Prostaglandin H Synthase and Vascular Function

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Abstract—Prostaglandin H synthase (PGHS) is a rate-limiting enzyme in the production of prostaglandins and thromboxane, which are important regulators of vascular function. Under normal physiological conditions, PGHS-dependent vasodilators (such as prostacyclin) modulate vascular tone. However, PGHS-dependent vasoconstriction (mediated by thromboxane and/or its immediate precursor, PGH₂) predominates in some vascular pathologies (eg, systemic hypertension, diabetes, cerebral ischemia, and aging). This review will discuss the role of PGHS-dependent modulation of vascular function in a number of vascular beds (systemic, pulmonary, cerebral, and uterine) with an emphasis on vascular pathophysiology. Moreover, the specific contributions of the different isoforms (PGHS-1 and PGHS-2) are discussed. Understanding the role of PGHS in vascular function is of particular importance because they are the targets of the commonly used nonsteroidal antiinflammatory drugs (NSAIDs), which include aspirin and ibuprofen. Importantly, with the advent of specific PGHS-2 inhibitors for treatment of conditions such as chronic inflammatory disease, it is an opportune time to review the data regarding PGHS-dependent modulation of vascular function. (Circ Res. 2001;89:650-660.)

Key Words: endothelium • cyclooxygenase • prostaglandin • thromboxane • eicosanoid

Prostaglandins (PGs) and thromboxane (Tx) are critical modulators of vascular tone in both physiological and pathophysiological conditions; therefore, the regulation of their production has been and continues to be an area of intensive study.

The production of these eicosanoids is regulated by the availability of arachidonic acid and the activity of prostaglandin H synthase (PGHS), also known as prostaglandin endoperoxide synthase or cyclooxygenase (reviewed in Smith et al³). Liberation of arachidonate from membrane phospholipids is mediated through phospholipases. Once arachidonate is released it is converted to PGH₂ by PGHS. PGHS is a rate-limiting enzyme that exhibits a cyclooxygenase activity that incorporates two molecules of oxygen into arachidonic acid to form PGG₂ and a peroxidase activity catalyzing a 2-electron reduction of PGG₂ to PGH₂. Cell-specific isomerization or reduction of PGH₂ produces biologically active endproducts, such as prostacyclin and thromboxane (Figure 1).

There are two known isoforms of PGHS: PGHS-1 and PGHS-2. The objective of this review is to provide an overview of the physiological roles for the PGHS isoforms in the regulation of vascular function. A brief introduction to the regulation of PGHS activity and expression is presented to provide a better understanding for the physiological function. Specific reviews regarding cellular and molecular regulation of the enzyme have been previously published.¹

Regulation of PGHS Activity and Expression

PGHS-1 and PGHS-2 derive from human chromosome 9 and 1, respectively. Although there is approximately 60% to 65%
PGHS Activity

An unusual kinetic feature of PGHS is the autoinactivation of the enzyme. Indeed, there is a finite turnover of approximately 1300 mol arachidonate/mol enzyme before it is inactivated. The activity of PGHS requires low levels of lipid peroxides for activation and then continued activation occurs autocatalytically by newly generated PGG2. Differences in the peroxidases for activation and then continued activation occurs (PGI2) is a potent vasorelaxant. In some conditions and vascular nates is converted to PGH2 by a cyclooxygenase activity and a

PGHS Activity

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In addition, there may be an important link between nitric oxide and the activity of PGHS. The effect of nitric oxide and its intermediates on PGHS activity has been previously reviewed. Briefly, a number of investigators have reported that nitric oxide increases PGHS activity in a variety of cell types (including vascular endothelial cells); however, a direct effect of nitric oxide on PGHS activity in an isolated enzyme preparation has not been demonstrated.5 In light of these observations, the activation of PGHS by nitric oxide may occur through intermediary pathways. Indeed, it was shown that peroxynitrite (produced from an interaction of nitric oxide and superoxide anions) can activate PGHS.6 Ultimately, the regulation of PGHS activity by nitric oxide is complex and may depend on the quantity/source of nitric oxide as well as the specific PGHS isoform.8 Indeed, exposure of bovine aortic endothelium to exogenous nitric oxide donors enhances PGHS-1 activity in nonactivated endothelial cells but suppresses PGHS-2 activity in cells activated by serum or phorbol ester.9 Conversely, however, PGHS-1 consumes nitric oxide via a peroxidase-dependent mechanism that further contributes to the proaggregatory activity of PGHS-1 within platelets.9

Interestingly, activation of PGHS is also a source of superoxide production because of its ability to cooxidize substances such as NAD(P)H.10 Indeed, it has been reported that the primary source of oxygen free radicals in piglet cerebral cortex with ischemia/reperfusion is due to the release of superoxide anions formed by the activation of PGHS.11 This could lead to a feed-forward loop (via lipid peroxidation and/or peroxynitrite formation) for the activation of PGHS (Figure 2). Although PGHS activation is a source of superoxide anions, it should be noted that specific induction of PGHS-2 limits oxidative damage (mediated by H2O2) in cardiomyocytes due to production of prostacyclin.12 Indeed, it has been suggested that PGHS-2 induction may be beneficial in sites of endothelial injury to replace the protective actions of PGHS-1.13

Therefore, in addition to PGHS activation, another important control of prostaglandin production is through the regulation of PGHS expression.

