

Nonsteroidal Anti-Inflammatory Drugs, Acetaminophen, Cyclooxygenase 2, and Fever

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used antipyretic agents that most probably exert their antifever effect by inhibiting cyclooxygenase (COX)-2. Thus, COX-2-selective drugs or null mutation of the *COX-2* gene reduce or prevent fever. Acetaminophen is antipyretic and analgesic, as are NSAIDs, but it lacks the anti-inflammatory and anticoagulatory properties of these drugs. This has led to the speculation that a COX variant exists that is inhibitable by acetaminophen. An acetaminophen-inhibitable enzyme is inducible in the mouse J774.2 monocyte cell line. Induction of acetaminophen-inhibitable prostaglandin E_2 synthesis parallels induction of COX-2. Thus, inhibition of pharmacologically distinct COX-2 enzyme activity by acetaminophen may be the mechanism of action of this important antipyretic drug.

From an historical viewpoint, the development of modern nonsteroidal anti-inflammatory drugs (NSAIDs) owes much to a seminal observation by Reverend Edward Stone, who in 1763 described the fever-reducing value of willow bark extract, a solution rich in salicylates [1–3]. Reverend Stone extolled the benefits of his extract for the treatment of agues, an antiquated synonym for fevers. For >100 years, willow and other plant extracts, or their active ingredient, salicylates, were a popular method for reducing not only fever and pain associated with feverish diseases but also the pain associated with arthritis. In an effort to make the unpleasant-tasting salicylates more palatable, in 1898 Felix Hoffman of Bayer made a simple acetylated derivative: acetylsalicylate, or aspirin (figure 1). This drug was more effective at inhibiting pain than previous salicylates and ruled the pharmaceutical industry for treatment of pain, fever, and inflammation for 80 years. However, in the 1960s and 1970s, numerous salicylate and nonsalicylate drugs were produced that were significantly more potent than aspirin. Examples are indomethacin, ibuprofen, and diclofenac (figure 1). Significantly, these drugs had the same beneficial effects (antipyresis, analgesia, and anti-inflammation) and the same potential side effects (stomach ulceration) as aspirin.

Phenacetin, a once-popular antipyretic and analgesic developed by Bayer and marketed in 1887 [3, 4], fell out of favor because of a variety of reported side effects, including hepatic toxicity and nephropathy [3, 4]. In 1948 and 1949, Brodie and Axelrod reported that acetaminophen, a drug that had been

used as early as 1893 as an antipyretic, was the major metabolite of phenacetin [3, 4]. Because of the less severe side effects of acetaminophen, it replaced phenacetin as an analgesic and antipyretic. Unlike aspirin, acetaminophen is considered to have little or no anti-inflammatory activity.

None of these drugs had a known mechanism of action until 1971, when John Vane made the pivotal discovery that aspirin, indomethacin, and salicylate were inhibitors of prostaglandin (PG) synthesis [5]. Specifically, these drugs inhibited cyclooxygenase (COX), the enzyme that catalyzes the rate-limiting steps governing the synthesis from arachidonic acid of PGH_2 , the precursor of all PG isomers. The next year, Flower and Vane [6] extended the concept of COX inhibition to the mechanism of action of acetaminophen by showing that this drug was an inhibitor of COX activity in the brain. However, unlike inhibition by aspirin, inhibition of COX activity by acetaminophen was biphasic and required glutathione and hydroquinone as cofactors. Because some subsequent studies showed that COX was insensitive to acetaminophen, the mechanism of action of acetaminophen has been unclear.

A critical piece of information missing during the peak of NSAID development and exploration was that there were 2 COX enzymes with distinct functional roles. In 1991 studies in cell division and neoplastic transformation converged with NSAID pharmacology when 3 laboratories independently cloned and identified an inducible COX now called COX-2 [7–9]. Unlike COX-1, which had been purified and studied extensively during the 1970s and 1980s, COX-2, with only a few exceptions, was found to be a rare protein found in mammalian tissues. Moreover, it is highly inducible in fibroblasts, macrophages, vascular endothelium, and other cell types by a wide variety of stimuli: cytokines, tumor promoters, oncogenes, and changes in cellular environment [7–9]. Expression of COX-2 was also found to be down-regulated by anti-inflammatory steroids, findings consistent with a role in inflammation. The di-

