic rat embryos to levels similar to those observed in control embryos (Higa et al., 2007).

Thus, deficient PGI_2 activity in placentas of diabetic pregnancies may alter placental blood flow and nutrient transfer to the fetus and thereby contribute to fetal programming and development of adulthood vascular disease. Further elucidation of the role of decreased PGI_2 and IP receptor in the altered placental blood flow and fetal nutrient transport in diabetic pregnancies should enhance our understanding of the mechanisms involved in diabetic embryopathy, fetal programming, and the origin of adult vascular disease and thereby provide novel approaches for early intervention.

VII. Prostacyclin Metabolism and Vascular Disorders in the Newborn

Placental PGI_2 increases sharply between 6 and 12 weeks of pregnancy, the period of fetal organogenesis, and is derived mainly from vascular tissue and to a lesser extent from trophoblasts (Walsh, 2004). Later in pregnancy, circulating levels of PGI_2 and PGE_2 increase gradually toward term, mainly because of increased production by the placenta (Wright et al., 2001). Because the lung is a major site of PG catabolism and pulmonary blood flow is very low in the fetal circulation, the high circulating levels of PGE_2 may also be due to its low rate of catabolism. PGs are important for maintaining the patency of the fetal ductus arteriosus (Smith, 1998) and the autoregulation of blood flow in the brain and the eyes of the growing fetus (Hardy et al., 1997). After birth, closure of the ductus arteriosus and the drop in pulmonary vascular resistance lead to separation of the pulmonary and systemic circulation. These vascular adaptive mechanisms may be influenced by changes in circulating levels of the vasodilator PGI_2 and PGE_2 after birth. In a full-term infant, PGE_2 and PGI_2 fall gradually after birth because of the absence of placental production and their increased catabolism in the now-functioning lungs (Schneider and Moore, 2006). However, prematurely born infants lack complete lung maturity and therefore have higher levels of circulating PGE_2 and are more exposed to its vasodilator effects. The pre-

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mature infant ductus arteriosus, and cerebral and retinal vessels are also more sensitive than those of term infants to the vasodilator effects of PGI_2 and PGE_2 because of incomplete developmental changes in vascular PRs and post-PR signaling mechanisms (Wright et al., 2001). Alterations in PGI_2 metabolism may contribute to neonatal disorders or neurodevelopmental disabilities especially in premature infants. PGI_2 excess in premature infants may contribute to patent ductus arteriosus and intraventricular hemorrhage, whereas PGI_2 deficiency in newborns with perinatal hypoxia or sepsis may cause PPHN. PGI_2 metabolism may also be altered in retinopathy of prematurity (ROP) and hypoxic ischemic encephalopathy of the newborn (Fig. 8).

