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Perspective

Since the discovery in 1991 of a second isoform of prostaglandin endoperoxide H synthase (PGHS, or cyclooxygenase), there has been considerable interest in the question of why two isoforms of this enzyme are necessary and what roles they might play. PGHS-1-deficient (1) and PGHS-2-deficient (2, 3) mice and isoform-specific inhibitors have been developed and used to investigate the physiological functions of PGHS-1 and PGHS-2. These studies suggest that there are processes in which each isozyme is uniquely involved (e.g., platelet aggregation for PGHS-1, ovulation for PGHS-2) and others in which both isozymes function coordinately (e.g., carcinogenesis, inflammation). There are also physiological events in which one PGHS isozyme normally functions but for which the other can compensate when the first is lacking (e.g., parturition and remodeling of the ductus arteriosus). Biochemical studies indicate that each isoform can function independently; namely, that there are distinct PGHS-1 and PGHS-2 prostanoid biosynthetic pathways. Thus, the unique physiological roles for each isozyme can be rationalized by what is known about the biochemistry of the enzymes. To facilitate discussion of the physiological functions of PGHS-1 and PGHS-2 and to point out those functions for which PGHS-1 and PGHS-2 can substitute for one another, we describe the following in sequence: (a) physiological processes that depend solely or primarily on PGHS-1, (b) physiological processes that depend solely or [...]

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Since the discovery in 1991 of a second isoform of prostaglandin endoperoxide H synthase (PGHS, or cyclooxygenase), there has been considerable interest in the question of why two isoforms of this enzyme are necessary and what roles they might play. PGHS-1-deficient (1) and PGHS-2-deficient (2, 3) mice and isoform-specific inhibitors have been developed and used to investigate the physiological functions of PGHS-1 and PGHS-2. These studies suggest that there are processes in which each isozyme is uniquely involved (e.g., platelet aggregation for PGHS-1, ovulation for PGHS-2) and others in which both isozymes function coordinately (e.g., carcinogenesis, inflammation). There are also physiological events in which one PGHS isozyme normally functions but for which the other can compensate when the first is lacking (e.g., parturition and remodeling of the ductus arteriosus). Biochemical studies indicate that each isoform can function independently; namely, that there are distinct PGHS-1 and PGHS-2 prostanoid biosynthetic pathways. Thus, the unique physiological roles for each isozyme can be rationalized by what is known about the biochemistry of the enzymes.

To facilitate discussion of the physiological functions of PGHS-1 and PGHS-2 and to point out those functions for which PGHS-1 and PGHS-2 can substitute for one another, we describe the following in sequence: (a) physiological processes that depend solely or primarily on PGHS-1, (b) physiological processes that depend solely or primarily on PGHS-2, and (c) processes in which both PGHS-1 and PGHS-2 are involved and act coordinately. We then summarize the biochemical evidence for distinct PGHS-1 and PGHS-2 biosynthetic pathways.

Physiological processes for which PGHS-1 is responsible

Platelet aggregation. Mature platelets are essentially non-nucleated cellular vesicles that mediate hemostasis by aggregating in response to arterial injury. Platelets can form thromboxane A₂ (TXA₂) from arachidonic acid (AA) supplied either exogenously or generated from endogenous phospholipid stores (see Brash, this Perspective series, ref. 4). TXA₂ exits the cells and acts via the platelet TXA₂ receptor (TP) to initiate platelet shape

changes and subsequent platelet aggregation (5). TXA₂ can also act via TP receptors to cause constriction of vascular smooth muscle (5). Platelets express PGHS-1 but not PGHS-2. Thus, TXA₂ is formed by platelets only via PGHS-1 (see Table 1) (6). Not surprisingly, platelets from PGHS-1-null mice fail to undergo AA-induced aggregation (1), indicating that PGHS-2 cannot compensate for an absence of PGHS-1 in platelets. Low-dose aspirin, used therapeutically as an anti-thrombogenic agent, targets platelet PGHS-1 (7).

