Prostanoids mediate a variety of cellular interactions in physiological and pathological processes, including hemostasis and thrombosis, glomerular filtration and water balance, ovulation, embryo implantation, and development; induction of labor or abortion; and inflammation and modulation of immune responses. Prostanoids are biologically active metabolites of arachidonic acid (AA). In response to different stimuli (i.e., physical, chemical, hormonal, cytokines, etc.), AA is mobilized from membrane phospholipids through the action of phospholipases (PL) and is then converted to prostaglandin (PGH)2 by cyclooxygenase (COX)-1 or COX-2 (Figure 1). COX iso-enzymes catalyze a two-step reaction, first cyclizing AA to form PGH2, and then reducing the 15-hydroperoxy group to form PGH2. Cell-specific isoenzymes or reductases catalyze the conversion of PGH2 to biologically active end-products including PGE2, PGF2α, PGD2, and PGI2 and thromboxane (TXA2). Known collectively as prostanoids (Figure 1). Prostanoids are produced as needed in the cell of origin, act primarily as autacoids on the parent cell and/or neighboring cells, and have very short half-lives.

COX-1 and COX-2 enzymes catalyze the same reactions, show approximately 60% identity in their amino-acid sequence within a given species, but are encoded by two different genes, located in different chromosomes. They seem to have different functions even within the same cell type (1, 2). COX-1-dependent prostanoids serve a number of physiologic "housekeeping" functions, such as modula- tion of platelet aggregation and cytoprotection in the gastrointestinal mucosa (1). In addition, the expression of COX-1 is developmentally regulated in many different tissues including thymus (1, 3), and small changes in expression (e.g., 1-2-fold increase) can occur after stimulation with hormones or growth factors (1). In contrast, COX-2 is induced in macrophages, fibroblasts, vascular endothelial cells, and smooth muscle cells by various cytokines, endotoxins, growth factors, or tumor promoters (1). Therefore, COX-2-depend-ent PGs play a major role in inflammation and cell proliferation; however, constitutive expression of COX-2 has been found in certain regions of the brain, reproductive tissues, kidney, and thymus (2, 3).

For many years, the rate-limiting step in the production of PGs was thought to be the activation of PLs to release membrane-bound AA (Figure 1). The discovery of COX-2 in the early 1990s and of different mechanisms through which the two COX isoforms are regulated added a new rate-limiting step in PGs biosynthesis through the inducible conversion of AA by COX-2 (Figure 1). Subsequently, it became evident that the protein expression of specific down-stream PG synthases, such as the PGE2 synthase (PGES), could be induced, leading to an overall increase in enzymatic activity PGE2 production (4, 5). Two isoforms of PGES have been described—one membrane-associated (mPGES) and one cytosolic (cPGES) (6, 5)— and COX-1 and COX-2 exhibit differential "functional" coupling to each isoform of the PGES in different cell types, including platelets (4-7). There has been no demonstrated physical binding between COX and PGES; rather, it is more likely that intracellular colocaliza- tion of these enzymes creates a local zone where AA is processed by physically close (but not coupled) enzymes. In addition, intracellular distribution of both COXs and PGES appears compartmentalized, in that COX-2 and mPGES are mainly membrane-associated and local- ized in the perinuclear envelope (8). Intracellular compartmentalis- tion and functional coupling create preferential and selective "routes" for AA in response to different biological demands. Indeed, PGE2 and PGD2 appear to be the main prostanoids produced by COX-2, whereas COX-1 can generate all of the PGs (6, 7), depending on the availability of other enzymes present. Finally, the complex regulation and tissue specificity of prostanoid actions is enriched by a variety of cell receptors which trigger different intracellular signaling (8).

