

Dominance of Cyclooxygenase-2 in the Regulation of Pancreatic Islet Prostaglandin Synthesis

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Dramatic, scientifically important discoveries in prostaglandin (PG) pharmacology and physiology have taken place over the past decade. Chief among these discoveries is the identification of two separate forms of cyclooxygenase (COX), a constitutive and an inducible form, both of which exist in most tissues. The pancreatic islet is an exception to this rule because it continually and dominantly expresses the inducible form, COX-2. It has also been learned that nonsteroidal anti-inflammatory drugs affect the two forms of COX with different potencies, a finding with far-reaching clinical implications. An equally important finding is that PGE₂, which is known to negatively modulate glucose-induced insulin secretion, has at least four different subtypes of receptors with different mechanisms of action and metabolic consequences. These recent changes in our understanding of the molecular regulation of PG synthesis call for a reconsideration of previous hypotheses involving PGE₂ as a regulator of β -cell function in physiological and pathophysiological states. *Diabetes* 47:1379–1383, 1998

For more than a century, drugs that inhibit synthesis of prostaglandins (PGs) have been known to improve glucose disposal. For more than two decades, PGE₂ has been known to negatively regulate pancreatic islet function. Yet, despite these facts and the flurry of publications about arachidonic acid metabolites and diabetes that began in the mid-1970s, a notable lull in progress settled in during the mid-1980s. What happened?

I think the explanation is tripartite. First, diabetes investigators grew weary of keeping up with the ever-proliferating array of PGs, thromboxanes (TXs), leukotrienes, and hydroxyecosatetraenoic acids (HETEs). Second, an uneasiness arose regarding the concept that PGs participate in islet dysfunction, because use of conventional nonsteroidal anti-inflammatory drugs (NSAIDs) to inhibit cyclooxygenase (COX) failed to improve glucose control in diabetic patients.

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COX, cyclooxygenase; IL, interleukin; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; TX, thromboxane.

Third, and most important, basic research in arachidonic acid metabolism in the 1980s failed to keep pace with the molecular biologic advances so pervasive in other areas. Consequently, development of synthesis inhibitors of specific steps in arachidonic acid metabolism was sluggish, and few new insights evolved about postreceptor mechanisms of action for PGs.

Fortunately, in the past decade, adaptation of molecular techniques to this area has made radical changes in our thinking about PG pharmacology and physiology (1). The most significant new developments are the identification of two separate forms of COX (a constitutive and an inducible form), redefinition of sites of action for PG synthesis inhibitors, and identification of subtypes of PGE receptors with different postreceptor mechanisms of action. These new research findings have broad implications for our understanding of the possible roles of PGs in pancreatic islet physiology and the pathogenesis of diabetes. It is tempting to include in this Perspective other important new information about enzymatic regulation of the other arachidonic acid pathways, the receptors for the products formed, and the drugs that influence them. However, I will not discuss these other issues for two reasons. First, this additional information, although abundant, is less immediately relevant to the islet and diabetes. Second, it seems wise in this case to choose discretion rather than to impose on the reader's valor, lest weariness should set in once again.

TWO FORMS OF COX

Until the late 1980s, it was accepted that one enzyme, COX, regulated the conversion of arachidonic acid into the intermediate endoperoxides that subsequently form PGD₂, PGE₂, PGF_{2 α} , PGI₂, and TXA₂, which are the prostanoids most relevant to human physiology. This dogma has changed for several reasons. It is now clear that two forms of COX exist (Fig. 1). COX-1 is the constitutive form, whose products are thought to play a role in modulating physiological, basal activity in tissues. COX-2, the regulated form, is associated with inflammatory or stimulated events in tissues and responds to specific inducers. One of the earliest clues that two forms of COX exist was provided by experiments in epithelial cells isolated from sheep tracheal mucosa; these experiments demonstrated that a 70-kDa COX protein and a 2.8-kb COX mRNA were coordinately expressed (2). The surprise was that the mRNA levels did not increase as expected when COX activity was stimulated. Rehybridization of Northern blots at lower stringency identified a 4.0-kb mRNA COX