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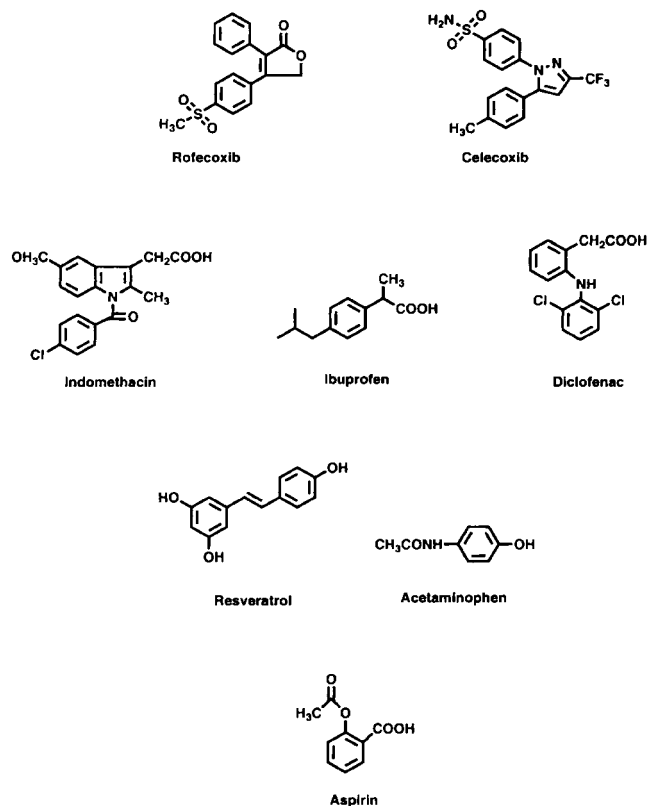


Figure 1. Structural comparison of aspirin, selected carboxylate-containing, competitively acting nonsteroidal anti-inflammatory drugs, nonacidic acetaminophen and resveratrol, and cyclooxygenase-2-selective inhibitors.

vergent regulation of constitutively expressed COX-1 versus inducible COX-2 gave rise to the working hypothesis that COX-1 was a “physiological form” of COX functioning to maintain tissue homeostasis, whereas COX-2 was an “inflammatory form” of COX [10]. Many excellent reviews have been written on the structure and function of COX-2 [11–13]. Here we describe the pharmacological progress made in developing selective inhibitors of COX-2, which, along with the results of studies of molecular genetics in mice, unequivocally prove the role of COX-2 in fever. Moreover, we explore the mechanism by which COX-2 may causatively effect fever in humans and other mammals and describe and discuss recent experiments that show that an acetaminophen-inhibitable COX activity can be induced in J774.2 cells. Induction of this activity parallels induction of COX-2.

COX-1/COX-2 Inhibition and Isoenzyme-Specific NSAIDs

The discovery of a second COX opened the possibility of the development of isoenzyme-specific drugs, which became a reality in the form of celecoxib [14] and rofecoxib [15], which

were marketed in 1999. As described later, COX-2-selective drugs are potent antipyretic agents. How NSAIDs function to inhibit PG synthesis is now known at the level of crystallographic resolution. Concepts necessary to the understanding of how NSAIDs bind and inhibit COX are briefly summarized here, and we refer the reader to more thorough treatments of the subject [16, 17]. At the primary sequence level, COX-1 and COX-2 share 60% amino acid identity. However, at the tertiary and quaternary levels, they show striking similarity. Both isoenzymes are homodimers with distinct domains for dimerization, membrane binding, and catalysis. Both enzymes colocalize to the lumen of the nuclear envelope and endoplasmic reticulum, where they tightly adhere to the membrane’s luminal surface by a series of 4 amphipathic helices [18, 19]. COX-2, but not COX-1, has also been shown to traffic into the cis-Golgi [20]. Intraluminal localization in these compartments allows the enzymes to contain 4 disulfide bonds because the nuclear envelope/endoplasmic reticulum environment is oxidizing. Additionally, each isoenzyme is glycosylated on 3 (COX-1) or 3 or 4 (COX-2) asparagines with high-mannose moieties [16–19].

