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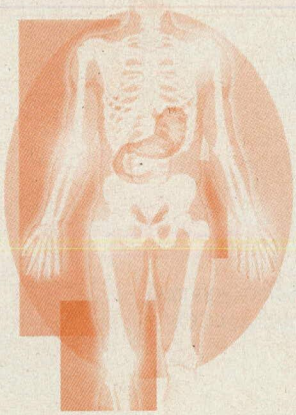

COX-1 and COX-2 in health and disease

RAYMOND A. ADELIZZI, DO, FACOI

Nearly 30 years ago, cyclooxygenase (COX) was identified as an enzyme that initiates the biotransformation of arachidonic acid to prostanoids. It is now known that COX exists as two distinct but similar isozymes, COX-1 and COX-2. Prostaglandins (PGs) formed by the enzymatic activity of COX-1 are primarily involved in the regulation of homeostatic functions throughout the body, whereas PGs formed by COX-2 primarily mediate pain and inflammation. Based on structural differences in the active sites of COX-1 and COX-2, a new class of drugs has been developed that specifically inhibits COX-2 but not COX-1 activity. By preserving the synthesis of homeostatic PGs, these specific inhibitors of COX-2 provide the clinical benefits of nonsteroidal anti-inflammatory drugs and minimize the consequences of nonspecific inhibition of PG synthesis.

(Keywords: celecoxib, cyclooxygenase, prostaglandins, rofecoxib)

In 1971, John Vane[1] elucidated a mechanism of action of aspirin and related compounds now called nonsteroidal anti-inflammatory drugs (NSAIDs). He noted that aspirin and indomethacin (and, to a lesser extent, salicylate) inhibit the synthesis of prostaglandins (PGs) in a dose-related manner. Vane hypothesized that the clinical action of NSAIDs as well as their toxic gastrointestinal (GI) effects are produced by competitive binding to one or more enzymes that convert arachidonic acid to PGs. At the time of Vane's hypothesis, these biotransformational enzymes had not yet been characterized.[1]

Vane's hypothesis was confirmed and the enzyme identified as cyclooxygenase (COX), also known as prostaglandin endoperoxide synthase.[2] COX is expressed in at least two isoforms: COX-1 is constitutively expressed in most tissues, whereas COX-2 is primarily an inducible enzyme, the expression of which is rapidly upregulated in many tissues in response to tissue damage or the presence of proinflammatory cytokines.[3]

Through utilization of a structural difference in the enzymatic sites of COX-1 and COX-2, several COX-2–specific inhibitors have been developed, and their potential usefulness studied in preclinical and clinical models.[4] On the basis of these studies, it is now believed that the analgesic and anti-inflammatory properties of NSAIDs are primarily a function of COX-2 inhibition, whereas the adverse effects of these agents are primarily a consequence of COX-1 inhibition.[5]