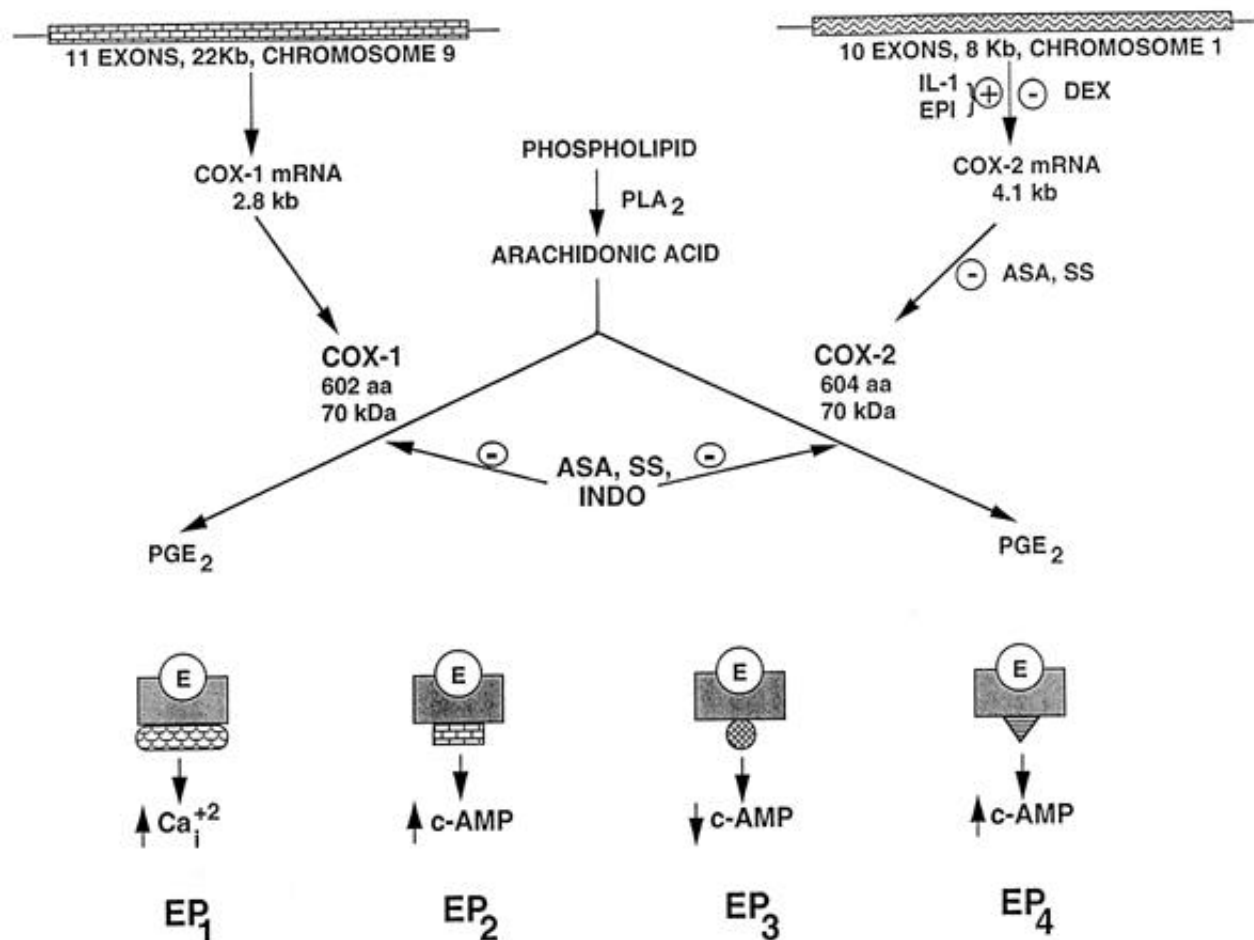


FIG. 1. Molecular regulation of PG synthesis. The COX-1 and COX-2 genes control synthesis of the COX-1 and COX-2 enzymes, respectively. The two genes are located on separate chromosomes and give rise to mRNAs of different sizes. The two enzymes are approximately the same size but have approximately 60% homology. Both enzymes use arachidonic acid as substrate to generate biologically active prostanoids (PGD₂, PGE₂, PGF_{2α}, PGI₂, and TXA₂), here represented by PGE₂. Once PGE₂ is formed, it can interact with any one subtype or all four subtypes of EP receptors. Cytokines, such as interleukin I (IL-1), and epinephrine (EPI) increase gene expression of COX-2 mRNA but do not affect COX-1 mRNA levels. Dexamethasone (DEX) diminishes the induction of COX-2 gene expression. Acetylsalicylic acid (ASA) and sodium salicylate (SS) decrease the mass of COX-2 enzyme translated and processed after COX-2 mRNA levels are increased. These two NSAIDs and others, represented by indomethacin (INDO), also inhibit activation of the COX-1 and COX-2 enzymes. None of the NSAIDs affect expression of the COX-1 gene or translation and processing of the COX-1 enzyme. Adapted from Robertson (1).

species whose expression did increase coordinately with COX activity. The authors concluded that two genes may exist that are responsible for synthesis of COX. At about the same time, work by Fu et al. (3) using endotoxin and human monocytes suggested the existence of two pools of COX. After several more years, separate COX-1 and COX-2 genes were cloned (4), thereby irrefutably establishing the existence of two forms of the enzyme and making essential a complete overhauling of our concepts of enzymatic regulation of PG synthesis.

PG SYNTHESIS INHIBITORS: REDEFINING SITES OF ACTION

During the 1970s and 1980s, it was accepted that NSAIDs decreased PG levels by inhibiting the activity of a single COX. Moreover, the action of corticosteroids to decrease PG levels was generally thought to be related to inhibition of arachidonic acid cleavage from phospholipid, a step regulated by phospholipase A₂.

These tenets began to change in 1991. Wu et al. (5) observed that interleukin (IL)-1-induced synthesis of COX