Regulation of PGHS Expression in Vascular Cells

PGHS-1 is a 69-kDa protein that is constitutively expressed but can also be induced. For example, shear stress induces PGHS-1 in human umbilical vein endothelial cells.14 In addition, there are a number of putative promoter response elements for PGHS-1, including shear stress elements and transcription factor sites.15

PGHS-2 is considered the inducible isofrom (72 kDa MW) whose expression is increased by a number of cardiovascular risk factors, such as cytokines, cholesterol, lipoproteins, and hypoxia. Lipopolysaccharides will induce expression that can be inhibited by antiinflammatory glucocorticoids or cytokines.16 Interleukins, such as IL1α, have been shown to increase both PGHS-2 and PLA2, leading to enhanced prostacyclin production in human vascular endothelial cells.17 IL1β has also been shown to induce PGHS-2 through a mechanism mediated by activation of the extracellular signal-regulated kinases (ERKs) JNK/SAPK and p38 MAPK.18 The signaling pathway(s) that mediates induction of PGHS-2 will depend on the stimulus. Lysophosphatidylcholine, a component of oxidized low-density lipoprotein, has
Potential Role of PGHS and Altered Vascular Function

Figure 2. Potential role of PGHS and altered vascular function. Superoxide anions (O2−) are released by the activity of PGHS. Superoxide anions (via production of hydroxyl radicals) can initiate membrane lipid peroxidation. Superoxide anions also react with nitric oxide (NO) to produce peroxynitrite (ONOO−) and thus reduce nitric oxide bioavailability. Lipid peroxides and/or peroxynitrite activates prostaglandin H synthase (PGHS) leading to increased prostaglandin endoperoxide (PGH2) and thromboxane (TxA2), as well as a feed-forward mechanism of further superoxide anion production. PGH2 and TxA2 bind to the same receptor causing a vasoconstriction. Isoprostanes are produced by a free radical–mediated cleavage of arachidonate that can be initiated by PGHS activity (perhaps via superoxide anion production). Prostacyclin (PGI2) is reduced as the result of extensive lipid peroxidation and/or peroxynitrite (which preferentially inhibits PGI2 synthase activity), resulting in reduced vasorelaxation. These pathways may represent mechanisms for impairment of the balance between relaxing and contracting factors thereby contributing to vascular dysfunction.

been shown to induce p38 MAPK and the transcription factors CREB and ATF-1 resulting in PGHS-2 upregulation in cultured endothelial cells.19 Alternatively, phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C (PKC), induced expression of PGHS-2 primarily through activation of ERK1 and ERK2.20

The nuclear factor (NF)-κB is a mediator of cytokine and hypoxia-induced transcription of PGHS-2.21-22 However, it has been reported that although NF-κB is necessary for transcription of PGHS-2, it alone was not sufficient to fully induce the enzyme in response to hypoxia.22 It was determined that with hypoxia, a member of the high-mobility-group (HMG) I(Y) protein family facilitated transactivation of PGHS-2 promoter.22 As well, high-density lipoproteins have been shown to synergize with cytokines (TNFα and IL-1β) to enhance expression of PGHS-2 in endothelial cells. However, the high-density lipoprotein did not influence the nuclear translocation or DNA binding of NF-κB, suggesting an influence of transactivating factors other than those of the NF-κB pathway itself.23

Another factor that contributes to PGHS-2 induction in vascular cells is the activity of antioxidant enzymes. Overexpression of the human catalase gene, but not Cu/Zn or Mn superoxide dismutase, increased the expression of PGHS-2, which was associated with a decreased intracellular level of peroxides in cerebral microvascular endothelial cells.24 Regulation of PGHS by cAMP (the second messenger for the primary product, prostacyclin) has also been tested using bovine aortic endothelial cells that revealed a complex regulation. Increasing cellular AMP inhibited PGHS-1 activity without affecting PGHS-1 expression, but PGHS-2 protein was upregulated.25 However, PMA-induced expression of PGHS-2 is inhibited by extracellular AMP in human pulmonary microvascular endothelial cells.26

A negative feedback loop that regulates PGHS levels may exist in vascular cells that involves the peroxisome proliferator-activated receptor (PPAR).27 PPARs are transcription factors that are ligand-dependent. A PGD2 metabolite (a product of PGHS) is a potent ligand of PPARγ that results in inhibition of transcription for PGHS in macrophages.27 Similarly, in human aortic smooth muscle cells, PPARα ligands inhibit interleukin-induced expression of PGHS-2 due to PPAR suppression of NF-κB signaling.28 However, the regulation of PGHS-2 in response to PPAR activation is likely tissue-specific. Indeed, direct activation of the peroxisome proliferator response element on PGHS-2 in epithelial cells of the mammary and colon enhances PGHS-2 expression.29