The catalytic domains of COX-1 and COX-2 each contain 2 distinct active sites. The COX active site binds arachidonic acid and cyclizes and oxygenates it to form an unstable intermediate PGG₂. This short-lived molecule diffuses from the COX active site to the peroxidase active site, where a hydroperoxyl moiety on PGG₂ is reduced to a hydroxyl. The resulting PGH₂ acts as a substrate for isomerase pathways that produce other PG, thromboxane, and prostacyclin isomers.

Crystallographic elucidation of COX-1 in 1994 by Picot et al. [18] defined the molecular architecture of the COX and peroxidase active sites. Only the COX site is bound and inhibited by aspirin and other NSAIDs. This site is a hydrophobic channel or tunnel whose exit is in the membrane-binding domain of the enzyme. Arachidonic acid diffuses into the COX active site from the membrane domain. Recent studies indicate that COX-1 and COX-2 bind arachidonic acid in different ways. COX-1 utilizes arginine 120 in its active site to form an ionic bond with the carboxylate group of arachidonate. Conversely, arginine 120 appears to form a hydrogen bond with arachidonate in the COX-2 active site, and this interaction contributes less to the binding energy than the ionic bond formation does in COX-1 [21]. Importantly, cocrystallization studies have shown that many carboxylate-containing NSAIDs (e.g., indomethacin, flurbiprofen, suprofen, and salicylate) also form ionic bonds with arginine 120 and that this interaction is essential for enzyme inhibition [21]. The position of this residue (at a constriction in the channel near the channel opening) allows these NSAIDs to occlude the channel and prevent arachidonate entry.

Arginine 120 also plays an important role in inhibition of COX-1 and COX-2 by aspirin. Formation of a weak ionic bond with the carboxylate of aspirin positions aspirin 5 Å below a reactive serine, serine 530, to which it diffuses and transfers its

acetyl group. The presence of the acetyl adduct to serine 530 is sufficient to sterically prevent cyclization and oxygenation of arachidonate to PGG₂. Thus, unlike all other commercially available NSAIDs, aspirin inhibits COX-1 and COX-2 by covalent modification resulting in permanent inhibition of the enzymes.

COX-2 has now been crystallized, an analysis that revealed a number of subtle differences in the COX active site that have been exploited for drug development. The core residues lining the COX active sites of the 2 enzymes are identical except for one: isoleucine 523 in COX-1 is changed to a valine in COX-2. The less bulky side chain in valine of COX-2 opens up a hydrophobic side pocket in the COX-2 active site that is not accessible in COX-1 [17, 19]. This and other subtle differences in the COX-2 active site have been exploited to produce COX-2 selective inhibitors celecoxib and rofecoxib. Depending on the assay used, these drugs are potent by a factor of ≥ 30 -fold in inhibiting COX-2 over COX-1.

COX-2 and the Febrile Response

Over the past 2 decades, much evidence for the mediating role of PGE₂ in fever has been reported (for reviews, see Dinarello et al. [22], Saper [23], and Cocceani and Akarsu [24]). With the discovery of COX-2, it was clear that the different COX isoforms might play unique roles in fever. Research in humans and animal models have shown that COX-2 plays a dynamic role in generating fever induced by lipopolysaccharide (LPS), yeast, and cytokines.

Fever induction studies in rodents gave the first indications that COX-2 causes fever and that fever-inducing COX-2 expression is localized in specific cell types in the brain. Fever induction in rats by ip injection of LPS, TNF- α , or IL-1 β has been found to be accompanied by COX-2 mRNA induction in rat brain vasculature [25–31]. Specifically, this induction was seen in vein/venule endothelial cells and, to a lesser extent, in arterial endothelial cells throughout the brain, subarachnoid space, and spinal cord [31]. Occasional induction in perivascular cells has also been reported [32]. Expression patterns for COX-2 mRNA produced by fever-inducing stimuli is similar in mouse, prepubertal pig, and rat brain [30, 33, 34]. That is, fever induction is accompanied by an increase in COX-2 expression throughout the vasculature of the brain.