protein could be diminished by pretreatment of cultured human umbilical vein endothelial cells with aspirin or sodium salicylate. In contrast, pretreatment with indomethacin had no apparent effect. They also reported that aspirin suppressed expression of the 2.7-kb form of COX mRNA. These observations added an important new dimension to our thinking about sites of actions for NSAIDs, emphasizing that aspirin and sodium salicylate can decrease COX synthesis as well as inhibit its activity (Fig. 1).

A new site of action for glucocorticoids was also identified by Crofford et al. (6) in experiments using synovial tissue from patients with rheumatoid arthritis. Using Western blot analysis, they demonstrated two forms of COX protein in freshly explanted rheumatoid synovial tissues. De novo synthesis of the inducible form of COX protein was enhanced by IL-1 or phorbol myristic acid and was markedly suppressed by dexamethasone. They further demonstrated that COX-2 mRNA was greatly increased by IL-1 or phorbol myristic acid and suppressed by dexamethasone. This report suggested that a major site of the inhibitory action of corticosteroids involves

TABLE 1
Nomenclature and mechanisms of action for receptor subtypes of prostanoids

Receptor subtype	Mechanisms of action
DP	Stimulation of adenylyl cyclase and increased cAMP levels via G _s
EP	
EP ₁	Stimulation of phosphatidylinositol turnover and elevation of intracellular free Ca ²⁺ via G _q
EP ₂	Stimulation of adenylyl cyclase and increased cAMP levels via G _s
EP ₃	Inhibition of adenylyl cyclase and decreased cAMP levels via G _{i/o}
EP ₄	Stimulation of adenylyl cyclase and increased cAMP levels via G _s
FP	Stimulation of phosphatidylinositol turnover and elevation of intracellular free Ca ²⁺ via G _q
IP	Stimulation of adenylyl cyclase and increased cAMP levels via G _s
TP	Stimulation of phosphatidylinositol turnover and elevation of intracellular free Ca ²⁺ via G _q

gene expression of COX-2 (Fig. 1), a timely observation given the mounting concern caused by the concept that the major site of action for glucocorticoids is inhibition of phospholipase A₂ activity (3,7).