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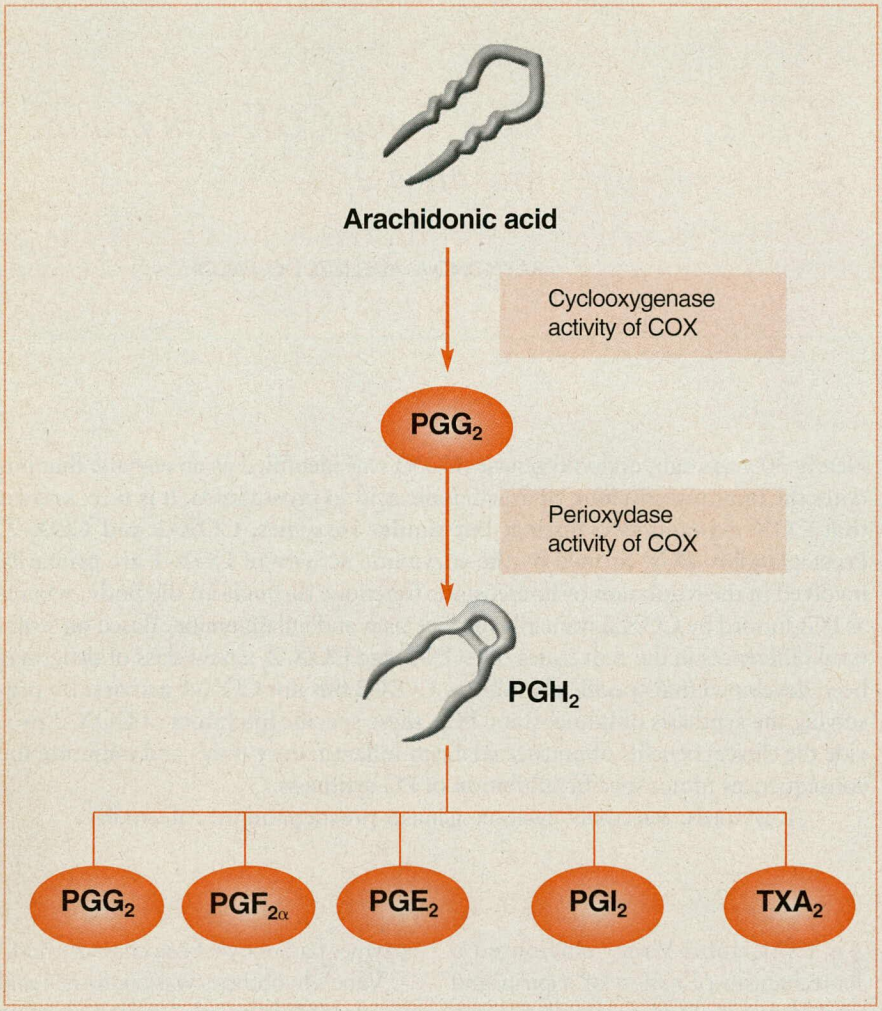

◀ **Figure 1.** *Roles of COX-1 and COX-2 in prostaglandin synthesis.*[6] (COX = cyclooxygenase enzyme; PGG_2 = prostaglandin G_2; PGH_2 = prostaglandin H_2; PGD_2 = prostaglandin D_2; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PGE_2 = prostaglandin E_2; PGI_2 = prostaglandin I_2 (prostacyclin); TXA_2 = thromboxane A_2.

Enzymatic activity of COX-1 and COX-2

The role of COX in prostanoid synthesis is illustrated in *Figure 1.*[6] In the first step, the cyclooxygenase activity of either COX-1 or COX-2 oxygenates and isomerizes arachidonic acid, creating the intermediate product prostaglandin G_2 (PGG_2). In the second step, which occurs at a different enzymatic site, peroxidase reduces PGG_2 to its hydroxyl analog, PGH_2. Distinct synthases or reductases then convert PGH_2 to one of several stable prostanoids: PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, or thromboxane A_2 (TXA_2).[7]

Structure of COX-1 and COX-2

COX-1 and COX-2, which are encoded by two separate genes, have slightly different amino acid sequences; these sequences are similar from species to species.[8] In humans, the COX-1 and COX-2 enzymes have roughly 60% overall amino acid homology[4,9] and have molecular weights of 62,500 to 72,000 and 72,500 to 74,000 Daltons, respectively.[10] In the region of their active sites, however, the amino acid homology is closer to 90%.[8]

The molecular structures of COX-1 and COX-2 as described by Kurumbail and associates[8] show that both isozymes are dimers, with the COX activation site located in a long, hydrophobic channel between the two subunits. A substitution of one amino acid in the activation site, from isoleucine in COX-1 to valine in COX-2, creates a second, larger NSAID-binding pocket; it is this additional binding site that confers selectivity of NSAIDs for one isozyme or the other. Smaller NSAIDs can bind to the pocket in the activation sites of either COX-1 or COX-2; the larger COX-