A current working hypothesis is that PGE₂ synthesized from vascular COX-2 is essential for fever; however, it has also been postulated that fever-causing PGs may originate from perivascular microglia and meningeal macrophages throughout the brain after systemic immune challenge [32, 35]. Evidence for a pivotal role of COX-2 production in brain vascular endothelial cells in fever is provided by a high correlation between the number of COX-2 mRNA-positive brain blood vessels and the intensity of the febrile response in LPS-challenged rats [29, 30]. Moreover, brain vascular endothelial cells cultured *in vitro* se-

creted PGE₂ preferentially on the basal side rather than the luminal side in a ratio of 4:1, further suggesting that PGE₂ synthesized in epithelial cells could conveniently cross into the CNS [36].

In addition to the brain vasculature, important signaling roles have been suggested for the organum vasculosum laminae terminalis, the vagus nerve system, the complement system, and Kupffer cells [37–40].

Effect of COX-2-Selective Inhibitors on Fever

Work done with genetically altered mice showed that wild-type mice and COX-1^{+/–} and COX-1^{–/–} mice all exhibited a normal fever response after challenge by LPS [33]. The responses of COX-2^{+/–} and COX-2^{–/–} mice to a challenge by LPS or by IL-1 β were quite different. COX-2-deficient mice were unable to mount a febrile response to exogenous pyrogens or endogenous pyrogens equivalent to that of wild-type mice. Instead, the COX-2-deficient mice exhibited a drop in body core temperature of between 0.5°C and 1.4°C after ip injection of LPS or IL-1. This result conclusively confirms the pivotal role of COX-2 in the development of fever. It would appear, however, that COX-1 does not participate in the febrile response. Similarly, mice that lacked the PGE₂-EP₃ receptors were unable to develop a normal febrile response to either an exogenous or an endogenous pyrogenic challenge [41].

Further illustrating a central role of COX-2 in febrinogenesis are studies in various animal systems that demonstrate the effectiveness of COX-2 selective or preferential inhibitors in reversing or suppressing the febrile response in animals challenged with exogenous or endogenous pyrogens. The experimental COX-2-selective drug L-745,337 has been shown to suppress or reverse the febrile response in both rats [42] and prepubertal pigs [43] challenged by LPS. The same results have been found when COX-2-preferential drugs such as nimesulide [44], etodolac [45], and meloxicam [46] are used. DUP-697, another experimental COX-2 selective inhibitor, has been shown to be a potent antipyretic in rats [47]. Similarly, the COX-2 selective inhibitor DFU has been shown to have antipyretic effects in squirrel monkeys [48] and rats [49] in LPS-induced fever models.

Two recently developed COX-2-specific inhibitors that have undergone extensive testing in humans are rofecoxib (MK-0966) and celecoxib (SC-58635). In human whole-blood assays for COX-1 and COX-2 that use arachidonic acid as a substrate, rofecoxib showed selectivity ratios for the inhibition of COX-2 to COX-1 of 36. Under the same experimental conditions, celecoxib showed selectivity ratios for COX-2 to COX-1 of 6.6 [50]. In clinical trials, rofecoxib has also been shown to reduce naturally occurring fever in humans, as well as reducing LPS-induced pyrexia in rats and squirrel monkeys [51].

COX-2 and Acetaminophen

From a structural standpoint, it is challenging to envision how acetaminophen would inhibit the COX site of COX because it lacks a carboxylic acid moiety for interaction with arginine 120 (figure 1). However, other simple, nonacidic compounds such as resveratrol are also known to inhibit both COX-1 and COX-2 [52, 53] (figure 1). Their mechanism of inhibition is currently unknown, and it is unclear whether they inhibit the COX or peroxidase active sites of the enzyme. The early work of Flower and Vane [6] established the possibility that acetaminophen exerted its action through inhibition of COX. Moreover, their work provided a mechanistic rationale as to why acetaminophen would possess part of the complement of therapeutic activities possessed by NSAIDs (analgesia and antipyresis) but lack anti-inflammatory and anticoagulatory activity. That is, that acetaminophen exerts its effect on a subtype of COX located in the brain. The finding of 2 forms of COX confirmed the notion of COX subtypes, but further investigation of COX-2 failed to find it to be significantly inhibited by acetaminophen [54].