A. Prostacyclin and Patent Ductus Arteriosus

The DA is a fetal vessel that shunts blood between the pulmonary artery and the aorta, and normally constricts within 24 to 48 h after birth. Failure of postnatal closure or PDA is inversely related to gestational age and reaches 50% in extremely-low-birth-weight infants. PDA results in a "left-to-right" shunt that can lead to pulmonary edema, respiratory problems, and chronic lung disease (Hamrick and Hansmann, 2010). On the other hand, maintaining the patency of DA at birth is critical for survival of infants with DA-dependent congenital heart disease until surgical correction of the congenital anomaly (Leonhardt et al., 2003). The increased incidence of PDA in premature infants is due to birth before the normal adaptations in the
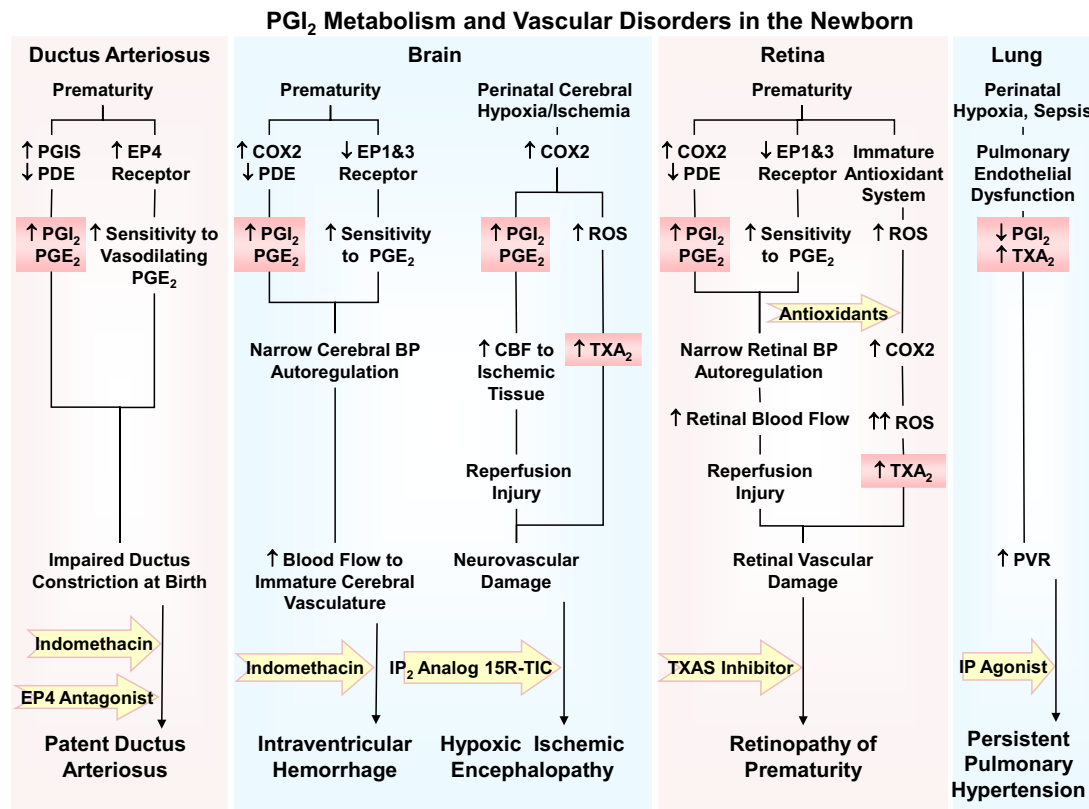
FIG. 8. PGI_2 metabolism and vascular disorders in premature and full-term newborn. In the premature newborn, PRs and endogenous modulators of PG metabolic pathways are not fully developed and thereby predispose to certain neonatal vascular disorders. In ductus arteriosus (DA) of premature newborn, the increased PGIS, decreased phosphodiesterase (PDE) and cAMP metabolism, and increased vasodilator EP4 receptor, together with decreased PGE_2 catabolism in the lung, lead to increased PGI_2 and PGE_2 availability and vasodilator sensitivity, impaired DA constriction at birth, and patent DA. In the brain, prematurity is associated with increased COX2, decreased PDE, and decreased vasoconstrictor EP1 and EP3 receptors, which, together with decreased PGE_2 catabolism in the lung, lead to increased PGI_2 and PGE_2 production and sensitivity in the cerebral circulation, narrow cerebral BP autoregulation, and increased blood flow to immature cerebral vasculature and result in intraventricular hemorrhage. In addition, in the brain, perinatal cerebral hypoxia and ischemia increase COX2 expression and PGI_2 and PGE_2 production, leading to increased CBF to ischemic tissue, reperfusion injury, neurovascular cell damage, and hypoxic ischemic encephalopathy. Increased COX2 activity also increases ROS that favors increased TXAS activity over PGIS, leading to increased TXA_2 production and further increase in neurovascular cell injury. In the retina, similar to the brain, prematurity could affect the PG pathway with increased COX2, decreased PDE, decreased vasoconstrictor EP1 and EP3 receptors, increased PGI_2 and PGE_2 production and vascular sensitivity to vasodilating PGE_2, resulting in narrow retinal BP autoregulation, and reperfusion injury, leading to retinopathy of prematurity. Because of the immature antioxidant system in the premature retina, the increased oxygen saturation accompanying the increase in retinal blood flow results in increased ROS that leads to increased TXA_2 synthesis, and further retinal vascular damage and aggravation of retinopathy of prematurity. In an ill newborn, perinatal hypoxia or sepsis causes pulmonary vascular damage and endothelial dysfunction, leading to deficient PGI_2 and increased TXA_2 production in pulmonary vessels, increased PVR, and PPHN. Modulators of PG synthesis/activity such as indomethacin and EP4 antagonist inhibit the increase in the vasodilator PGI_2 and PGE_2 production and activity in PDA. Indomethacin could decrease the increased cerebral production of PGI_2 and PGE_2, contributing to intraventricular hemorrhage. A specific IP2 analog 15R-TIC is cytoprotective in hypoxic ischemic encephalopathy. Antioxidants and TXAS inhibitors decrease ROS and TXA_2 in reperfusion injuries of the neonatal retina. IP receptor agonists can compensate for the deficient PGI_2 activity in PPHN.

DA take effect toward term to promote postnatal DA constriction (Smith, 1998). PDA may occur in a premature infant as a result of failure of DA intimal cushion formation, a process involving NO-mediated fibronectin synthesis and activation of EP4 receptor, which in turn promotes production of hyaluronic acid, an ECM component used by DA SMCs to migrate inward (Hamrick and Hansmann, 2010). Other theories suggest prematurity-associated reduction in sensitivity to oxygen-induced closure of DA at birth (Hamrick and Hansmann, 2010) or increased DA sensitivity to vasodilator PGs, which maintain patency of fetal DA (Wright et al., 2001; Leonhardt et al., 2003).