Parturition. The PGHS isozymes play distinct physiological roles in female reproduction. Early studies demonstrated that PGHS-1-null female mice produced litters of normal size but had difficulty with parturition (1), whereas other aspects of the reproductive process in PGHS-1-null mice were normal (8). Gross et al. (9) also found that the onset of parturition was delayed in PGHS-1-null mice and observed that administration of PGF_{2α} resulted in the onset of labor, suggesting that PGHS-1 is the source of the PGF_{2α} (Table 1). However, treatment with LPS, a bacterial component that induces cellular responses associated with septicemia, induces PGHS-2 and allows parturition to proceed, even in PGHS-1-null mice. Use of SC-236, a PGHS-2-selective nonsteroidal anti-inflammatory drug (NSAID), blocks the onset of parturition in these LPS-treated mice (10). Thus, under certain pathological conditions, PGHS-2 can also produce the PGF_{2α} needed to initiate parturition.

Physiological processes for which PGHS-2 is responsible

Ovulation and implantation. PGHS-2-null female mice are infertile (3). Although follicular development is observed in PGHS-2-deficient mice, ovulation is greatly reduced and, of the eggs released, very few are fertilized (8). Ovarian PGE₂ production is increased by pituitary gonadotropins in wild-type and PGHS-1-null mice, but not in PGHS-2-null mice, indicating that PGHS-2 is responsible for this increased ovarian PGE₂ production. Because ovulation in PGHS-2-null mice can be restored by PGE₂ administration (11), PGE₂ formed via PGHS-2 is believed to be the product that is responsible for ovulation. In addition to affecting ovu-

lation, PGHS-2 deficiency also impedes blastocyst implantation and decidualization. PGI₂ has been demonstrated to be the prostanoid involved in this process, and it is also formed through PGHS-2 (Table 1) (12). Interestingly, although prostanoids appear to act primarily via G protein-linked receptors (5), the nuclear peroxisomal proliferator-activated receptor PPAR δ appears to mediate the action of PGI₂ in implantation (12), suggesting that alternative routes may exist by which PGI₂ can influence gene expression.

Neonatal development. Although it is widely believed that PGHS-1 acts in development, there are few reports available to support this claim, and recent studies suggest that PGHS-2 plays a more important role, at least in neonatal development (13, 14). PGHS-2-deficient mice develop a severe renal pathology that is not mimicked by administration of NSAIDs to adult mice (2, 3). Initially, some investigators believed that this phenotype represented a compensatory artifact of the PGHS-2 knockout, but Komhoff et al. (13) showed recently that postnatal treatment with a PGHS-2-selective NSAID caused a severe reduction in glomerular diameter in the neonatal mouse kidney. This was the same renal pathology as seen in PGHS-2-null mice (13) and could not be caused by treating adult mice with the PGHS-2-selective inhibitor.

Another neonatal event in the mouse in which PGHS-2 has a key role is in the closure of the ductus arteriosus (14). Although the ductus closes normally in PGHS-1-null mice, about 35% of PGHS-2-null mice die with a patent ductus within 48 hours of birth. In wild-type mice, PGHS-2, but not PGHS-1, is seen by immunohistochemistry to be significantly induced in the smooth muscle cells of the ductus during closure; however, the fact that 65% of COX-2-null mice survived to weaning suggests that PGHS-1 can play a compensatory role. Indeed, a reduction in *PGHS-1* gene dosage from wild-type to heterozygosity further increases

the incidence of patent ductus arteriosus and decreases the 48-hour survival of PGHS-2-null mice to about 20%. Because a role for PGHS-1 is only evident when PGHS-2 is absent, it is unlikely that PGHS-1 is involved in ductus closure in wild-type mice.

These examples indicate that PGHS-2 has key roles during postpartum development. Because the birthing process involves a number of physiological changes and stresses that could induce PGHS-2, and because PGHS-2-null mice have decreased survival at all ages, it may be useful to search for other neonatal tissues in which PGHS-2 induction promotes normal neonatal development.

Processes involving both PGHS-1 and PGHS-2

Inflammation and wound healing. The relative contributions of PGHS-1 and PGHS-2 to inflammatory responses are incompletely resolved. Since the identification of the PGHS-2 isoform, the hypothesis has been that PGHS-2 is the primary source of prostanoids that contribute to inflammation. However, several recent studies show that prostanoids formed via PGHS-1 are also involved (1, 15).