Along with the transcriptional regulation of PGHS-2, there is posttranscriptional control of PGHS-2 at the 3′ untranslated region of its mRNA because of multiple copies of AU-rich elements, which regulate mRNA turnover and translational inhibition.30

Disruption of the PGHS-1 or PGHS-2 gene in mice has demonstrated that the PGHS-1–deficient mice survive normally, whereas PGHS-2–deficient animals develop nephropathy and may die early.31 Another vascular developmental complication that occurs with PGHS-2–deficient mice is that 35% of the mice die within 48 hours after birth with a patent ductus arteriosus (an arterial connection that directs blood flow away from the pulmonary circulation in fetal life that must close at birth). Moreover, 100% of the mice deficient of both isoforms die with a patent ductus arteriosus within 12 hours of birth.32 Specific studies that have used PGHS-1–or PGHS-2–deficient mice for understanding vascular control in adult mice are addressed in the following detailed sections on vascular function.

Localization of PGHS Within the Vasculature

Both the endothelium and smooth muscle cell contain PGHS; however, endothelial cells contain up to 20 times more PGHS than smooth muscle cells.33 In regard to subcellular localization of PGHS, immunogold-labeling microscopy has demonstrated that both PGHS-1 and PGHS-2 are present in equal proportions in the luminal surface of endoplasmic reticulum and in the inner and outer membranes of the nuclear envelope in human umbilical vein endothelial cells.2 There does not appear to be a different subcellular localization of PGHS-1 versus PGHS-2, although separation of their activities could be because of coupling to different phospholipases.34 Interestingly, in bovine aortic endothelial cells, prostacyclin synthase and PGHS-1 were colocalized to the nuclear envelope and endoplasmic reticulum. However, there was a lack of colocalization of the PGHS-2 with prostacyclin synthase.35
PGHS Products of the Vasculature

In general, prostacyclin is a potent vasorelaxant produced by PGHS-1 and prostacyclin synthase in nonactivated endothelial cells. However, after IL-1β stimulation of human umbilical vein endothelial cells, prostaglandin E₂, F₂α, and D₂ were all produced via PGHS-2 while prostacyclin synthase is inactivated. The inactivation of prostacyclin was likely owing to peroxynitrite and hydroxyl radicals. Thromboxane is a vasoconstrictor that is primarily produced by platelets, but can also be synthesized from endothelial cells. Indeed, thromboxane synthase has been cloned in endothelium. In addition, PGH₂ and thromboxane both bind to the same receptor on vascular smooth muscle cells to produce a vasoconstriction.

An interesting interaction between platelet and endothelial cells can occur regarding eicosanoid production. Karim et al demonstrated that endothelial cells stimulated by thrombin restore thromboxane production of aspirin-treated platelets. Indeed, there is transcellular formation of thromboxane by platelets from PGH₂ released by endothelial cells. However, recent data indicate that PGHS activity needs to be enhanced (via an overexpression of PGHS-2) and prostacyclin synthase reduced substantially before there can be significant transcellular synthesis of thromboxane.

Other potential vascular products that may be produced by PGHS activation are the isoprostanes such as 8-iso-PGF₂α. Isoprostanes are a family of oxygenated arachidonate products that are structurally similar to eicosanoids. Under conditions of oxidative stress, release of isoprostanes from arachidonate is a free radical–mediated process that does not require PGHS. However, it has also been demonstrated that in conditions that induce PGHS-2 there is a coinciding release of 8-isoprostanes that could be inhibited with the PGHS inhibitor indomethacin. However, there is no evidence for enzymatic generation of isoprostanes in vivo. One interpretation for the PGHS-2–dependent production of isoprostanes is that oxygen free radicals are produced from the activity of the enzyme that leads to free radical–mediated release of isoprostanes. Therefore, PGHS-2 may affect vascular function by enhancing the free radical environment within the cell.

PGHS and Vascular Function

Under normal physiological conditions, eicosanoids (primarily prostacyclin) produced by the PGHS pathway generally induce vasorelaxation. Moreover, PGHS-dependent vasodilation can compensate for the deficiency of other vasorelaxants such as nitric oxide synthase as demonstrated by flow-dependent vasodilation that is PGHS-dependent in mutant mice deficient of endothelial nitric oxide synthase.