PG receptor subtypes were first characterized pharmacologically and classified based on their sensitivity to five primary prostanoids (i.e., PGE2, PGF2α, PGI2, and PGD2) and their affinity for EP1, EP2, EP3, and EP4 receptor subtypes, respectively (8). Among prostanoids, PGE2 has the most receptors: four subtypes of EPs have been characterized so far: EP1, EP2, EP3, and EP4, defined on the basis of their pharmacological profiles (8). EPs are encoded by distinct genes and have divergent amino-acid sequences, but all bind PGE2 with higher affinity than other prostanoids. Thus, based on multiple receptor subtypes, PGE2 can trigger several different intracellular signal transduction paths and has diverse final effects, which sometimes seem to be even functionally opposing within the same cell or organ.

Activation of the EP1 receptor most likely increases intracellular Ca2+, through Gs, phospholipase C (PLC)/inositol trisphosphate signaling, and protein kinase C (PKC) activity. EP1-mediated Ca2+ increases, however, might not be solely dependent on Gq activity (8). EP2 and EP4 stimulate adenylyl cyclase via Go, leading to the production of adenosine 3′,5′-monophosphate (cyclic AMP, cAMP), which then activates the cAMP-dependent protein kinase (PKA) (9). Stimulation of EP4 is also known to activate phosphoinositide 3′-kinase (PI3K (9). At variance with other EPs, the EP3 has multiple splice variants, each having a unique C-terminal cytoplasmic tail (8), which adds more complexity to EP3-mediated signaling. EP3 generally inhibits adenylyl cyclase through the activation of Gi (a pertussis toxin-sensitive G protein), however, EP3 is likely to signal through G-protein-Blo interactions as well (8).

The complexity of the final response to PGE2 is further com- plicated by evidence that multiple EPs are often coexpressed or induced in the same cell or organ. The biological significance and regulation of this coexpression is currently unknown, but it surely indicates that the response to PGE2 is tightly modulated and hardly predictable, based on the activation of different pathways by dif-
ferent EP subtypes. The “plasticity” of the final PGE2-triggered response in a given cell, may also vary depending on the local PGE2 concentration. The diversity of receptors, of intracellular signaling, and the possible coexpression of more than one isoform altogether call for the need of extensive investigation on PGE2 to assess its final biological effects and the metabolic pathways in which it is involved in different cell types and tissues. The pleiotropic effects of PGE2 are reflected in the complexity of phenotypes generated by the disruption of each EP in mice (Table 1) (10–28). Overall, data from knockout animals further confirm that more than one EP is often involved in the same physiological or pathological process (e.g., immunity, inflammation, carcinogenesis, or brain damage) with different or even opposing contributions.

Given the extraordinary complexity, fine regulation, and tissue-specificity of the final effects of the ubiquitous system of prostanoid—and of PGE2 in particular—it is not surprising that blocking the total PGE2/prostanoid production in each organ, through the pharmacological inhibition of upstream COX-1 and/or -2, might potentially be beneficial and deleterious at the same time.