Next, an evaluation of the concentrations of various NSAIDs required to inhibit the activity of COX-1 and COX-2 appeared (8). Aspirin, indomethacin, ibuprofen, acetaminophen, and sodium salicylate were reported to be more potent as inhibitors of COX-1 than of COX-2, whereas diclofenac and naproxen were more potent in inhibiting COX-2 than COX-1. These observations carry important therapeutic implications, because aspirin and indomethacin, often used to combat inflammation, were far more effective in inhibiting COX-1, which is thought not to mediate inflammation but rather to modulate normal physiological processes, possibly accounting for some of the undesirable side effects of anti-inflammatory drugs not specific for COX-2. Another twist in this tale of two enzymes involved sodium salicylate, aspirin, and the transcription factor NF-κB. This factor plays an important induction role in the expression of many genes involved in inflammation and infection. Sodium salicylate and aspirin were reported to inhibit the activation of NF-κB (9), a contention of clear relevance to molecular regulation of PG synthesis because the COX-2 gene has an NF-κB binding site (10). Thus, NSAIDs may have an additional site of action on PG synthesis, namely, the COX-2 promoter.

PG RECEPTOR SUBTYPES

Since 1972, PGs have been known to have specific binding sites on cell plasma membranes (11). Radiolabeled PG binding studies using tritiated PGD₂, PGE₁, PGE₂, PGF_{2α}, and PGI₂ have been reported by many investigators (12). The examined tissues include cellular derivatives of fat, liver, adrenal cortex and medulla, ovary (corpus luteum), uterus, kidney, stomach, ileum, thymus, skin, brain, lung, pancreatic islets, and various blood products, including platelets, red blood cells, neutrophils, macrophages, and monocytes. Increasingly, G-proteins were found to contribute signifi-

cantly to PG postreceptor mechanisms of action, an observation relevant to the islet (13,14).

In the mid-1990s, a new classification, involving five types of receptors corresponding to the five naturally occurring prostanoids (PGD₂, PGE₂, PGF_{2α}, PGI₂, and TXA₂), was proposed (15,16). In this nomenclature, binding sites are referred to as P receptors, and a preceding letter indicates the prostanoid to which each receptor is most sensitive. Consequently, the terms DP, EP, FP, IP, and TP are used (Table 1). EP receptors have been grouped into four subtypes and termed EP₁, EP₂, EP₃, and EP₄, based on the relative sensitivities of the receptors to various selective agonists and antagonists.

In this system of classification, the mechanism of action of DP receptors involves stimulation of adenylyl cyclase. FP receptors, like EP and TP receptors, are coupled to stimulation of phosphoinositide hydrolysis. The mechanism of action of IP, EP₂, and EP₄ receptors also involves stimulation of adenylyl cyclase activity. Only the EP₃ receptor is associated with inhibition of adenylyl cyclase. Thus, although there are specific receptors for specific PGs, and at least four different receptor subtypes in the case of PGE₂, some of these different receptors are coupled and share biochemical pathways.

Although the genes responsible for synthesis of the various receptor subtypes have been cloned, less is known about regulation of expression of these genes compared with our knowledge of regulation of the COX-2 gene. Moreover, achievement of a complete understanding of the molecular biology of prostanoid receptors is complicated by the existence of receptor subtypes. For example, two groups of investigators have reported two isoforms of the EP₃ receptor subtype, namely, EP_{3α} and EP_{3β} (17,18), and different EP receptor cDNAs have been reported to arise by alternatively spliced variants from a single gene (19,20).

DOMINANCE OF COX-2 IN THE PANCREATIC ISLET: AN EXCEPTION TO THE RULE

Intrigued with these exciting developments, my laboratory staff set out in 1996 to ascertain how well the pancreatic islet, and the β-cell specifically, conform to the new rules of PG pharmacology. We were surprised to find only one COX mRNA in various nonstimulated and stimulated preparations, including HIT-T15 cells, Syrian hamster islets, and human islets (21). This observation led us to the bigger surprise that COX-2 was the sole form present, and that COX-2 gene expression was present to a great degree under nonstimulated conditions, yet it increased modestly with IL-1 treatment and diminished with dexamethasone treatment (Fig. 2). Control experiments with 3T3 cells and non-islet tissues from Syrian hamsters revealed the expected predominance of COX-1 mRNA under basal conditions. Dominance of COX-2 gene expression in islet tissue was found consistently using Northern blot analysis, reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot analysis, PGE₂ enzyme immunoassay, and a COX-2 specific inhibitor (19).