Although DA generates more PGI_2 than PGE, PGE_2 is the main PG that maintains patency of fetal DA (Wright et al., 2001). Comparing the effects of EP and IP analogs on DA from rabbit fetus showed that EP4 followed by IP receptor mediates dilation of DA precontracted with indomethacin or potassium. Both PGE_2 and cicaprost dilate DA, but cicaprost is 100-fold less potent (Smith et al., 1994). Toward term, there is a gradual decrease in DA total EP receptors (Bhattacharya et al., 1999), relative increase in EP3 receptors, and a decrease in EP4 receptors, leading to decreased sensitivity to the dilator action of PGE_2 (Fan et al., 2010). In addition, in term infants, PGI_2 availability in DA decreases as a result of increased local phosphodiesterase activity and decreased local PGIS activity as a result of increased oxygen tension after birth (Hamrick and Hansmann, 2010). Compared with term infants, premature infant DA has more total EP receptors (Bhattacharya et al., 1999), increased sensitivity to vasodilator PGE_2 as a result of up-regulation of EP4 receptor, increased PGI_2 synthesis, decreased phosphodiesterase, and increased cAMP availability, which, together with the increased levels of PGE_2 caused by decreased catabolism in the immature lung, will impair DA constriction (Fig. 8).

PGE_1 has the same affinity as PGE_2 for EP4 receptor and one-third the affinity of PGI_2 for IP receptor and has been used to keep a DA open after birth (Leonhardt et al., 2003). On the other hand, indomethacin has been used to decrease the synthesis of the vasodilator PGI_2 and PGE_2 and close PDA in prematurely born infants (Smith, 1998). Antenatal administration of indomethacin to pregnant women is paradoxically associated with increased risk of PDA in the newborn (Smith, 1998). This has been explained by the experimental observation that exposure of fetal sheep DA to indomethacin in utero is associated with reduced blood flow through the vasa vasorum, leading to hypoxia and death of medial DA SMCs and impaired constriction after birth (Goldbarg et al., 2002). The efficacy of indomethacin in closing PDA depends on gestational age, being less effective in extremely preterm infants, probably because of decreased synthesis of PGE_2 and its activation of EP4 receptors, leading to failure of intimal cushion formation and immature DA development (Yokoyama et al., 2006; Hamrick and Hansmann, 2010).

In addition to its use as a treatment option, prophylactic intravenous indomethacin administered to premature infants within the first few hours after birth may decrease the risk of developing PDA. A systematic review of 2872 preterm infants showed that prophylactic indomethacin reduces the incidence of symptomatic PDA and the need for surgical closure. Indomethacin showed few side effects except for mild transient renal impairment (Fowlie et al., 2010). Ibuprofen is as effective as indomethacin in closing PDA with less risk of the necrotizing enterocolitis and transient renal insufficiency associated with indomethacin. The rate of PDA closure with both indomethacin and ibuprofen reaches 60 to 80% in mixed populations of premature infants aged between 24 and 32 gestational weeks (Hamrick and Hansmann, 2010). Therefore, ibuprofen, either orogastric or intravenous, is currently the drug of choice (Ohlsson et al., 2010). It has been proposed that targeting the specific PRs in DA would be more effective and less toxic than COX inhibitors. EP4 receptor antagonists would block the dilator effect of PGE_2, reduce the potentiation of the effects of PGI_2 by PGE_2, and preserve vasoconstrictor effects of TP and EP3 receptors. However, EP4 and IP receptors are expressed in many tissues, and their blockade may shift the prostanoid balance toward activation of vasoconstrictor EP1 and EP3 receptors and cause more adverse effects than COX inhibitors (Smith, 1998).