However, in vascular pathologies, there may be an imbalance where PGHS-dependent vasoconstrictors become more predominant (Figure 2). Moreover, in a number of vascular complications there are common cardiovascular risk factors, such as oxidative stress and dyslipidemia, which are known modulators for PGHS-dependent function that may lead to impairment of vascular function. For instance, in vitro exposure of oxidative stress (induced by tert-butyl hydroperoxide) causes vasoconstriction in isolated aortic rings from both normotensive and hypertensive rats, in part, because of enhanced PGHS-2 expression. The vasoconstrictor products are likely not thromboxane (because thromboxane synthase inhibition did not alter the response) but rather PGH₂ or isoprostanes. An in vivo model of oxidative stress using a rat model of vitamin E deficiency also demonstrated an increased PGHS-dependent vasoconstrictor that modified endothelium-dependent vascular responses via the PGH₂/TxA₂ receptor.

In obese subjects, assessment of endothelium-dependent function using acetylcholine indicated impaired vascular function compared with the control group. Infusion of either the antioxidant vitamin C or the PGHS inhibitor indomethacin at the time of acetylcholine administration enhanced forearm blood flow in the obese group, suggesting a role for oxidative stress and PGHS-dependent vasoconstriction to modify vascular function. Similarly, in hypercholesterolemic patients, vasodilation to acetylcholine as assessed by forearm blood flow is enhanced by pretreatment with the PGHS inhibitor aspirin. Indeed, cholesterol has been shown to induce vasoconstriction in rat aortic rings that was prevented by inhibitors of PGHS-2 or p38 MAPK. In addition, smooth muscle cells treated with oxysterol 25-OHC (an oxidized derivative of cholesterol that is present in atherosclerotic plaques) specifically increased PGHS-2 in coronary arteries.

In contrast, high-density lipoproteins (HDLs) synergize with cytokines to enhance PGHS-2 to increase prostacyclin synthesis in endothelial cells. HDLs may evoke vascular protection via production of prostacyclin, whereas oxidized LDLs and/or cholesterol impairs endothelial-dependent vascular function. The shift to predominately PGHS-dependent vasoconstrictors with oxidized derivatives of lipids could be caused by elevated lipid peroxide levels that would inhibit prostacyclin synthase, thus allowing for more PGH₂ and/or thromboxane to mediate vasoconstriction.

Overall, in vascular pathologies, there may be an imbalance where PGHS-dependent vasoconstrictors become more predominant. However, the role for PGHS-dependent modulation of vascular function will depend on the type of vascular complication (and animal model studied) and the vascular bed.

Hypertension

Shear stress increases prostacyclin production via enhanced expression of PGHS-1, PGHS-2, and prostacyclin synthase. In the arterioles of rat cremaster muscle, shear stress in normotensive rats results in vasodilation due to endothelium-derived vasoconstrictor prostaglandins. However, in hypertensive rats, shear stress in cremaster arterioles increases the PGHS-dependent vasoconstrictor, PGH₂, that reduces the vasodilatory response. Moreover, modulation of endothelium-dependent relaxation has been associated with PGHS-dependent vasoconstriction in the aorta, mesenteric, and renal arteries of the spontaneously hypertensive rat (SHR). Specifically, the expression of PGHS-1 was enhanced in endothelial-intact aorta of these SHR animals. In addition, the PGHS-dependent vasoconstriction is not affected by thromboxane synthase inhibition. However, a PGH₂/TxA₂...
receptor blocker inhibited the contractile responses of the renal arteries, whereas inhibition of superoxide production improved relaxation in the mesenteric arteries. Interest-

ingly, before the development of overt hypertension in the SHR animals, inhibiting PGHS activity or scavenging superoxide anions restored endothelial-dependent function but inhibition of the thromboxane synthease or the PGH_{2}/TxA_{2} receptor did not. The authors suggested that superoxide anions from PGHS activation impaired the vascular response. Because superoxides are known scavengers of nitric oxide, the reduced nitric oxide–mediated vasodilation that is observed in this model may originate from PGHS activation.

Other models of hypertension have also noted enhanced PGHS-dependent vasoconstriction. Pressor response in the renal circulation is increased in the rat hypertensive model of aortic coarctation. In particular, PGHS-1 activity and expression are increased in this model that leads to vasoconstriction via the PGH_{2}/TxA_{2} receptor. Similarly, in the Lyon strain of hypertensive rats, which exhibit enhanced renal vascular resistance, salt loading increased medullary PGHS-1 expression.

The development of angiotensin-dependent hypertension in rats is also caused, in part, by PGHS-dependent vasoconstrictors acting on the PGH_{2}/TxA_{2} receptor. However, it is noted that the degree of increased blood pressure was dependent on the balance of PGHS-dependent vasodilators with the PGHS-dependent vasoconstrictors. In a mouse model of hypertension (double transgenic mice that overexpress human renin and human angiotensinogen), carotid artery relaxation was impaired due, in part, to a PGHS-dependent vasoconstrictor. In another animal model where hypertension ensues after prolonged nitric oxide synthase inhibition, PGHS inhibition with indomethacin for 1 week reduced the blood pressure elevation, indicating that the PGHS-dependent vasoconstrictor(s) predominates in this model. However, selective inhibition of PGHS-2 (via administration of celecoxib for 3 weeks) in both normal and hypertensive (due to prolonged nitric oxide inhibition) rats further enhanced blood pressure, indicating inhibition of a PGHS-2–dependent vasodilator. Similarly with portal hypertension, there is an amplified role for the PGHS-dependent vasodilator, prostacyclin, that promotes splanchnic hyperemia. By comparison, in deoxycorticosterone acetate (DOCA)-hypertensive rats, increased sensitivity to serotonin is not altered by PGHS inhibition.