Interestingly, some recent studies have indicated that PGHS-2 acts in both the initiation of the inflammatory response and in the resolution phase. Gilroy et al. (16) recently reported that PGHS-2 expression and PGE₂ levels increased transiently early in the course of carrageenan-induced pleurisy in rats. Later in the response, PGHS-2 was induced again to even greater levels and generated anti-inflammatory prostaglandins, such as PGD₂ and 15-deoxy- Δ^{12-14} -PGJ₂, but only low levels of the proinflammatory PGE₂ (Table 1). Further support for an anti-inflammatory role of PGHS-2 in this model was the finding that late administration of the PGHS-2-selective inhibitor NS-398 exacerbates the inflammatory response. Furthermore, Wallace et al. (15) observed that in the paw carrageenan inflammation model, the

Table 1
Isoform-specific physiological functions of PGHS-1 or PGHS-2

Physiological process	PGHS-1	PGHS-2	Prostaglandin involved
Ovulation	Not essential	Essential (8, 11)	PGE ₂
Implantation	Not essential	Essential (8)	PGI ₂
Parturition	Essential (1, 9)	Compensatory (10)	PGF _{2α}
Inflammation resolution	Not essential	Essential (16)	PGD ₂ , 15-deoxy-PGJ ₂
Platelet aggregation	Essential (1)	No role	TXA ₂
Perinatal kidney development	Not essential (1)	Essential (13)	Not determined
Ductus arteriosus remodeling	Compensatory	Essential (14)	TXA ₂ /PGH ₂
T cell development	Stage-specific	Stage-specific (37)	PGE ₂
Gastric ulceration	Inhibition of both isoforms necessary (17)		Not determined
Ulcer healing	Not essential	Essential (15, 18)	Not determined
Intestinal cancer	Both isoforms have essential roles (20)		Not determined
Crypt stem cell survival	Essential (21)	Compensatory (38)	PGE ₂

inflammation resolves within 7 days in wild-type mice but is unmitigated over this period in PGHS-2-deficient mice. Thus, PGHS-2 appears to have two roles in the inflammatory process, initially contributing to the onset of inflammation and later helping to resolve the process.

Gastric ulceration. The inhibition of PGHS-1 by NSAIDs has for many years been considered the biochemical event responsible for gastric ulcer development. It was therefore surprising that PGHS-1-deficient mice had a 99% reduction in gastric PGE₂ levels but did not spontaneously develop ulcers (1). Initially, many researchers attributed this absence of gastric ulceration in PGHS-1-null mice to compensatory mechanisms. However, more recently, a novel PGHS-dependent mechanism of NSAID-induced gastric ulceration has been reported (17). In these studies, neither the PGHS-1-selective inhibitor SC-560 nor the PGHS-2-selective inhibitor celecoxib sufficed to induce ulcers, but gastric damage occurred when the two NSAIDs were administered in combination. These studies may explain why PGHS-1-deficient mice do not spontaneously develop ulcers. Presumably, if inhibition of PGHS activity is the sole mechanism of NSAID-induced ulceration, administering PGHS-1 inhibitors to PGHS-2-null mice or PGHS-2 inhibitors to PGHS-1-null mice would be ulcerogenic.

In addition to its role in resolving inflammation (15, 16), PGHS-2 has been shown to promote ulcer healing. Indomethacin-induced ulcers heal poorly in PGHS-2-null mice compared with wild-type mice and ultimately prove fatal (15). Like the PGHS-2-deficient mice, PGHS-2 selective NSAIDs prolong the healing of ulcers in rats and mice (18, 19). Thus, as in inflammation, there are roles for PGHS-2 in both the onset and resolution of a disease state, a matter that may be relevant when considering chronic use of PGHS-2-selective drugs.

Carcinogenesis. To date, most studies have implicated PGHS-2, rather than PGHS-1, as the isoform involved in colon carcinogenesis. In a recent study, however, we determined that deficiency for either PGHS-1 or PGHS-2 reduces polyp formation in *Min*^{+/+} mice about equally (~80%; ref. 20). These studies raise the question of whether PGHS-1 and PGHS-2 contribute to tumorigenesis through common or different mechanisms. Only a speculative answer to this question can be given at present. Based on recent work by Houchen et al. (21), it is possible that PGHS-1 functions in early stages of tumorigenesis by protecting initiated stem cells from DNA damage-induced death, whereas PGHS-2 contributes to tumor promotion after loss of heterozygosity of the *APC* gene has occurred (22). Studies by Chulada et al. (20) indicate that at the adenoma stage of tumor development, PGHS-2 is present in the interstitial cells rather than the epithelial cells of the tumor, and

thus, that prostanoids formed via PGHS-2 exert a paracrine rather than an autocrine effect to promote tumor growth. Work with PGHS null mice indicates that both isozymes contribute to the increased PGE₂ production seen in polyps. Overall, these results suggest that both PGHS-1 and PGHS-2 play key roles in intestinal tumorigenesis and that PGHS-1 may also be an effective chemotherapeutic target for NSAIDs.