B. Prostacyclin Metabolism and Persistent Pulmonary Hypertension of the Newborn

Factors interfering with the ventilatory and circulatory changes that occur normally at birth may prevent the postnatal decrease in pulmonary vascular resistance (PVR) leading to PPHN and consequently persistent right-to-left shunting across the DA and/or foramen ovale and persistent fetal circulation (Ostrea et al., 2006). PPHN is a serious neonatal disease characterized by severe hypoxemia shortly after birth in the absence of cyanotic congenital heart disease. PPHN can be primary or secondary to antenatal or immediate postnatal hypoxia caused by many conditions, including neonatal asphyxia and congenital diaphragmatic hernia (Ostrea et al., 2006). The pathogenesis of PPHN may be hypoxia, inflammation, and/or mechanical strain on the pulmonary vessels as a result of increased blood flow to the lungs. Hypoxia causes EC dysfunction, an imbalance between the vasodilators NO and PGI_2 and the vasoconstrictors ET-1 and TXA_2, and VSMC phenotypic change from a contractile to matrix secreting and proliferative phenotype. Inflammation, mainly in the form of sepsis or meconium aspiration, triggers increases in ET-1 and TXA_2 from pulmonary ECs and downstream signaling pathways favoring contraction and VSMC proliferation (Dakshinamurti, 2005). Mechanical injury to the pulmonary vessels may occur after birth as a result of obstruction of cardiac outflow, such as in congenital coarctation of the aorta (Dakshinamurti, 2005) or in utero as a result of fetal DA constriction, leading to increased blood flow to the lung, pulmonary vascular remodeling, and PPHN (Leffler et al., 1984). Prenatal ligation of the DA in fetal lambs

produces the same hemodynamic and structural features of PPHN (Alano et al., 2001). In humans, in utero DA constriction may occur as a result of maternal use of NSAIDs such as indomethacin, which decreases the production of the vasodilator PGI_2 and PGE_2 (Vermillion et al., 1997). The use of indomethacin as a tocolytic agent could cause DA constriction, especially at or later than 31 weeks of gestation, and is therefore restricted to pregnancies <32 weeks (Vermillion et al., 1997; Haas et al., 2009).

During normal transition to extrauterine life, ventilatory changes, including lung expansion and increased oxygen tension, and circulatory changes induced by the release of vasodilator PGs and NO contribute to the fall in PVR. Disturbances in these two physiological mechanisms result in persistent increase in PVR and PPHN. PGI_2 plays a role in pulmonary vasodilation at birth, and its release is triggered by rhythmic distention of the lungs and not by increased oxygenation (Velvis et al., 1991; Ostrea et al., 2006). The levels of 6-keto-$PGF_{1\alpha}$ are higher in lung biopsies obtained from newborn lambs after cesarean section than in biopsies taken in utero just before delivery (Abman and Stenmark, 1992). PGI_2 also contributes to adequate lung expansion and ventilation in the transitional period from fetal to neonatal life by increasing stretch-induced surfactant secretion by type II alveolar epithelial cells (Rose et al., 1999). As has been shown in adult PAH, altered PGI_2/TXA_2 balance may contribute to the pathogenesis of PPHN. In neonatal calves, chronic hypobaric hypoxia induces severe PPHN accompanied by decreased PGI_2 synthesis in pulmonary arteries from the PPHN group and in cultured ECs derived from these arteries (Geraci et al., 1999). In lung tissue from a lamb model of PPHN induced by surgical ligation of fetal DA, TXA_2 content, as measured by its metabolite TXB_2, was 6-fold higher than in control tissues (Abman and Stenmark, 1992). In addition, in a hypoxia-induced model of PPHN in newborn piglets, TXA_2 is increased and PGI_2 is decreased in pulmonary arteries from the hypoxic piglets compared with control piglets. In addition, in this model, PGIS is reduced, TXAS and COX2 are unaltered, but COX1 is unexpectedly reduced, suggesting that the increased TXA_2 is probably mediated by COX2 (Fike et al., 2003). Transgenic mice overexpressing PGIS in pulmonary epithelial cells (Tg+) and exposed to chronic hypobaric hypoxia have lower PVR than do Tg− mice. In addition, histologic examination of the lungs revealed nearly normal pulmonary arteriolar vessels in PGIS Tg+ mice but hypertrophy of the vessel wall in PGIS Tg− mice (Geraci et al., 1999). These observations support the notion that PGI_2 may play a role in modifying the pulmonary vascular response to chronic hypoxia.

Management of PPHN includes several approaches and pharmacologic measures to increase pulmonary vasodilation and decrease PVR (Ostrea et al., 2006). Inhaled NO is a standard pharmacologic therapy for PPHN, but its beneficial effects are transient or minimal in 40% of infants. PGI_2 and IP analogs administered intravenously or by inhalation have been used in term and preterm infants with PPHN and are effective in infants not responding to inhaled NO. In a small group of infants with PPHN, the intravenous PGI_2 dose was titrated according to the response. Although an initial PGI_2 dose at 20 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} failed to reduce pulmonary arterial pressure or systemic BP, PGI_2 at 60 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} decreased pulmonary arterial pressure within 4 to 12 h and improved oxygenation at a median time of 87 h (Eronen et al., 1997). Because of the cost of inhaled NO, some centers have used inhaled PGI_2 (epoprostenol) and iloprost. Clinical trials and case reports supported the use of inhaled PGI_2 or iloprost for treating PPHN (Bindl et al., 1994; Soditt et al., 1997; Weinberger et al., 2001; Chotigeat and Jaratwashirakul, 2007). Disadvantages of inhaled PGI_2 include the short duration of action (Chotigeat and Jaratwashirakul, 2007), absence of an ideal ventilator delivery system (Ivy, 2010), and the high pH of epoprostenol solution. A major advantage of inhaled PGI_2, in addition to the drug efficacy, is that higher doses can be reached without the drop in systemic BP associated with the intravenous route (Soditt et al., 1997). Human and experimental studies have also shown synergistic effects of PGI_2 in combination with PDE inhibitors (Weinberger et al., 2001), inhaled NO (Kelly et al., 2002), milrinone (Lakshminrusimha et al., 2009), and Rho-kinase inhibitors (Rhodes et al., 2009), and the usefulness of these combined approaches in PPHN warrants further study.