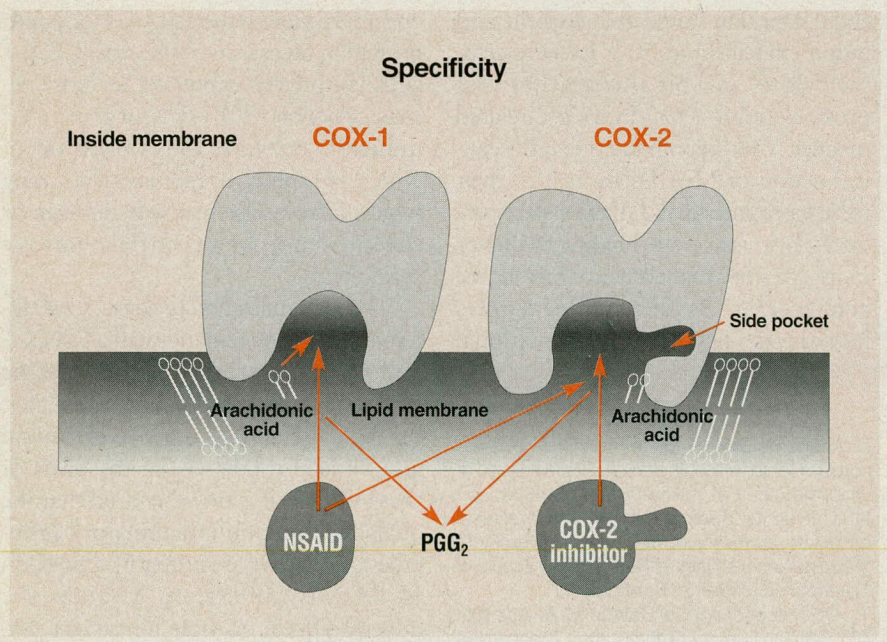

Figure 2. *Author's conceptualization of structural basis of NSAID isoforms.*

Table 1
Physiologic and Pathologic Functions of Cyclooxygenase (COX)-Derived Prostanoids*

Prostanoid	Physiologic action	Pathologic action
Thromboxane A_2	Platelet aggregation	Thrombosis
Prostaglandin D_2	Induces slow-wave sleep Neuromodulation Vascular tone modulation Inhibits platelet aggregation	 Hypotension Mastocytosis
Prostaglandin E_2	Vasodilation Renal blood flow modulation Decreases water/salt reabsorption Protects gastrointestinal mucosa Mobilizes macrophages Modulates renin release	Hypotension Inflamed synovium
Prostaglandin $F_{2\alpha}$	Longitudinal smooth muscle contractor Vasoconstriction Triggers follicular ovulation Promotes uterine implantation Stimulates uterine contractions	Stimulates gastrointestinal motility Induces labor
Prostaglandin I_2	Inhibits platelet aggregation and adhesion to vascular endothelium Modulates renin release Vasodilation	Hypotension

*Sources: References 7 and 15 through 17.

2–specific inhibitors only fit into the larger binding pocket found on the COX-2 isozyme (*Figure 2*).

Anatomic distributions of COX-1 and COX-2

COX-1 and COX-2 differ in anatomic distribution. On a subcellular level, both isozymes are membrane bound, COX-1 to the lumen surface of endoplasmic reticulum, and COX-2 to both the endoplasmic reticular and nuclear membrane.[10,11] On a cellular level, quantitative analysis of mRNA in murine tissues has found constitutive expression of COX-1 throughout the body—in stomach, colon kidney, liver, brain, heart, lung, spleen, GI tract, platelets, and endothelium.[3,12,13] When the same tissue samples were assayed for COX-2, no measurable mRNA was found, aside from small amounts in the lung and liver.[12] Other studies have found constitutive expression of COX-2 in discrete areas of the kidney, brain, and uterine tissues during gestation.[2]

Expression of COX-2, however, is predominantly induced by proinflammatory or mitogenic stimuli, mediated by cytokines or mitogens.[7] Rapid upregulation of COX-2 occurs in various components of the immune system (eg, monocytes and macrophages), synoviocytes, endothelial cells, and chondrocytes. The COX-2 but not COX-1 gene is down-regulated by anti-inflammatory agents such as glucocorticoids.[13]

The nonprimate anatomic distribution of COX-1 and COX-2, at least in the kidney, may not completely correlate with the distribution found in primates. While the anatomic distribution of COX-1 and COX-2 in human kidneys was found to be similar to that seen in nonhuman primates in a recent study, some clinically significant differences were found when their distribution in primate kidney was compared with that found in other species.[14] A study of human, monkey, rat, and dog renal tissue in nonhypovolemic animals found that in primate tissue, unlike rat or dog, there was a predominant expression of COX-1 but limited COX-2 expression in the maculae densa and papilla.[14]

Role of prostaglandins in regulating homeostasis

Prostanoids, produced through the action of the constitutively expressed COX-1 isozyme, function through cell surface receptors to mediate numerous developmental and physiologic functions throughout the body.[7] In 1994, Vane and Botting[15] provided a comprehensive overview of the physiologic functions of PGs (*Table*[7,15-17]).