Recently, our laboratory identified a way to induce murine J774.2 cells to produce a COX activity that was more sensitive to acetaminophen inhibition than is COX-1 or COX-2. Ironically, the inducer of this activity was diclofenac, a potent inhibitor of COX-1 and COX-2. The remainder of this review summarizes those studies.

Induction of COX-2 by NSAIDs

Identification of COX-2 as a *v-src*-inducible protein led our laboratory to investigate the effect of NSAIDs on neoplastic transformation initiated by this oncogene. Our study system used chicken embryo fibroblasts transformed with a temperature-sensitive mutant of Rous sarcoma virus [55–57]. Treatment of these cells with NSAIDs and analgesics showed that 18 of 26 drugs tested inhibited focus formation of transformed cells at concentrations of 10–100 μM (table 1). Drugs that were ineffective were prodrugs (e.g., sulindac) or weak inhibitors of COX (e.g., acetaminophen, acetophenetidin, and salicylamide). Conversely, active metabolites such as sulindac sulfide were highly effective at preventing focus formation (unpublished data). The cytotoxic effects of 8 of the drugs most effective in preventing focus formation were investigated in detail. All 8 were found to induce apoptosis. Relatively rapid induction of apoptosis by diclofenac, the most thoroughly studied drug, occurred at concentrations as low as 32 μM . Members of apoptosis signaling pathways essential for NSAID induction of programmed cell death in these cells were later defined [57].

In the process of characterizing NSAID-induced apoptosis in *v-src*-transformed chicken embryo fibroblasts, the effect of these drugs on COX-1 and COX-2 expression was also assessed. Unexpectedly, it was found that COX-2, but not COX-1, was highly induced by all of the apoptosis-inducing drugs we in-

Table 1. Morphological inhibition of transformation in response to treatment with nonsteroidal anti-inflammatory drugs (NSAIDs).

NSAID	Effect	
	100 μM	10 μM
Oxicam		
Isoxicam	N	N
Piroxicam	P	N
Salicylate		
Aspirin	N	N
Diflunisal	C	N
Acetaminophenol	N	N
Acetophenetidin	N	N
Salicylamide	N	N
Acetic acid		
Indomethacin	C	C
Acemetacin	C	P
Tolmetin	P	N
Sulindac	N	N
Diclofenac	C	P
Zomepirac	P	N
Fenamate		
Mefenamic acid	C	P
Flufenamic acid	C	P
Niflumic acid	C	P
Propionic acid		
Ketoprofen	P	N
Naproxen	P	N
Indoprofen	P	N
Ibuprofen	C	N
Flurbiprofen	P	N
Suprofen	P	N
Fenbufen	P	N
Carprofen	C	N
Pyrazole		
Phenylbutazone	N	N
Oxyphenbutazone	N	N

NOTE. Chicken embryo fibroblasts (CEF) were transformed with Rous sarcoma virus (RSV). C, complete inhibition of focus formation; N, no effect; P, partial inhibition, which was characterized by cell rounding and formation of small clumps of cells.

vestigated (figure 2). Induction resulted in both elevated COX-2 mRNA and protein levels. Doses for maximum induction of COX-2 by diclofenac were 25–50 μM ; however, doses of 200 μM or more suppressed induction of COX-2.

Numerous immortalized and nonimmortalized mammalian cells in culture were evaluated to identify an analogous mammalian model to chicken embryo fibroblasts. One cell system that exhibited a profound incidence of apoptosis, although at higher concentrations of NSAID than those used in chicken embryo fibroblasts (CEF), was the murine J774.2 cell line. This monocytic/macrophage European cell line was isolated from a tumor that arose in a BALB/c mouse in 1968 and is related but not identical to the J774A.1 cell line used in the United States. As with CEF, NSAIDs also induced COX-2 but not COX-1 in J774.2 cells (figure 3). Unlike in CEF, this induction required maximally effective doses of diclofenac of 500 μM and has been reported to occur without a concomitant increase in COX-2 mRNA [58, 59]. Also, COX-2 induction in J774.2 cells required 48 h to occur as opposed to 24 h in CEF [56, 58].