In humans with essential hypertension but not renovascular or aldosterone-induced hypertension, forearm blood flow in response to the acetylcholine-mediated vasodilation is increased in the presence of indomethacin, suggesting that PGHS-dependent vasoconstrictors predominate in essential hypertensive patients. In a more recent study by the same group, they evaluated aging normotensive and essential hypertensive patients and determined that PGHS-dependent vasoconstrictors impair endothelial-dependent vasodilation in both aging and hypertension. Moreover, inhibition of PGHS improves vascular function, in part because of restoration of the bioavailability of nitric oxide, suggesting the possibility that in essential hypertension the PGHS pathway could be a primary contributor of superoxide anions.

### Pulmonary Hypertension

Prostacyclin is an important mediator of pulmonary vascular function early in life. The ontogeny of PGHS-1 and -2 have been assessed in lungs from late-gestation fetal lambs and 1-week-old newborn lambs. Interestingly, PGHS-1 is predominant in endothelial cells at all gestational ages; in contrast, PGHS-2 protein was absent. Furthermore, there is a postnatal rise of PGHS-1 that is due, in part, to release of inhibition by glucocorticoids that decrease after birth. In studying the role of PGHS, however, it is important to note species variations. For instance, in human pulmonary arteries PGHS-dependent vasorelaxants predominate, whereas in porcine pulmonary arteries, PGHS-dependent vasoconstrictors are released.

Under a pathological condition, porcine pulmonary microvascular reactivity to serotonin is increased after cardiopulmonary bypass due, in part, to a PGHS-2–dependent vasoconstrictor. Similarly, impaired endothelial-dependent relaxations in conduit pulmonary arteries isolated from rats with hypoxic pulmonary hypertension was due, in part, to activation of the PGH_{2}/TxA_{2} receptor. In humans, although the role of PGHS-dependent vasodilation is evident in normal pulmonary function, the modulation of PGHS-dependent vascular function in pulmonary hypertension is not clear and therefore may be species-dependent.

Overall, the data indicate that the role for PGHS and the specific isoforms in hypertension is complex. The role for PGHS to induce vasodilation or vasoconstriction may depend on the animal model and/or type of hypertension as well as the vascular bed studied. In addition, the specific regimen for PGHS inhibition (drug, dose, and/or duration) will affect outcome in these studies. Nevertheless, there is a balance in PGHS-dependent vasodilators and vasoconstriction. In some types of systemic hypertension, impaired endothelium-dependent function is mediated, in part, by PGHS-dependent vasoconstrictors.

### Preeclampsia

Preeclampsia is a syndrome of pregnancy that is characterized by hypertension, proteinuria, and platelet aggregation. There is considerable evidence that dysfunction of vascular endothelial cells contributes to the pathophysiology of preeclampsia and that prooxidants are mediators in altering endothelial function. In regard to the PGHS pathway, there are data to suggest that there is a greater thromboxane production compared with prostacyclin (as measured by their stable metabolites) in the placenta as well as in the urine and plasma of women with preeclampsia compared with women with uncomplicated pregnancies. Indeed, trials were conducted to evaluate the use of low-dose aspirin therapy to inhibit platelet-derived PGHS in pregnant women to prevent the onset of preeclampsia. However, in two large multicenter studies, aspirin afforded only a slight improvement in the incidence of preeclampsia in a small subset of women and had no effect on perinatal morbidity. However, as discussed in this review, a PGHS-dependent vasoconstriction may be derived from the vasculature, and therefore, low-dose aspirin that primarily inhibits platelet PGHS may not be effective.
Interestingly, the expression and activity of PGHS-2 are enhanced in placental trophoblasts from women with pre-eclampsia, which may alter fetalplacental function. In addition, it was recently reported that overexpression of the thromboxane receptor in the vasculature of mice resulted in intrauterine growth restriction (which is a pregnancy complication for women with preeclampsia). Therefore, a better understanding of the control and pathophysiology of both isoforms of PGHS in the vasculature and placenta of women with preeclampsia may lead to new therapeutic strategies.