C. Prostacyclin Metabolism and Cerebral Intraventricular Hemorrhage of the Newborn

Intraventricular hemorrhage (IVH) is bleeding into the microvascular tissue lining the brain ventricles, observed in 25% of very-low-birth-weight preterm infants. Severe IVH is associated with high mortality, neurodevelopmental disability, mental retardation, and cerebral palsy. The pathologic features of IVH involve multiple genetic and environmental factors that affect cerebral blood flow (CBF), oxidative stress, angiogenesis, inflammation, and coagulation (McCrea and Ment, 2008).

CBF in the newborn is autoregulated over a more narrow range of BP than in adults, and therefore hypoxemia and changes in systemic BP lead to marked fluctuations in CBF (Hardy et al., 1997). PGs play a role in determining CBF (Wright et al., 2001). PGE_2 and $PGF_{2\alpha}$ induce cerebral vasoconstriction in adults, but in the newborn, $PGF_{2\alpha}$ exerts minimal constriction, and PGE_2 causes cerebral vasodilation as a result of decreased density of vasoconstrictor FP and EP (mainly EP1) receptors (Li et al., 1997). On the other hand, the effects of PGI_2 on cerebral vessels do not change with age, thus shifting the balance of prostanoid action toward vasodilation (Wright et al., 2001). Although excess dilator PGs would maintain brain perfusion in a full-term newborn, especially during labor, in preterm newborns, they could impair cerebral vasoconstriction required at the upper limit of CBF autoregulation, leading to hemorrhage into the immature germinal matrix microvas-

culature and IVH (Hardy et al., 1997). Experimental studies support that PGI_2 contributes to the increase in CBF under conditions of hypovolemia (Leffler et al., 1986). In newborn beagle pups, induction of hemorrhagic hypovolemia followed by rapid volume re-expansion is associated with neonatal IVH. Acute hypercapnia or hypertension may be the triggering factors for IVH in this model and likewise in critically ill preterm infant (Ment et al., 1984).

Indomethacin inhibits the production of vasodilator PGE_2 and PGI_2 and prevents IVH in the newborn beagle pup model by lowering baseline CBF and blunting the BP changes in response to hemorrhagic hypovolemia/volume re-expansion insult. In addition, ethamsylate, a nonsteroidal drug that reduces capillary bleeding time, prevents IVH in the newborn beagle pup by inhibiting PGIS and TXAS and decreasing PG synthesis (Ment et al., 1984). In humans, indomethacin prophylaxis given to premature infants on day 1 of life reduces the incidence of severe IVH. However, a meta-analysis found no effect of indomethacin on mortality or long-term neurodevelopmental outcomes on follow-up at 18 to 36 months of age (Fowlie et al., 2010). Two major clinical trials studied the use of ethamsylate for prophylaxis against IVH (Benson et al., 1986; EC Ethamsylate Trial Group, 1994). The first trial in 360 preterm newborns showed a reduction in incidence of IVH, especially the severe form. The second trial, in which 334 premature infants received ethamsylate in the first 4 h after birth, showed no difference in incidence of IVH compared with placebo group. In both trials, long-term patient follow-up showed no benefits in neurodevelopmental outcomes and neuromotor or cognitive function (Elbourne et al., 2001; Schulte et al., 2005). Failure of indomethacin and ethamsylate to improve the IVH global outcome is probably attributable to the fact that although COX inhibition may prevent the initial increase in CBF causing IVH, it could lead to harmful neuronal effects. For instance, COX inhibition could decrease the production of PGE_2, which plays a neuroprotective role via nuclear EP2 receptors (Wright et al., 2001), and PGI_2, which has shown neuroprotective effects in various stroke models, leading to deleterious effects on the neurons of preterm infant independent of cerebral perfusion (Harding et al., 2007). Genetic factors may also explain the lack of long-term neurodevelopmental benefits of COX inhibition in some patients with IVH. For example, prematurely born subjects with COX2 C-765 polymorphism and low COX2 have decreased cognitive performance at ages 2 and 5.5 years compared with the G allele peers. Therefore, patients with the C allele would show different responses to indomethacin compared with the G-allele patients (Harding et al., 2007).