Prostaglandins are synthesized in every part of the GI tract. In the stomach,

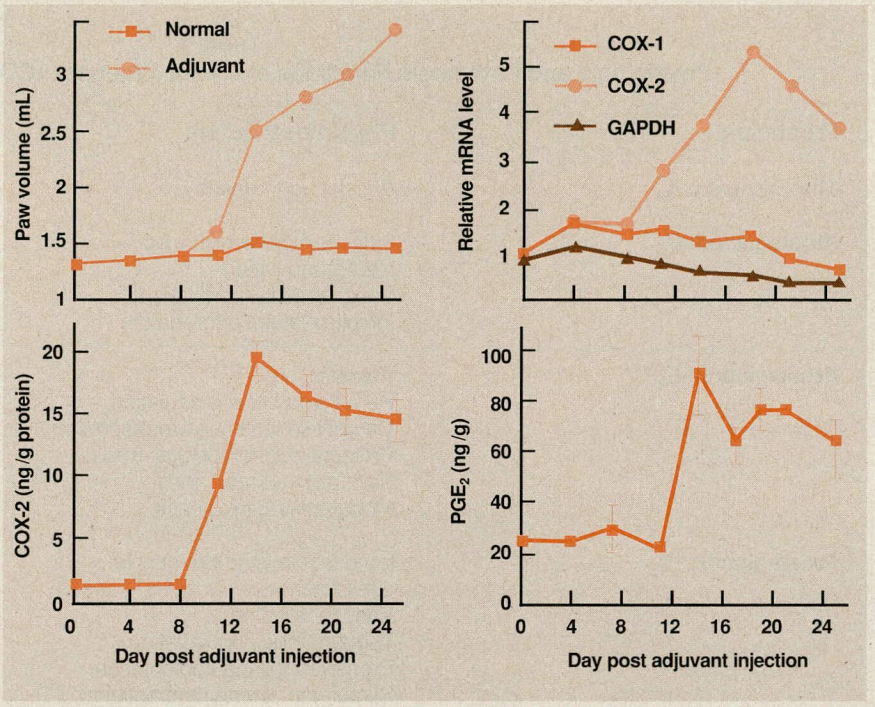

Figure 3. *Temporal progression of response to adjuvant injection. The timing of COX-2 mRNA upregulation, COX-2 protein expression, the development of edema, and PGE_2 production during adjuvant-induced arthritis. Data are presented as the group mean±SEM (n=3 animals per group). (Reproduced with permission from The American Society of Clinical Investigation, from Anderson GD, Hauser SD, McGarity KL, et al. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis.* J Clin Invest *1996;97:2672-2679.)*

PGE_1 and PGE_2 formed in parietal cells exert potent antisecretory action, decreasing the release of gastric acid and pepsin and reducing the volume of fluid in the gastric lumen. A major action of constitutively expressed COX-1–derived gastric PGs is cytoprotection of the gastric mucosa, mediated predominantly by PGE_2 and prostacyclin (PGI_2).[9] Prostaglandins alter intestinal muscle tone: PGE_1 and $PGF_{2\alpha}$ contract the longitudinal muscles, PGE_1 and PGE_2 relax the circular muscles, and PGI_2 relaxes the longitudinal muscles. The combined actions of PGE_1 stimulate GI motility, accelerating the transit rate. Fluid and electrolyte transport across the intestinal mucosa are altered by PGE_1 and $PGF_{2\alpha}$, promoting the retention of fluid in the lower intestines.[17,18]

In blood, a balance of fluidity is achieved to two vascular prostanoids with opposing actions: TXA_2 is a potent trigger of platelet aggregation, and PGI_2 a potent inhibitor of platelet aggregation.