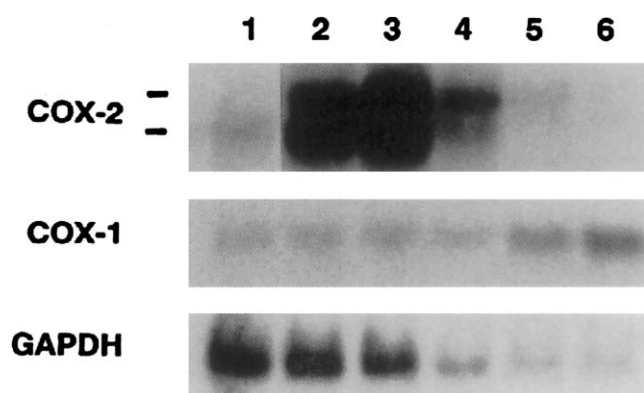


Figure 2. Effect of concentration on diclofenac induction of cyclooxygenase (COX)-1 and COX-2. For Northern blot analysis, 20 μ g of total RNA from Rous sarcoma virus (RSV)-transformed chicken embryo fibroblasts (CEF) with and without treatment with various doses of diclofenac 29 h after shift to 37°C was hybridized to COX-1, COX-2, or glyceraldehyde phosphate dehydrogenase (GAPDH) probes. Lane 1, Serum-starved RSV-transformed cells without drug treatment. Lanes 2–6, Diclofenac treatment at 25 μ M, 50 μ M, 100 μ M, 200 μ M, and 400 μ M, respectively. COX-1, 7-day exposure; COX-2, 20-h exposure; GAPDH, 6-h exposure. From Lu et al. [56].

Induction of a Novel COX Activity

The results of these 2 experiments in different cell models suggested that prolonged exposure to NSAIDs at concentrations high enough to completely inhibit COX-1 and COX-2 resulted in induction of COX-2. This induction may have been an attempt by the cell to salvage itself from the lethal effects of complete inhibition of COX activity. If this were the case, it seemed possible that the COX induced may have specific and distinct enzymatic activities. To test this, J774.2 cells were exposed to various doses of diclofenac for 48 h, after which the diclofenac-containing mediums were removed and COX activity was measured by exposing the cells to 30 μ M arachidonic acid. With regard to purified COX-1 and COX-2, or these enzymes in cell homogenates, diclofenac acts as a pseudoirreversible inhibitor. Diclofenac enters the COX active site in a time-dependent fashion, lodges there tightly, and washes out with a half-life of hours or days [60, 61]. Consistent with these properties of binding, diclofenac at 5–50 μ M, when given to J774.2 cells for 48 h as described above, had a dose-dependent inhibitory effect on COX activity (figure 4). Unexpectedly, however, at doses above 50 μ M, where diclofenac began to induce apoptosis and COX-2, there was a dramatic, dose-dependent increase in enzyme activity. This induction was particularly obvious when COX activity was normalized to cellular protein concentrations (figure 4). After these studies, NSAID-inducible activity was also identified by others by use of the COX-2 selective drug NS398. This COX-2-selective inhibitor, like diclofenac, is a time-dependent inhibitor of COX-2 [62]. However, chronic treatment of rat fetal hepatocytes with NS398 induces

COX-2 and COX activity that is revealed upon removal of the NSAID [62].

The finding of this unusual NSAID-induced COX activity allowed it to be characterized pharmacologically. The increased COX activity in J774.2 cells induced by diclofenac was more sensitive to acetaminophen than either COX-2 or COX-1 in the same cells (figure 5). The 50% inhibitory concentration (IC_{50}) values for acetaminophen inhibition were 0.125–1 mM, depending on the experiment. The reason for this experimental variation is currently unknown but is presumed to be subtleties in cell treatment that have yet to be identified. Additionally, the NSAID-induced activity was found to be inhibited by diclofenac itself and by other NSAIDs, but at significantly higher concentrations than those needed to inhibit either COX-1 or COX-2 in the same cells. For example, COX-2 in J774.2 cells had been shown to be inhibited by diclofenac, flurbiprofen, or tolafenamic acid with IC_{50} values of 1, 0.1, and 0.2 μ M, respectively [54]. In contrast, the COX activity induced by diclofenac was inhibited with IC_{50} values for same drugs of 10, 230, and 220 μ M, respectively, or an increase of 1–5 orders of magnitude. Perhaps most significant was that aspirin, a relatively weak inhibitor of COX-2, failed to inhibit the induced NSAID-induced activity.