D. Prostacyclin Metabolism and Vasculopathic Retinopathy of Prematurity

Premature infants lack complete retinal vascularization, especially in the periphery, and are more sensitive to oxidative stress because of an immature antioxidant system. As a result, retinas of premature infants are highly susceptible to ischemia/reoxygenation damage and ROP. ROP involves an initial vaso-obliterative ischemic phase followed by an angiogenic response with excessive preretinal neovascularization and ultimately retinal detachment and loss of vision (Hardy et al., 2005).

Similar to CBF in the newborn, retinal blood flow is autoregulated over a very narrow range of perfusion pressure, and choroidal blood flow at the outer layer of the retina is almost devoid of the autoregulatory mechanism. Prostanoids play a role in regulating choroidal blood flow in response to changes in BP and oxygen. PGE_2 causes choroidal vasoconstriction in the adult but vasorelaxation in the newborn because of decreased vasoconstrictor EP1 and EP3 receptors in the neonatal choroid. In addition, PGI_2 and PGD_2 cause greater choroidal vasodilation in the newborn than in the adult, possibly as a result of augmented coupling to adenylyl cyclase. Thus, in severely ill preterm newborns in the presence of a dominant PG vasodilator effect, increased BP and hyperoxia increase blood flow and oxygen delivery to the retina. The increase in oxygen results in the generation of ROS and isoprostanes in the immature retina, which is partially devoid of antioxidants; as a result, normal vascular development ceases and large areas of newly formed immature retinal vessels are obliterated, leading to hypoperfusion and ischemia (Hardy et al., 2005). ROS also decrease the vasodilator effects of NO and convert it to peroxynitrite, which in turn causes nitration and inactivation of PGIS but not TXAS. Decreased NO also abolishes more than 75% of vasodilation mediated by NO-stimulated PGI_2 production (Hardy et al., 2000). ROS and peroxides also increase COX expression, which further stimulates prostanoid synthesis. Both PGI_2 and TXA_2 are produced, but as peroxidation progresses, PGIS is inactivated by ROS and TXA_2 increases, favoring vasoconstriction (Hardy et al., 2000). This is supported by the observation, in a newborn pig asphyxia-reoxygenation model of ROP, that ocular blood flow increases 5 min after asphyxia but decreases at 60 min. In addition, malondialdehyde, TXB_2, PGE_2, and 6-keto-$PGF_{1\alpha}$ increase 5 min after asphyxia; at 60 min, however, PGE_2 and 6-keto-$PGF_{1\alpha}$ return to nearly preasphyxia levels, and malondialdehyde and TXB_2 continue to increase (Chemtob et al., 1995). In addition to causing retinal vasoconstriction, TXA_2 mediates neuromicrovascular degeneration, which is caused by EC death and further contributes to the vaso-obliteration in ROP (Beauchamp et al., 2001).

The initial vaso-obliterative ischemic phase is followed by the compensatory angiogenic phase, with excessive intravitreal preretinal neovascularization (Hardy et al., 2005). COX2-derived PGE_2 acting through EP3 and to less extent EP2 receptor have been shown to contribute to the preretinal neovascularization in different models of ischemic retinopathies. However, whether PGI_2 contributes to this pathological angiogenesis is unclear (Sennlaub et al., 2003).

In the newborn pig asphyxia/reoxygenation model of ROP, COX inhibition prevents the asphyxia-induced rise

in the peroxidation marker malondialdehyde, and free radical scavengers prevent the rise in prostanoids (Chemtob et al., 1995). However, a meta-analysis of the use of prophylactic indomethacin versus placebo in premature infants did not demonstrate a statistical difference in ROP outcome (Fowlie et al., 2010). Targeting the prostanoid molecules that contribute to the pathogenesis of ROP using selective PG inhibitors rather than COX inhibitors may spare the desirable effects of other COX products such as PGI_2. For example, TXAS inhibitors and selective EP3 or EP2 antagonists could control vaso-obliteration and vitreal neovascularization, respectively.

E. Prostacyclin Metabolism and Hypoxic Ischemic Encephalopathy of the Newborn

Asphyxia at birth with the development of hypoxic ischemic encephalopathy (HIE) is one of the causes of neurological disabilities in children, especially those prematurely born (Gücüyener et al., 1997). In animal models of asphyxia of different ages and species, postasphyxial cerebral hyperemia has been observed (Pourcyrous et al., 1988), and metabolic changes continue after reperfusion of ischemic tissues causing more severe and maintained cell injury (Perlman, 2006). The mechanisms of cellular energy failure that follows reperfusion may involve mitochondrial dysfunction secondary to extended reactions from primary insults such as ROS, RNS, Ca^{2+} influx, and excitatory neurotoxins (Perlman, 2006).