Vascular tone is regulated by locally produced PGs that modulate vascular smooth muscle contraction or relaxation, depending on the location of the blood vessel. In most vascular beds, PGE_2 is a potent vasodilator and $PGF_{2\alpha}$ is a potent vasoconstrictor.

In the renal cortex, PGE_2 and PGI_2 stimulate the release of renin, triggering release of aldosterone, which subsequently promotes potassium secretion in distal tubules and collecting ducts.[19] Medullary PGE_2 and PGI_2 promote vasodilation, increasing renal blood flow and thus the glomerular filtration rate; inhibit tubular reabsorption of sodium; inhibit chloride transport across the loop of Henle; trigger the release of antidiuretic hormone; and increase urine flow by attenuating the osmotic effect of antidiuretic hormone in the collecting tubule. In the renal medulla, TXA_2 modulates these effects, promoting vasoconstriction and inhibiting tubular reabsorption of sodium.

In the brain, several actions triggered by PGD_2 balanced by the effects of PGE_2 appears to regulate the sleep/wake cycle. Nanomolar amounts of PGD_2, thought to act as a neuromodulator in the brain, induce slow-wave sleep when microinjected into the preoptic area of conscious rats[20]; when injected into the posterior hypothalamus, PGE_2 prolongs the wake cycle.

Role of prostaglandins in pathologic states

COX-2 initiates the synthesis of PGs that mediate inflammation, as in arthritis, or mitogenesis, as is believed to occur with colorectal cancer.[7]

In a study using a standard animal model, coadministration of a monoclonal antibody highly specific for PGE_2 reversed the shortened painful-stimulus–withdrawal time and blocked increase in edematous paw volume following the injection of the irritant carrageenan.[21] In a separate protocol using this animal model, the investigators found that treatment with naproxen also increased the stimulus-withdrawal time, and indomethacin decreased paw volume following carrageenan injection.[21] The investigators concluded that the pain and inflammation measured in this model were mediated by PGE_2 and that the NSAIDs studied alleviated the inflammatory reaction by suppressing the production of PGE_2.

In another study using the rat paw injection of carrageenan, treatment with increasing doses of the COX-2–specific inhibitor celecoxib were comparable to the protective effects of indomethacin, which was used as a positive control.

Using a similar animal model, researchers documented the progression of events following an injection of Freund's adjuvant into a rat paw, from upregulation of COX-2 mRNA to expression of the COX-2 protein and concomitant or immediate edema, local production of PGE_2, inflammation, and eventual joint destruction (*Figure 3*).[22]

There was no change in tissue levels of COX-1 mRNA following injection of the irritant Freund's adjuvant. Control of the inflammation reaction was achieved by treatment with dexamethasone, and an equivalent degree of control was achieved with either indomethacin or an experimental COX-2–specific inhibitor, SC-58125.[22]

Animal studies provide compelling evidence that COX-2 mediates the production of PGE_2, which results in an inflammatory response that can be blocked by inhibiting the activity of COX-2. Human studies have supported this conclusion. The expression of COX-2 in synovial tissue, but not of COX-1, is increased in patients with inflammatory arthritis, and COX-2 expression appears to be responsible for the production of inflammatory PGs that contribute to joint injury in arthritis.[23]

A link between COX-2 expression and tumorigenesis has been suggested, especially in colorectal cancer. Oshima and colleagues,[24] using a murine model of familial adenomatous polyposis, found strong evidence for a key role for COX-2 in the development of colon cancer. The mice, with a genetically engineered defect in the *Apc* gene, typically develop benign adenomas in the colon in a process that resembles adenoma development observed in humans with a similar genetic defect. In studying COX-2 expression, and inhibition with specific COX-2 inhibitors, strong support was found for the hypothesis that increased levels of COX-2 expression are associated with an increased risk of colon cancer, and that inhibiting COX-2 reduces that risk.