Thus far, we have been unable to disassociate the induction of NSAID-induced enzyme activity from induction of COX-2 in that whenever we have found the elevated activity, we have found COX-2 to be elevated as well. However, as described below, there are cell lines in which we can induce COX-2 in the presence of NSAIDs and not obtain any activity. Our cur-

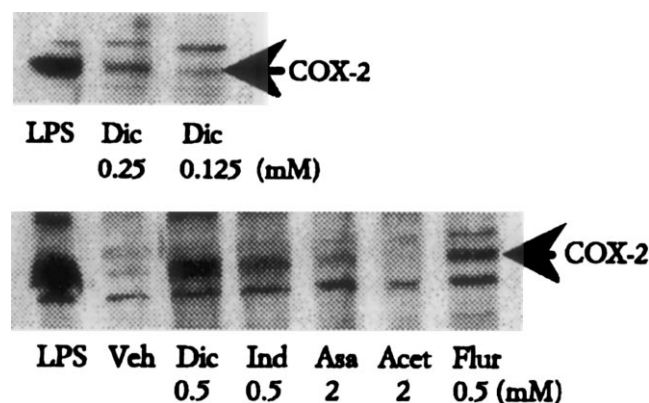


Figure 3. Induction of cyclooxygenase (COX)-2 protein expression by nonsteroidal anti-inflammatory drugs (NSAIDs). J774.2 cells were treated with lipopolysaccharide (LPS; 1 μ g/mL for 12 h), vehicle (Veh), or NSAIDs (for 48 h) at the doses indicated. Cellular proteins were electrophoresed and probed by Western blot with isoenzyme-specific anti-COX-2 sera. COX-2 was induced 10–30-fold by 0.5 mM indomethacin (Ind 0.5%), 0.5 mM flurbiprofen (Flur 0.5), and 0.5 mM diclofenac (Dic 0.5), but was not detectably induced by 2 mM acetaminophen (Acet 2) and was only marginally induced by 2 mM aspirin (Asa 2). Induction of COX-2 by diclofenac was dose dependent. From Simmons et al. [58].

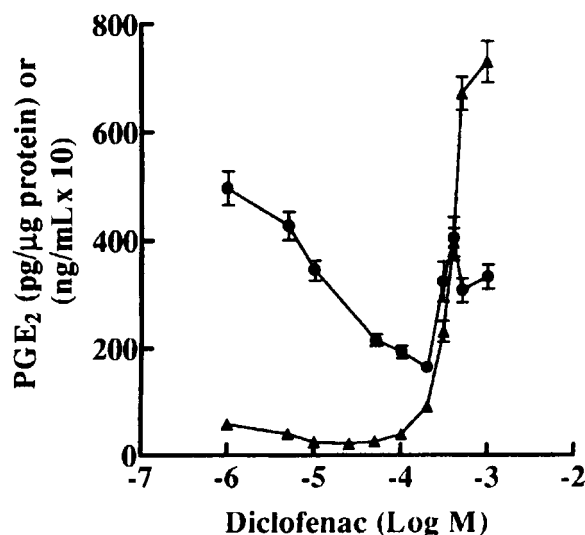


Figure 4. Induction of cyclooxygenase (COX) activity by diclofenac in J774.2 cells. ●, J774.2 cells were treated with diclofenac at the concentrations indicated for 48 h. After treatment cells were washed twice to remove drug and then exposed to 30 μ M arachidonic acid for 15 min, the amount of prostaglandin E₂ (PGE₂) released was estimated by radioimmunoassay, expressed here as nanograms of PGE₂ per milliliter of media times a factor of 10. ▲, Values have been recalculated as picograms of PGE₂ per microgram of protein to normalize for the large decrease in cell number and mass occurring at higher NSAID concentrations (≥ 200 μ M). From Simmons et al. [58].