### Table 1. The Pleiotropic Action of PGE2: Phenotypes of EP Knockout Mice

<table>
<thead>
<tr>
<th>Genetic deletion</th>
<th>Inflammation/Immunity</th>
<th>Carcinogenesis</th>
<th>Reproduction</th>
<th>Kidney</th>
<th>Lung</th>
<th>Central or peripheral nervous system</th>
<th>Bone</th>
<th>Gastrointestinal tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP1/−/−</td>
<td>No effect on MNL (13)</td>
<td>↓ Early and AOM-induced colon cancer (24)</td>
<td>↓ Airway responsiveness (27)</td>
<td>↓ Inflammation-dependent fever (26)</td>
<td>↓ NMDA-induced stroke (28)</td>
<td>↓ Gastrointestinal integrity (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP2/−/−</td>
<td>↓ PGE2-dependent MNL inhibition (10)</td>
<td>↓ skin and colon tumors (12.13)</td>
<td>↓ Ovulation and fertilization (11)</td>
<td>↓ Hypertensive effect of PGE2 (9)</td>
<td>↓ Salivary hypertension (9)</td>
<td>↓ Basal secretion rate (9)</td>
<td>↓ Spinal PGE2-evoked hyperalgesia (16)</td>
<td>↑ Stroke after artery occlusion or NMDA (17, 18)</td>
</tr>
<tr>
<td>EP3/−/−</td>
<td>↑ Allergic inflammation (21)</td>
<td>No effect on MNL (10)</td>
<td>↓ Colon cancer development induced by AOM (22)</td>
<td>↓ Capacity of urinary concentration (23)</td>
<td>↓ Pyrogen-dependent fever (23)</td>
<td>No effect on gastric mucosal protection (23)</td>
<td>Acid induced bicarbonate secretion from duodenum (23)</td>
<td></td>
</tr>
<tr>
<td>EP4/−/−</td>
<td>↓ Arthritis (9)</td>
<td>↓ PGE2-dependent MNL inhibition (10)</td>
<td>↓ Migration of APC cells (9)</td>
<td>↓ Skin hyper-responsiveness (9)</td>
<td>↑ Infarct in ischemia-reperfusion injury (20)</td>
<td>↓ Bone mass (9)</td>
<td>↓ Intestinal mucosal barrier (25)</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate references. MNL, mixed lymphoid reaction; AOM, azoxymethane; NMDA, N-Methyl-D-Aspartate; PTH, parathyroid hormone. ↓ indicates reduction; ↑ indicates enhancement.
which are among the most widely used agents worldwide, inhibit COX activity, preventing the formation of prostanoids. Individual NSAIDs show variable potencies against COX-1 compared to COX-2, although none of them shows greater than 20-fold preference for COX-2. Highly selective COX-2 inhibitors, called Coxibs, have been marketed in late nineties and showed comparable anti-inflammatory and analgesic activities with fewer gastrointestinal complications than traditional NSAIDs (2). This therapeutic profile is compatible with the inhibition of COX-2-dependent PGE2 synthesis, which is involved in inflammation and pain, as previously shown by in vitro, animal, and human studies; however, Coxibs have been associated with a 2- to 3-fold excess of cardiovascular events (29–32). Although the extent of this excess needs further definition, and ad hoc placebo-controlled studies are lacking, this phenomenon appeared influenced by the pre-existing profile of a patient’s cardiovascular risk (29–32). The hazardous cardiovascular effect mediated by the Coxibs likely arises from inhibition of COX-2-dependent PGI2 released from endothelium [which is protective for vessel injury (33)] without a concomitant inhibition of COX-1-dependent platelet activation. Alternatively, it may be an indirect effect of increased blood pressure (34), or it might be related to the inhibition of COX-2 dependent anti-inflammatory pathways (35). It is unknown whether this profile applies to Coxibs only or to NSAIDs as well, for clinical trials versus placebo are lacking. Furthermore, as shown by the Therapeutic Arthritis Research and Gastrointestinal Event (TARGET) study (36), the excess of cardiovascular complications was nonsignificant in short-term studies (37). Regardless of the final outcome of this issue, altogether, these data strongly encourage the development of new therapeutic strategies [beyond a generic COX-1/COX-2 inhibition, either selective (Coxibs) or non-selective

![Diagram of the arachidonic acid cascade and its physiological and pharmacological modulation](https://via.placeholder.com/150)

**Figure 1.** Arachidonic acid cascade and its physiological and pharmacological modulation. The metabolism of arachidonic acid to prostanoids is represented. Physiological regulations (right side of the figure) and pharmacological modulators (left side of the figure) are shown at different steps of the pathway. Enzymatic pathways, products, regulation, and drugs preferably mediated by COX-1 are indicated in green, whereas items preferably mediated by COX-2 appear in blue. Substrates, modulators, products, or drugs common to both isoenzymes are shown in red. See text for detailed explanations of abbreviations.
of PGE2-dependent MMPs in resident macrophages might cause plaque destabilization. Furthermore, EP4 overexpression is associated with enhanced inflammatory reactions in human atherosclerotic plaques, suggesting a contribution of the EP4 receptor to plaque destabilization (39). Recently, Paradis et al. have directly proved that in macrophages, COX-2-dependent MMP expression is effectively blocked by the administration of selective EP4 antagonists or by silencing EP4 expression via small interfering RNAs (siRNAs) (50). Therefore, in patients with advanced atherosclerosis, targeting the EP4 might be a better strategy than COX2 blockade in preventing symptomatic plaque rupture.