PGI_2 and PGE_2 levels undergo significant fluctuations during asphyxia in the newborn and in turn affect CBF. This is supported by the observation that the cerebral vasodilation after asphyxia/reventilation in asphyxiated piglets is associated with increased PGI_2 and PGE_2 in the cerebrospinal fluid surrounding cerebral vessels (Pourcyrous et al., 1988). However, during the reperfusion phase of cerebral ischemia, an increase in COX activity would also lead to the release of ROS as a byproduct of the peroxidase activity of COX (McCullough et al., 2004) (Fig. 8), and ROS, in turn, contributes to brain edema, alteration in cerebral vascular tone, and postasphyxial hypoperfusion. The increases in ROS and other metabolic factors rather than alterations in CBF during the reperfusion phase may contribute more to vascular pathology in the ischemic brain (Hardy et al., 2000). This is supported by the observation that treatment of asphyxiated newborn pigs with indomethacin inhibits the generation of ROS (Pourcyrous et al., 1993).

There is ongoing debate regarding the role of PGI_2 in HIE of the newborn. PGI_2 may have harmful effects in the initial phase of HIE, where it may contribute to postasphyxial cerebral reperfusion and in turn leads to increased ROS and aggravation of vascular pathology in the ischemic brain (Pourcyrous et al., 1993). Studies in a beagle pup model of perinatal asphyxia have shown that treatment with the TXAS inhibitor CGS-13080 (pirmagrel), which increases PGI_2 activity and CBF in early phases of neonatal HIE, failed to show improvement in the ischemic met-

abolic changes. In addition, the pups had an increased risk of developing IVH (Ment et al., 1989). Although PGI_2 is among the prostanoids that could contribute to increased CBF in the early reperfusion phase of cerebral ischemia in newborns with HIE, a delayed increase in PGI_2 production or activity in later stages of HIE may have more beneficial effects. For example, CSF samples taken later at 36 to 72 h after birth in newborns with HIE showed elevated PGI_2 and TXA_2 levels but a net decrease in PGI_2/TXA_2 ratio that correlate with disease severity, and may be used as a prognostic biomarker in asphyxiated infants (Liu et al., 2003). In addition, in the adult rat model of focal ischemia/reperfusion, overexpression of PGIS by adenoviral gene transfer, early before transient ligation of the right middle cerebral artery, caused a delayed increase in PGIS mRNA in the ischemic cortex at 24 to 72 h after ischemia, enhanced PGI_2 production at 72 h after ischemia, more favorable PGI_2/TXA_2 ratio, and decreased ischemic brain damage (Fang et al., 2006). In addition, beraprost reduces the neurological deficit score and infarct volume after both transient and permanent middle cerebral artery occlusion in an adult mouse model. In these animal models of brain ischemia, PGI_2 probably protects neurons by enhancing intracellular cAMP production and reducing Ca^{2+} overload in neurons to mitigate subsequent neuronal cell death (Saleem et al., 2010). Studies have also examined the potential IP receptor involved in the actions of PGI_2 after brain ischemia. 15R-TIC, a specific ligand to CNS-type IP2 receptor, has shown neuroprotective effects independent of cerebral perfusion in models of transient forebrain ischemia in gerbils, permanent middle cerebral artery occlusion in rats, transient middle cerebral artery occlusion/reperfusion in cynomolgus monkeys, and antiapoptotic effects on primary cultured hippocampal neurons (Cui et al., 2006). A few clinical trials in adults have also suggested a promising role of PGI_2 analogs in attenuating the anatomical and functional damage associated with focal brain ischemia or stroke, probably through vasculoprotective and neuroprotective effects (Bath, 2004).

In comparison with PGI_2, PGE_2 could have both protective and toxic effects on neuronal survival, probably because of the different EP receptor subtypes and functions in the brain. Gene deletion and in vitro studies suggest that EP2 and EP4 are neuroprotective, whereas EP1 and EP3 are excitotoxic during ischemia/reperfusion injury in the brain (McCullough et al., 2004; Saleem et al., 2009). Intraventricular injection of PGE_2 in a global ischemia rat model could act through EP3 receptor and escalate neural damage (Thornhill and Asselin, 1999; Saleem et al., 2009). Head cooling reduces ischemic damage in this rat model, and induction of hypothermia and reducing the metabolic demands may protect against further neuronal injury in infants with HIE.