rent hypothesis is that the NSAID-induced activity either represents a variant of COX-2 or a third COX. Induction of this COX enzyme may occur by activation of peroxisome proliferator activated receptors by NSAIDs as has been shown for COX-2 [63, 64]. However, if induction does not require induction of COX-2 mRNA, as reported [59], this would appear to be unlikely because peroxisome proliferator-activated receptors function as transcription factors. We have shown that NSAID treatment results in a COX-2-luciferase fusion protein localized in the cytosol, as opposed to membrane [58]. This suggests that chronic treatment with NSAIDs may result in mobilization of COX-2 in which COX-2 is either not translocated efficiently into the lumen of the nuclear envelope/endoplasmic reticulum or loses its high affinity for membrane. During the apoptosis that occurs concomitantly with NSAID treatment, large rearrangements and mixing of cellular compartments occur, and this may be the mechanism by which mobilization of COX-2 occurs. It is interesting to note that in nonapoptotic cells, extralumenal COX-2 subpopulations have been reported in the nucleus matrix and cytosol [65, 66].

Inhibition of NSAID-induced COX activity by acetaminophen but not aspirin, coupled with reduced sensitivity to competitively acting NSAIDs, suggests that large changes in the COX-2 active site or sites have occurred because of chronic treatment with high concentrations of NSAIDs. Supporting this

concept is the observation that diclofenac readily washes out of the active site of the enzyme in cells treated with high, but not low, concentrations of NSAIDs. What these changes are is currently unknown. However, it is clear that the factors or processes that produce this change are cell specific. This is indicated by clear differences in the degree to which this activity can be induced in different cell types. For example, J774A.1 cells, although derived from the same tumor as J774.2 cells, show substantially reduced induction relative to J774.2 cells, and some cells show no detectable induction of activity by diclofenac, even though COX-2 is present in the cells.

Conclusion

We have identified a COX activity that is more susceptible to inhibition by acetaminophen than is either COX-1 or COX-2 in the same cell. The increased susceptibility appears to be due to cell-specific factors that produce an altered COX-2 or induction of a new COX-3. However, concentrations of acetaminophen needed to evoke a 50% reduction in COX activity are still relatively high, and the activity is not inhibited by aspirin. Further research will be needed to determine if this

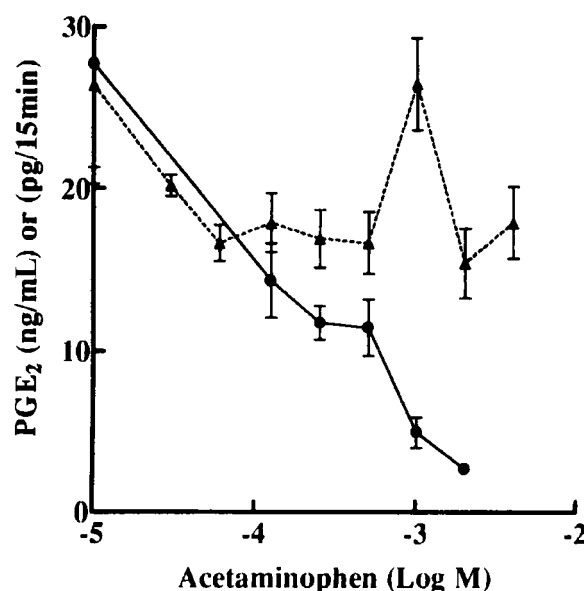


Figure 5. Sensitivity of diclofenac-induced cyclooxygenase (COX)-2 but not lipopolysaccharide (LPS)-induced COX-2 to inhibition by acetaminophen. ●, J774.2 cells were treated with 0.5 mM diclofenac for 48 h to induce COX-2 and apoptosis. After this treatment, diclofenac was removed by washing, and the cells were exposed to acetaminophen for 30 min at the doses indicated. After acetaminophen treatment, cells were exposed to 30 μ M arachidonic acid for 15 min, and the amount of prostaglandin E₂ (PGE₂) released was measured as nanograms of PGE₂ per milliliter of media. ▲, J774.2 cells were treated with LPS (1 μ g/mL for 12 h) to induce COX-2. Cells were treated with acetaminophen, and PGE₂ released was measured as picograms of PGE₂ per 15 min. From Simmons et al. [58].

activity is found in vivo and whether its inhibition is responsible for the antipyretic and analgesic properties of acetaminophen.

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