This consistent finding of COX-2 dominance led us to conduct experiments assessing promoter activity of the COX-2 gene, which revealed that IL-1 causes parallel changes in promoter activity and COX-2 mRNA. We sought to account for the exaggerated nonstimulated levels of COX-2 mRNA by using HIT-T15 cells, electrophoretic mobility shift assays for

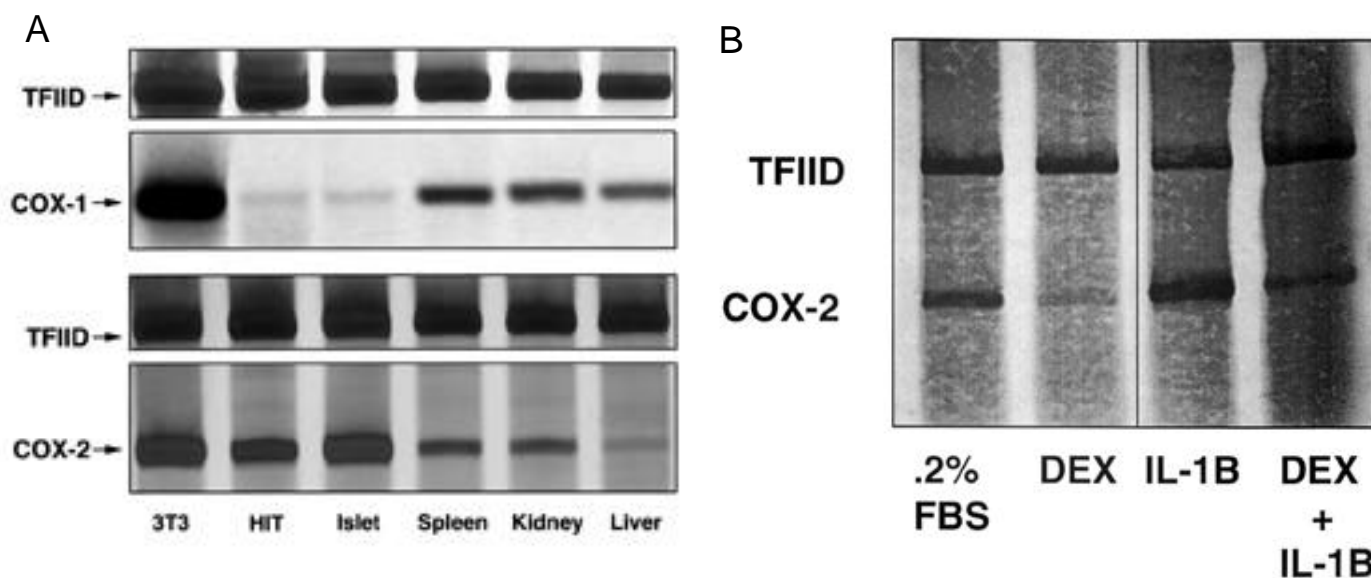


FIG. 2. *A:* Reverse transcriptase–polymerase chain reaction (RT-PCR) for COX-1 and COX-2 expression in Syrian hamster tissues. Total RNA was extracted from 3T3 and HIT-T15 (HIT) cells cultured with 10% fetal bovine serum (FBS) and from Syrian hamster islets, spleen, kidney, and liver. Single-stranded cDNA transcribed from total RNA was used for polymerase chain reaction amplification with COX-1-, COX-2-, and transcription factor II D (TFIID)-specific primers. Amplified cDNAs were analyzed by 6% nondenaturing PAGE with visualization by silver staining. Detection of COX-1 mRNA in HIT cells and Syrian hamster islets was barely detectable, whereas COX-1 mRNA was readily detectable in 3T3 cells and in Syrian hamster spleen, kidney, and liver in this study and in two other separate experiments. In contrast, RT-PCR readily demonstrated COX-2 mRNA in HIT cells and hamster islets, whereas smaller amounts were observed in the other tissues. *B:* RT-PCR for COX-2 expression in human pancreatic islets. Human islets were cultured in RPMI-1640/0.2% FBS for 18 h and then incubated for 3 h in one of the following conditions: 1) control, 2) dexamethasone (DEX) (1 $\mu\text{mol/l}$), 3) IL-1 (5 ng/ml), or 4) DEX followed by an additional incubation for 2 h with IL-1 (5 ng/ml). RT-PCR readily detected COX-2 mRNA in human pancreatic islets cultured in media containing 0.2% FBS; greater levels were observed in human islets cultured in media containing 10% FBS in this study and in two other separate experiments. COX-2 mRNA levels also increased when islets were cultured in media containing 0.2% FBS and IL-1. Pretreatment with DEX suppressed levels of COX-2 mRNA cultured with either 0.2% FBS or IL-1 present in the media. Adapted from Sorli et al. (21).