Thus, the vasodilator actions of PGI_2 and PGE_2 and their effects on early and late neurological outcome in both the newborn and adults with brain ischemia are not well defined (Sumanović-Glamuzina et al., 2008). PGI_2 may

contribute to reperfusion in the initial phases of HIE but may be cytoprotective in later stages of ischemic injury independent of changes in CBF. Because the PR distribution and prostanoid functions in the brain differ with age, and because most research was done in adult brain ischemia/reperfusion models, the role of prostanoids and specifically PGI_2 in the pathogenesis of HIE in the newborn needs to be further studied. In this respect, studies using centrally acting IP2 receptor analogs would provide insights into the potential benefits of PGI_2 analogs acting on CNS-type IP2 receptors as local cytoprotective agents without having untoward effects on systemic BP.

VIII. Conclusions and Future Implications

Prostanoids are AA products involved in a wide range of biological events, including vascular homeostasis and hemostasis. The biological effects of prostanoids are regulated at different levels including cellular expression, activity, and preferential coupling of PLA_2, COX, and specific prostanoid synthases. Prostanoids function through different PRs localized both at the cell surface to promote rapid effects and in the nuclear envelope to regulate gene transcription. PGI_2 is a major prostanoid in the vascular system, where it maintains EC integrity and modulates the inflammatory response and leukocyte adhesion. PGI_2 also inhibits platelet aggregation, promotes VSMC relaxation and differentiation, and inhibits VSMC proliferation and migration.

COX2 inhibitors have been useful in understanding the balance between PGI_2 and TXA_2 in vascular homeostasis, and their imbalance in pregnancy-associated and neonatal vascular disorders. However, NSAIDs have a wide-spectrum action and their use as anti-inflammatory agents has been associated with cardiovascular side effects. In addition, the use of low-dose aspirin in PE and indomethacin for prophylaxis against PDA and IVH is not universally accepted. This prompted the search for specific modulators of the PG system that could be used efficiently and safely in pregnancy-associated and neonatal vascular disease. TXAS inhibitors or IP agonists could be useful in PE. Selective EP4 antagonists could be used instead of indomethacin to close a PDA in premature infants. Selective IP antagonists could be used in closing PDA depending on the relative roles of PGI_2 and PGE_2 (Smith et al., 1994). Specific IP2 analogs that cross the blood-brain barrier, such as 15R-TIC, may have neuroprotective effects with minimal systemic or cerebral vasodilating effects that traditional IP analogs may exert in HIE (Cui et al., 2006).

In selecting a PG modulator in pregnancy-related disorders it is paramount to consider the interactions in the maternal and fetal circulation. For example, a higher aspirin dose above 60 mg may be beneficial in PE but could increase fetal risks and bleeding tendency (Østensen et al., 2006). In addition, the use of indomethacin as a tocolytic agent may be associated with impaired fetal renal function, oligohydramnios, intrauterine fetal DA constriction,

and increased risk of PPHN. Selective antagonists of EP1 and EP3, the contractile receptors for PGE_2 in human uterus could be useful as tocolytic agents with little risk of constricting fetal DA (Smith et al., 1994).

In addition to PGI_2, NO, ET-1, and other eicosanoids have prominent cardiovascular effects, and targeting PGI_2 alone may not be sufficient in treating CVD that involve other vascular mediators. In PE, other bioactive factors such as sFlt-1 and other eicosanoids such as HETEs and isoprostanes are increased (Walsh et al., 2000; Pearson et al., 2010). In addition, deficient 15-deoxy-PGJ_2/PPARγ-signaling may be involved in the initial abnormal placentation in PE (Helliwell et al., 2004b). Likewise, vascular disorders of the newborn involve disturbed not only PGI_2, TXA_2, and PGE_2 metabolism but also changes in other eicosanoids (Ostrea et al., 2006). Isoprostanes have been associated with increased morbidity of neonatal PPHN (Gong et al., 2010), ROP (Hardy et al., 2000), and HIE (Rogers et al., 2005). On the other hand, in addition to the cytoprotective role of PGI_2 in later stages of ischemic injury associated with HIE, PGD_2 acting through DP1 receptors may protect the neonatal brain against EC degeneration (Taniguchi et al., 2007).

Thus, although evidence suggests a role for PGI_2 and other prostanoids in pregnancy, fetal, and neonatal adaptation, further investigation of the molecular events involved in these adaptive mechanisms and in pregnancy-related and neonatal vascular disorders is needed. These studies will help design specific agonists or antagonists of PG receptors, specific enzyme modulators, and genetic approaches that effectively target the defective prostanoid pathway while preserving the beneficial physiological processes during pregnancy and in the newborn.

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Wrote or contributed to the writing of the manuscript: Majed and Khalil.

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