**Estrogen and Aging**

During normal pregnancy, uterine blood flow increases, in part, because of prostacyclin. Indeed, PGHS-1 (but not PGHS-2) expression (mRNA and protein) are increased in the endothelium and to a lesser extent in the vascular smooth muscle of the ovine uterine artery in the follicular phase and in pregnancy where uterine blood flow is highest. These data suggest that control of PGHS-dependent relaxation is owing, in part, to the hormonal milieu. In the ovine fetal pulmonary endothelial cells, estrogen has been shown to upregulate PGHS-1 expression. Additionally, fetal estrogen levels rise dramatically during late gestation, and prostacyclin is an important pulmonary vasodilator during the perinatal period. In agreement with its functional role, there is a maturational increase in PGHS-1 but not PGHS-2. In general, in reproductive and pulmonary vascular beds, PGHS-dependent vasodilation predominates under conditions of elevated estrogen levels. In contrast, estradiol replacement in ovariectomized rats suppresses endothelium-derived PGHS-dependent vasoconstriction in systemic mesenteric arteries. Indeed, animal studies of aging females (both conditions relevant to menopausal women) have shown increased PGHS-dependent vasoconstriction acting through the TxA2/PGH2 receptor. A more recent study has described PGHS-2 as the predominant isoform in aging that contributes to vasoconstriction. These data agree, in part, with the results obtained in aorta of aging male rats where both PGHS-1 and PGHS-2 contribute to altered vascular responses.

These studies of specific increases in PGHS-2 modulation of vascular function in aging provide intriguing possibilities for alternative therapies for estrogen replacement. Further studies in human subjects are warranted.

**Atherosclerosis**

The role of thromboxane and prostacyclin in atherosclerosis has been reviewed over the years. However, the specific roles of vascular PGHS-1 versus PGHS-2 and their contribution to the pathophysiology continue to be studied extensively. Studies have demonstrated that both mRNA and protein for PGHS-2 are present in atherosclerotic plaques, macrophages, and smooth muscle cells, whereas PGHS-1 and -2 were present in the endothelium of atherosclerotic and healthy vessels. In addition, in an atherosclerotic plaque, it has also been shown that there is increased coexpression for PGHS-2 and MMP-9, an enzyme that is involved in destabilization of atherosclerotic plaques. Indeed, PGHS-2 inhibitors are suggested as one possible therapy to prevent atherosclerotic plaque formation. However, the outcome for such a therapy may not be clear because PGHS-2 induction may afford vascular protection by limiting adhesion molecule expression on human vascular smooth muscle cells.

In a patient population of atherosclerosis, a recent study determined, using aspirin and/or nimesulide and measuring product excretion, that both PGHS-1 and PGHS-2 contributed to prostacyclin production, whereas thromboxane production was dependent on PGHS-1. However, functional studies were not conducted and PGHS-dependent vasoconstriction may not be caused by thromboxane production but rather PGH2 and/or isoprostanes. Further studies are necessary to better understand the specific roles of PGHS-1 and PGHS-2 in the modulation of vascular function as well as their role in plaque formation in atherosclerotic patients.

**Diabetes**

The role of PGHS in diabetes goes beyond its role in vascular function. Insulin-dependent diabetes mellitus is a result of an inflammatory response in the pancreas leading to destruction of β cells. Administration of PGHS-2 inhibitors prevents the onset of diabetes in streptozotocin (STZ)-treated mice. However, the link between diabetes and vascular disease, in particular the role of the PGHS pathway to alter vascular function, is not completely understood.

Endothelial-dependent PGHS-mediated vasoconstriction has been reported in aorta of diabetic rats and rabbits as well as the pial arteriole of diabetic rats, although others have not demonstrated a specific role for PGHS in endothelial cell dysfunction. Moreover, studies have demonstrated both enhanced and impaired PGHS-dependent vasodilation in diabetic animals. In coronary arteries of diabetic dogs, PGHS inhibition reduced acetylcholine responses but did not affect the control dogs. These data indicate enhanced PGHS-dependent vasodilation in the diabetic animals. On the other hand, adenosine-induced renal vasoconstriction is enhanced in STZ diabetic rats. Indomethacin induced a potentiation of vasoconstriction in control rats that is attenuated in the STZ rats, suggesting impaired PGHS-dependent vasodilation. The role of PGHS-dependent modulation of vascular function in diabetes remains to be clarified. Conflicting data in animal models could be because of species, vascular bed, and the model of diabetes (eg, genetic and STZ-induced).

In subjects with noninsulin-dependent diabetes, impaired responses to acetylcholine were not improved by aspirin treatment. In normotensive type 1 diabetic and matched nondiabetic control patients, there was no difference in forearm blood flow in response to acetylcholine. However, indomethacin infusion reduced the vasodilatory response to a greater extent in the diabetic patients, suggesting a potential compensatory role for a PGHS-dependent vasodilator in modulating vascular responses. There is, however, an association of increased 8-iso-PGF2α levels and impaired glycemic control that could lead to impaired vascular function in diabetic patients. Overall, the data are conflicting as to the role of PGHS in modulating vascular function in diabetes. Nevertheless, a role for oxygen-derived free radicals has been proposed in diabetes, and it may be the...
cumulative effects of oxidative stress (see previous section) along with hyperglycemia that create a complex interrelationship of PGHS activity that alters vascular function.