two transcription factors (NF-IL6 and NF- κ B), and construction of mutant reporter genes deficient in binding sites for these two factors. We found surprisingly high levels of NF-IL6, and only modest amounts of NF- κ B, in the nonstimulated state. That basal COX-2 gene expression might depend on high basal NF-IL6 levels was suggested by our reporter gene experiments, in which promoter activity was greatly reduced when using the NF-IL6, but not the NF- κ B, mutant. On stimulation with IL-1, levels of these transcription factors changed reciprocally and corresponded temporally with biphasic changes in COX-2 mRNA. By the end of these studies, we concluded that COX-2 is dominantly expressed in the islet under both nonstimulated and stimulated conditions, a situation reported thus far in only rat brain (22), renal tissue (23), bronchial tissue (24), and granulosa cells (25) and recently confirmed in the islet (26). We hypothesized that this unusual situation in the islet may be related to high basal levels of NF-IL6 (21).

IMPLICATIONS FOR ISLET FUNCTION AND DIABETES PATHOGENESIS

The new knowledge that there are two forms of COX, that NSAIDs have more complicated effects on the PG synthetic pathway than originally envisioned, and that there are at least four (if not more) PGE₂ receptors with different postreceptor consequences has enormous implications for the interpretation of previously published data involving PGs and islet function. Attempting to reanalyze the 100 or more publications in this research area in light of this new infor-

mation is obviously beyond the scope of this Perspective. However, it needs to be pointed out that pivotal past experiments claiming to seat or unseat PGs as major players in islet function need to be repeated with the use of more modern technology and our greater knowledge of sites of action of drugs that influence PG synthesis.

What implications might the dominance of COX-2 in the islet have for islet function and the pathogenesis of diabetes? It seems reasonable to assume that under physiological circumstances the islet tonically synthesizes PGE₂, a process known to be stimulated by glucose (27). Because PGE₂ is an inhibitor of glucose-induced insulin secretion (28), it follows that a high basal activity of COX-2 might serve to modulate insulin release during fuel stimulation in the physiological state. On the other hand, should COX-2 activity be upregulated excessively, consequent overproduction of PGE₂ might lead to glucose intolerance and play a role in the pathogenesis of type 2 diabetes, as has been suggested (29). In previous assessments of this hypothesis, various NSAIDs were used to inhibit PGE₂ synthesis, and they usually provided mixed or, at best, modest beneficial effects on insulin secretion in type 2 diabetic patients (30). However, we now know that these older drugs are primarily directed against COX-1 and that COX-1 is virtually absent in the islet. It follows that these experiments should be repeated using newer COX-2-specific inhibitors to ascertain whether they improve insulin secretion in type 2 diabetes, which would favor the hypothesis that excessive endogenous release of PGE₂ contributes to the pathogenesis of this disease.

In the case of type 1 diabetes, which seems to involve IL-1, it seems reasonable to assume that the high basal COX-2 activity present normally in the islet is stimulated even higher by this cytokine. The consequence of inhibiting insulin release in this scenario could be viewed as either bad or good for the patient. On the one hand, increased PGE₂ would inhibit insulin release and worsen the developing hyperglycemia. On the other hand, to the extent that β -cell rest staves off the development of type 1 diabetes, IL-1-stimulated PGE₂ production might represent a host defense mechanism. Again, these speculations are readily subject to evaluation through use of the newer COX-2-specific antagonists.

Whatever conclusions emanate from renewed work in this area, further research on the crossroads where islet function and PG action converge is bound to clarify old scientific conundrums as well as introduce interesting new twists in the physiology and pathophysiology of the islet.

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