A similar connection between COX-2 and colon cancer may occur in humans. It has been hypothesized that production of COX-2 linked with the *Apc* mutation prevents the apoptosis of mutant adenoma cells, enhancing their transformation to cancer.[25] The expression of COX-2 in human colon cancer cells has been reported to increase their metastatic potential.[26]

The expression of COX-2 may also play a role in neuropathologic processes. COX-2 in rat brain appears to be involved in postsynaptic signaling of excitatory neurons in structures involved in cognitive functions.[27] In another study of rat brain, the regional distribution of constitutive COX-2 mRNA was highest in the cortex and hippocampus.[28] Animal studies have also demonstrated an upregulation of COX-2 expression in the brain in response to stimuli such as pain or seizure.[29]

In Alzheimer's disease, amyloid β-peptides found in neuronal plaques are believed to be elaborated during an inflammatory cascade. The central expression of COX-2 has been positively correlated with the presence of amyloid β-peptide in patients with Alzheimer's disease.[30] The expression of COX-2 in activated microglia cells, a rich source of prostanoids, has been demonstrated in rat cerebrum during the inflammatory process.[31] Research conducted to date suggests that COX-2–mediated inflammation may play a role in the development or progression of Alzheimer's disease. Both retrospective analyses and prospective clinical studies have reported a decreased incidence or reduced rate of progression of Alzheimer's disease among individuals who were taking NSAIDs on a long-term basis.[32-34]

Richard A. Pascucci, DO, FACOI, provides information about the clinical application of COX-2–specific inhibitors in the treatment of nonarthritic conditions, beginning on page S18.

Specific inhibition of COX-1 or COX-2

To varying degrees, all NSAIDs inhibit the cyclooxygenase activity of COX-1 and COX-2. An ideal NSAID would be one that provides COX-2 inhibition with little or no COX-1 inhibition at therapeutic dosages. COX-2 specificity is a characteristic of the new family of agents collectively referred to as COX-2–specific inhibitors. The first of these agents to be approved for clinical use are celecoxib and rofecoxib.

COX inhibitors can be classified in a number of ways—for example, by biochemical or pharmacokinetic properties, by relative degree of COX-1 and COX-2 inhibition in in vitro enzyme systems, or by response in biological models thought to reflect the relative importance of the two isozymes (eg, effects on stomach lining or localized sites of inflammation[3,10]). Recently, a classification of COX inhibitors based on a more complete analysis of biochemical, pharmacologic, and clinical data has been proposed.[35] According to this classification, COX inhibitors can be considered to belong to one of four groups:

- A *COX-1–specific* inhibitor is an agent with no measurable inhibition of COX-2. At present, low-dose aspirin is the only drug that falls into this category.
- A *COX-nonspecific* inhibitor inhibits both COX-1 and COX-2, perhaps with small pharmacologic differences in activity between the two isozymes, but with no clinically relevant differences in specificity.
- A *COX-2–preferential* inhibitor is an agent that inhibits COX-2 with little inhibition of COX-1 at therapeutic dosages.
- *COX-2–specific* inhibitors may be defined as agents that produce no clinically significant inhibition of COX-1, even at the highest therapeutic dosages.

Comment

The predominant inhibition of COX-1 by aspirin is the basis for its use as an antithrombotic agent; the anti-inflammatory and analgesic effects of NSAIDs are largely a function of their ability to inhibit COX-2. Because COX-1-derived prostanoids are involved in many physiologic functions, inhibition of COX-1 by nonspecific NSAIDs is associated with a characteristic pattern of adverse effects: dyspepsia, gastroduodenal ulcers, hemorrhage, salt and water retention and subsequent edema, headaches, delay in cartilage repair, and asthma attacks.

The data reviewed here suggest that the development of COX-2–specific inhibitors provides a means of controlling pain and inflammation with a more favorable safety profile than that of conventional NSAIDs. For patients who require continuing therapy, this may represent a significant advance. (Beginning on page S13, Elizabeth A. Tindall, MD, more completely discusses COX-2 inhibition in the management of pain and

inflammation.) Other potential therapeutic indications for COX-2–specific inhibitors are also currently under investigation. In the future, inhibition of COX-2 may be a useful therapy for controlling polyp development in the colon. Also, the inhibition of PG-directed apoptosis through COX-2 inhibition may provide a mechanism for minimizing the consequences of neuronal cell injury associated with Alzheimer's disease. The clinical utility of COX-2 inhibition in these therapeutic areas remains to be established in controlled clinical trials.

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