High glucose has been shown to enhance IL1β-induced PGHS-2 expression in rat vascular smooth muscle cells; however, the resulting effect on vascular function is not clear. Although acute hyperglycemia (25 mmol/L) causes vasorelaxation, in part via PGHS-dependent vasodilatations, chronic hyperglycemia can impair vascular function through formation of advanced glycation endproducts (AGEs). Interestingly, the receptor for AGEs mediates high-mobility-group 1-dependent migratory responses. As previously mentioned, a member of the high-mobility group facilitates induction of PGHS-2; therefore, there may be a role of AGE-dependent alteration of vascular function via the PGHS pathway that has yet to be elucidated. It is known that AGE induces oxidant stress on cells that has implications for vascular dysfunction, including via the PGHS pathway, as previously discussed. Indeed, one study demonstrated reduced prostacyclin production from human microvascular endothelial cells in response to AGE; however, the expression/activity of the specific isoforms of PGHS was not determined.

Cerebral Blood Flow and Ischemia

Prostaglandins act as modulators in neuronal as well as vascular function in the brain. In regard to the cerebral vascular system, expression and activity of both PGHS-1 and PGHS-2 are known modulators of cerebral blood flow. Increased blood flow due to hypercapnia can be inhibited with PGHS inhibition. In premature human infants given indomethacin for patent ductus arteriosus, cerebral blood flow velocities are decreased, suggesting that there is a predominant PGHS-dependent vasodilator that modulates vascular tone. Therefore, prophylactic PGHS inhibition has been proposed for prevention of intraventricular hemorrhage in very low birth weight infants.

In the human studies, the contributions of the specific PGHS isoforms are not known. Interestingly, in the cerebral microvasculature of newborn pigs, PGHS-2 is the predominant isoform (>90%) that contributes to prostaglandin production. Whether there is species variability or PGHS-2 is the predominant enzyme for vascular relaxation remains to be determined. Indeed, recent studies by Niwa et al. demonstrated that in mice, hypercapnia-mediated or endothelial-dependent increases in cerebral blood flow are attenuated by selective inhibitors of PGHS-1 but not PGHS-2. They confirmed these data using PGHS-1 and PGHS-2 knockout mice. Overall, their data indicate that PGHS-2 mediates vascular responses initiated by neural activity, whereas PGHS-1 is important for vascular (endothelial)-mediated relaxation.

Although vasodilator prostaglandins are important to reduce cerebral resistance, substance P causes PGHS-dependent vasoconstriction in canine cerebral arteries. In addition, pressure-induced vasoconstriction in newborn cerebral vasculature is caused, in part, by PGHS-dependent vasoconstrictors. Therefore, PGHS-dependent vasoconstriction may predominate in the cerebral vasculature under pathological conditions such as cerebral ischemia.

There is abundant evidence that there is a PGHS-dependent mechanism(s) of ischemic brain injury, which is beyond the scope of the present review. However, relevant to the present review, ischemic stress alters PGHS expression in the cerebral vasculature. In the neonatal pig, global ischemic stress increases PGHS-2 in the cerebral vasculature. In addition, in a rat model of focal ischemia, there were increased mRNA and protein for PGHS-2 (but no change in PGHS-1) in the cortical neurons and endothelial cells in the infarct zone. In acute cerebral ischemia but not chronic infarction, there is a marked increase in PGHS-2. In human brains from patients who died 1 to 2 days following infarction, there was increased PGHS-2 immunoreactivity in neutrophils, vascular cells, and neurons near the infarct. Therefore, data indicate that PGHS-2 contributes to the late stages of brain ischemia.

Indeed, PGHS-2 may have a critical role in coupling synaptic activity to blood flow in the brain. The overall impact of PGHS on cerebral blood flow remains to be elucidated. Importantly, there are numerous vasoactive mediators altering cerebral blood flow. These systems do not act in isolation, and their interactions may vary depending on the pathophysiological state. For example, nitric oxide is a potent vasodilator of the cerebral circulation, but it has been hypothesized that this vasodilatory pathway is disrupted during ischemia as a result of PGHS-derived superoxide anions. Further studies are necessary to decipher the complexity of these systems.