Biochemical evidence for unique PGHS-1 and PGHS-2 biosynthetic pathways

For there to be isozyme-specific effects, PGHS-1 and PGHS-2 must be able to function independently at the biochemical level. This can be achieved simply by segregating the isozymes into different cells. However, in many instances PGHS-1 and PGHS-2 are coexpressed in the same cells. Under the latter conditions, the independent functioning of PGHS isozymes appears to involve metabolic rather than physical segregation of these enzymes. The two PGHS isozymes are found predominantly in the same organelles, at the luminal surfaces of the endoplasmic reticulum and nuclear envelope of cells (23), although it is conceivable that each isoform is localized to a specific subdomain of these membranes. Neither enzyme appears to interact directly with cPLA₂ or sPLA₂s, the phospholipases that mobilize AA. It is possible that PGHS-1 or PGHS-2 can bind to the downstream synthases, such as TXA₂ synthase, PGI₂ synthase, and/or microsomal (m) PGE synthase (all of which enzymes are associated with the endoplasmic reticulum; refs. 24, 25); however, to date, there is no evidence for such interactions.

PGHS-2-independent functions of PGHS-1

When PGHS-1 and PGHS-2 are both expressed in the same cells, supplying AA either exogenously or via activation of cellular lipases always leads to the functioning of PGHS-2. Only when PGHS-2 is absent does PGHS-1 appear to function independently; but because PGHS-1 is a constitutive enzyme, whereas PGHS-2 is inducible, this is actually the usual situation. PGHS-2 is generally present in cells only during early stages of cell differentiation or replication (25). There is considerable literature on the regulation of PGHS-2 gene expression, but there is little information about how the expression of PGHS-1 is controlled (25).

PGHS-1-independent functions of PGHS-2

In vitro at high substrate/enzyme ratios, PGHS-1 and PGHS-2 have quite similar kinetic properties including their K_m values for AA (~5 μM; ref. 25). However, the K_m value of PGHS-1 for AA appears to be higher than that of PGHS-2 when the enzymes are studied in cultured cells (~2 μM and 10 μM, respectively; refs. 26, 27). Thus, in intact cells expressing both isoforms, PGHS-2 can function in the presence of a nonfunctioning PGHS-1

if the AA concentrations are kept relatively low. This mechanism may account for the fact that prostanoids formed in the so-called “late phase,” a period defined as occurring 2–6 hours after treating cells with tumor promoters, cytokines, or growth factors, are formed primarily via PGHS-2.

The intracellular concentration of free AA depends on competing actions of various lipases, which mobilize arachidonate from phosphoglycerides, and CoA ligases, which scavenge free fatty acids. There is a consensus that cPLA₂ is involved in mobilizing AA for PGHS-1 but that prostaglandin formation during the late phase and involving PGHS-2 depends on both cPLA₂ and one or more sPLA₂s (see Fitzpatrick and Soberman, this Perspective series, ref. 28; see also refs. 27 and 29–31).