So far, only few prostanooid receptor agonists or antagonists have been tested or are currently used in human disorders. Thromboxane receptor (TP) antagonists have been studied for their utility in preventing cardiovascular disorders. The potential advantages of potent TP antagonists compared with low-dose aspirin are related to the discovery of aspirin-insensitive agonists of the platelet receptor, such as TXA2, derived from the COX-2 pathway and 8α-TXα2 (an F2α-isoprostane that is a product of the free radical–catalyzed peroxidation of arachidonic acid) (51). The latter can synergize with subthreshold concentrations of other platelet agonists to evoke a full response, thus amplifying platelet activation in those clinical settings associated with enhanced lipid peroxidation (51). The TP agonist, S-18806 (tenofoax) is currently being compared to low-dose aspirin in a large randomized trial (PERFORATE) in patients with a recent cerebrovascular event (i.e., ischemic stroke and transient ischemic attack (TIA)) (52). Tioprost and other PGs, analogs are used in pulmonary arterial hypertension (53). Among EP agonists, misoprostol, a non-selective EP2/EP3/EP4 agonist, is used systemically as gastroprotective agent (54) and both locally or systemically in obstetrics (albueful use) for termination of pregnancy (55), induction of labor (56), and prevention of post-partum hemorrhage (57). Lower abdominal pain, diarrhea, chills, and fever are the most common side-effects (up to 50%) of sublingual misoprostol, which is associated with better bioavailability than oral administration; and 4) the timing and length of administration should be carefully considered. Nonetheless, more refined research on the targeting of prostanooid receptors may very well increase the clinical pharmacology of targetting prostanoid receptors may very well increase the capacity to control prostanoid receptors.
specify and decrease the side-effects associated with pharmacological regulation of the prostaglandin pathways in human disorders and may lead to more promising therapeutic options (as opposed to direct COX-2 inhibition) in the future. doi:10.1146.t2.3

Acknowledgments

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References

8. Nakamura, M., Naraba, T., Tanioka, N. et al. Regulation of prostaglandin synthesis in adult T-cell development. J. Clin. Invest. 100, 1469–1477 (1998). These authors were the first to molecularly clone and characterize the inducible isoform of PGES.
10. Nataraj, C., Thomas, D.W., Tilley, S.L., Nguyen, M.T., Mannon, R., Koller, B.H., and Coffman, T.M. Receptors for prostaglandin E2 that regulate cellu-

11. Kennedy C.R., Zhang, Y., Brandon, S. et al. Salt-sensitive hypertension (RBNE01A882_005) and by the “EICOXANOX” grant (project num-

15. Somishima, M., Takaki, K., Ohashi, M., Sugihara, K., and Taketo, M.M. Acceleration of intestinal polyps through prostaglandin E receptor EP2 knock-

16. Reinhold, R., Ahmad, S., Depner, U.B. et al. Spinal inflammatory hyper-


28. Ishii, S., Ishii, K., Iizuka, K., and Ishii, S. Characteristics of thermoregulatory and motile responses in mice deficient in prostaglandin EP1 and EP3 recep-

29. Bresalier, R.S., Sandler, R.S., Quan, H. et al. Adenomatous polyp pre-

30. Jones, C.S. Relative thromboembolic risks associated with COX-2 inhibi-

31. Chang, S.H., Ai, Y., Breyer, R.M., Lane, T.F., and Hla, T. The prostaglan-

32. Sonoshita, M., Takaku, K., Oshima, M., Sugihara, K., and Taketo, M.M. Acceleration of intestinal polyps through prostaglandin E receptor EP2 knock-

34. Shoji, Y., Tachibana, M., Kilmus, T. et al. Downregulation of prostagland-


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