Cancer

The literature concerning PGHS and cancer is vast and is beyond the scope of this review on vascular function. However, the role for PGHS in cellular proliferation and angiogenesis is an important area and, therefore, requires a brief synopsis. In brief, PGHS has been implicated in various cancers, in particular colorectal cancer. Indeed, a recent study demonstrated that PGHS-2 is upregulated in colorectal cancer and is inversely related to survival suggesting that it may have a role in tumorigenesis. Moreover, lung metastasis in mice injected with Lewis lung carcinoma was inhibited by thromboxane synthase inhibitors. PGHS-2–induced production of thromboxane promotes endothelial migration and corneal angiogenesis. Indeed, the literature demonstrating a contribution of PGHS-2 in tumor angiogenesis is vast and has been recently reviewed. In contrast, however, it has also been demonstrated that thromboxane inhibits migration and tube formation in human umbilical vein endothelial cells, which the authors suggest may be important relative to ischemia-induced angiogenesis. Therefore, understanding PGHS regulation of angiogenesis under various circumstances (ie, response to ischemia versus tumor growth), as well as the impact on vascular function, is important in regard to future therapeutic potentials for PGHS inhibitors.

Clinical Implications

PGHS is the target for nonsteroidal antiinflammatory drugs (NSAIDs), which include aspirin, indomethacin, and ibuprofen. NSAIDs are widely used for pain, arthritis, colon cancer, Alzheimer disease, and, relevant to this review, cardiovascular disease. A rationale for the use of low-dose aspirin in
protecting against cardiovascular disease was to irreversibly inhibit the cyclooxygenase activity of PGHS to inhibit platelet production of thromboxane. With this perturbation, new enzyme synthesis is required before more eicosanoids can be produced. Endothelial cells can synthesize new protein to replace aspirin-inactivated PGHS, which was initially thought to continue to produce the vasorelaxant prostacyclin. Platelets cannot synthesize new enzyme, thereby resulting in a preferential inhibition of thromboxane. However, as many of the studies presented in this review have proposed, there is a component of vascular PGHS that can produce vasoconstriction; therefore, a better understanding of the control and pathophysiology of the PGHS pathway in the vasculature is necessary when developing new therapeutic strategies to prevent PGHS activity in a number of pathologies.

Specifically, PGHS-2 inhibitors are being developed as a new class of inhibitors in conditions such as preventing angiogenesis for cancer growth and for chronic inflammatory diseases such as arthritis (reviewed in Schnitzer132). The specific inhibitors for PGHS-2 have been proposed to have the advantage of not causing gastric ulceration as is observed with the other NSAIDs, such as aspirin. NSAIDs also impair the healing of gastric ulcers; however, PGHS-2 inhibitors will also delay gastric healing because both PGHS-1 and PGHS-2 are involved in angiogenesis.133 These data have, therefore, challenged the rationale for the benefits of selective PGHS-2 inhibitors in not affecting the gastrointestinal tract. As presented in this review, a number of impaired vascular responses can be attributed specifically to PGHS-2, such as those in ischemia, inflammation, and aging vasculature. Therefore, specific PGHS-2 inhibitors may have beneficial effects on specific vascular pathologies, but this remains to be determined. Indeed, the incidence of myocardial infarction was greater in rheumatoid arthritis patients treated with a PGHS-2 inhibitor (rofecoxib) compared with patients treated with a nonselective inhibitor (naproxen), although the overall mortality rate was similar between the groups. Interestingly, the higher rate of myocardial infarction was primarily accounted for by the group of patients for whom low-dose aspirin therapy was indicated (the study, as designed, had required discontinuation of NSAIDs before randomization into the trial).134 Therefore, any potential beneficial actions of specific PGHS-2 inhibition will need to be balanced with nonspecific PGHS-1 inhibition for patients at risk for thrombosis.

Along with specific PGHS inhibitors, other strategies have been used, such as adenovirus-mediated transfer of PGHS-1. This was conducted in injured porcine carotid arteries to augment prostacyclin synthesis and inhibit thrombosis. The antithrombogenic effect, however, required a relatively high titer of the adenoviral vector.135,136 However, when incorporating the strategy of transfer of PGHS into the vasculature, a clear understanding of the physiological consequences is imperative because PGHS can contribute to either vasorelaxation or constriction depending on the circumstances.

Summary
The overall impact of PGHS expression/activity on vascular function is vast and complex. Under normal physiological conditions, primarily PGHS-dependent vasodilation modulates vascular tone. In vascular pathologies, there may be an imbalance where PGHS-dependent vasoconstrictors become more predominant (Figure 2). Moreover, in a number of vascular complications there are common risk factors such as oxidative stress and dyslipidemia, which alters PGHS-dependent function leading to impairment of vascular function.

Importantly, there are numerous vasoactive mediators that alter the vascular function. These systems do not act in isolation and their interactions may vary depending on the pathophysiological state. For instance, superoxide anions from PGHS activation may impair vascular function through scavenging of nitric oxide, thereby reducing nitric oxide–mediated vasodilation. Therefore, a clear understanding of the role of PGHS in vascular physiology and pathology is important for understanding its impact on other vasoactive pathways. Moreover, the specific roles for PGHS-1 and PGHS-2 in regulating vascular function are still emerging, which is imperative to consider when contemplating the use of specific PGHS-2 inhibitors for the treatment of a number of chronic conditions, such as inflammatory diseases and cancer.

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