The basis of the apparent K_m differences between PGHS-1 and PGHS-2 in intact cells is not clear. However, this situation can be mimicked in vitro with purified PGHSs when measuring rates of product formation at low concentrations of both AA and lipid hydroperoxide. The biochemical explanation for this rather complex type of regulation is as follows: PGHSs catalyze both a cyclooxygenase reaction, in which AA plus two O₂ molecules are converted to PGG₂, and a peroxidase reaction, in which PGG₂ is reduced to PGH₂ (ref. 25; see also Serhan and Oliw, this Perspective series, ref. 32). For the cyclooxygenase activities of PGHS-1 and PGHS-2 to function, the heme group at the peroxidase site must undergo a lipid peroxide-dependent oxidation. The oxidized heme then oxidizes a tyrosine in the cyclooxygenase site that, in turn, abstracts a hydrogen atom from AA, yielding an AA radical that reacts with O₂ to form PGG₂ (25) (itself a lipid peroxide). The concentration of lipid peroxide required to activate the cyclooxygenase activity of PGHS-1 is about ten times higher than that necessary for PGHS-2 in the presence of low AA concentrations (33). Thus, a combination of (a) low endogenous peroxide concentrations within the lumen of the endoplasmic reticulum, where PGHS-1 and PGHS-2 are predominately localized in intact cells (23) and (b) AA concentrations below 2 μM (i.e., at relatively high enzyme/substrate ratios; refs. 33, 34) would favor the operation of the cyclooxygenase activity of PGHS-2 over that of PGHS-1. Regardless of the mechanism, it appears that the cyclooxygenase activities of PGHS-1 and PGHS-2 are controlled differentially by regulating the amount of lipid peroxide and AA available to the enzymes.

Alternative substrates for PGHS-2. There are subtle differences in the fatty acid substrate specificities between PGHS-1 and PGHS-2 (25), but these are probably of little biologic significance beyond ensuring that both enzymes act on AA in preference to other fatty acid substrates. However, PGHS-2 but not PGHS-1 can use esterified fatty acid substrates (35). One example is 2-arachidonyl glycerol, which can be generated from

phosphatidylinositol by the sequential actions of phospholipase C and diglyceride lipase (35). 2-Arachidonyl glycerol is formed by a mouse macrophage line and converted to 2-PGD₂ glycerol via PGHS-2 and PGD synthase (35). This provocative observation raises a host of questions regarding the relative importance of the classical and alternative pathways. For example, it is possible that AA is normally not even the physiologically relevant substrate for PGHS-2. Considerable further work needs to be done on this topic, including determining whether 2-PGH₂ glycerol can be converted to 2-prostanoid glycerols other than 2-PGD₂ glycerol (e.g., 2-PGI₂ glycerol).

Coupling of PGHS-1 and PGHS-2 to downstream synthases. A final way in which PGHS-1 and PGHS-2 biosynthetic pathways may be dissociated metabolically is by a preferential coupling of the isoforms to various downstream synthases (see Fitzpatrick and Soberman, this Perspective series, ref. 28). PGHS-2 appears to be coupled metabolically to PGI₂ formation in the uterus in association with decidualization and implantation (12). There also appears to be an mPGE synthase that is coordinately induced with PGHS-2 and seems to function preferentially with PGHS-2, rather than PGHS-1 (24, 36).

Concluding remarks

The data obtained with PGHS-1 and PGHS-2 deficient mice clearly show differences in some physiological actions of the two isoforms, and some observed physiological roles are quite different from those originally expected. For example, it was quite surprising that mice deficient in PGHS-1, the constitutive isoform believed to be responsible for homeostasis, are healthy and live a normal life span, whereas mice lacking PGHS-2 expression, the inducible isoform thought to be expressed primarily in disease states, display various developmental problems, female reproductive disorders, and a shortened life span. Most recent studies have demonstrated a concordance between the physiological effects of the genetic deficiency of the PGHS isoforms and isoform-specific or -non-specific effects of NSAIDs. However, both the drug studies and the studies of PGHS deficient mice have their limitations. Whereas NSAIDs may cause some of their effects by mechanisms other than PGHS inhibition, compensation mechanisms or developmental changes may influence the responses of PGHS-deficient mice. Inflammation, for example, cannot be fully elucidated with PGHS-2-deficient mice, as PGHS-2 acts at two different steps in this process, initiation and resolution. Other prostaglandin-regulated responses may also require PGHSs to function at multiple steps. On the other hand, PGHS-null mice probably do model the development of certain diseases caused by PGHS inhibitors. Furthermore, in the PGHS-null mice there are examples of the remaining isoform being upregulated and compensating for the missing isoform; further studies will be needed to deter-

mine whether such upregulation contributes to the normal physiological process as it occurs in wild-type animals. Future biochemical studies will likely focus on the mechanisms by which different lipases and downstream synthases are coupled physically or metabolically to PGHS-1 and/or PGHS-2, why the K_m values for PGHS-1 and PGHS-2 are different in intact cells, and whether AA, 2-arachidonyl glycerol or another lipid is the preferred substrate for PGHS-2.

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