

Prostaglandin receptor EP2 in the crosshairs of anti-inflammation, anti-cancer, and neuroprotection

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Modulation of a specific prostanoid synthase or receptor provides therapeutic alternatives to nonsteroidal anti-inflammatory drugs (NSAIDs) for treating pathological conditions governed by cyclooxygenase-2 (COX-2 or PTGS2). Among the COX-2 downstream signaling pathways, the prostaglandin E₂ (PGE₂) receptor EP2 subtype (PTGER2) is emerging as a crucial mediator of many physiological and pathological events. Genetic ablation strategies and recent advances in chemical biology provide tools for a better understanding of EP2 signaling. In the brain, the EP2 receptor modulates some beneficial effects, including neuroprotection, in acute models of excitotoxicity, neuroplasticity, and spatial learning via cAMP–PKA signaling. Conversely, EP2 activation accentuates chronic inflammation mainly through the cAMP–Epac pathway, likely contributing to delayed neurotoxicity. EP2 receptor activation also engages β-arrestin in a G-protein-independent pathway that promotes tumor cell growth and migration. Understanding the conditions under which multiple EP2 signaling pathways are engaged might suggest novel therapeutic strategies to target this key inflammatory prostaglandin receptor.

Overview

Cyclooxygenase (COX) is the rate-limiting enzyme in the synthesis of biological mediators termed prostanoids, consisting of prostaglandin PGD₂, PGE₂, PGF_{2α}, prostacyclin PGI₂, and thromboxane TXA₂. Prostanoids function via activation of nine G-protein-coupled receptors (GPCRs): DP1 and DP2 receptors for PGD₂; EP1, EP2, EP3, and EP4 for PGE₂; FP for PGF_{2α}; IP for PGI₂; and TP for TXA₂ (Figure 1). As the inducible COX isoform, COX-2 is generally regarded as a pro-inflammatory enzyme and contributes to tissue injury [1,2]. However, the deleterious cardiovascular and cerebrovascular side effects of sustained inhibition of COX-2 point to beneficial actions of some COX-2 downstream prostanoid signaling [3]. The Jekyll and Hyde nature of COX-2 signaling pathways suggests that modulation of a specific prostanoid synthase or receptor could be a superior therapeutic strategy com-

pared to generic block of the entire COX-2 cascade. The rapid induction of COX-2 by cell injury or excessive neuronal activity is often associated with induction of membrane-associated PGE synthase-1 (mPGES-1 or PTGES), which produces PGE₂ from COX-2-derived PGH₂ [4]. Among the multiple COX-2 downstream signaling pathways, prostaglandin PGE₂ signaling via its EP2 receptor subtype appears to be a major mediator of inflammatory and anaphylactic reactions within both the periphery and brain. EP2 signaling pathways engage protein kinase A (PKA), the exchange protein activated by cAMP (Epac), and β-arrestin. Here, we highlight our current understanding of EP2 receptor signaling and summarize its pathophysiological roles in disparate disease conditions involving inflammation, such as chronic pain, cancer, and brain injury, with an emphasis, where possible, on recent *in vivo* experimental data.

PGE₂–EP2 signaling

As a stimulatory Gs GPCR, EP2 activation by PGE₂ stimulates adenylate cyclase (AC), resulting in elevation of cytoplasmic cAMP levels to initiate multiple downstream events via its prototypical effector PKA. PKA directly phosphorylates and activates transcription factors such as the cAMP-responsive element binding protein (CREB), which mediates neuronal plasticity, long-term memory formation, neuronal survival, and neurogenesis in the brain (Figure 2) [5]. In the past decade, Epac has emerged as an alternative cAMP sensor [6]. Two Epac isoforms have been identified so far: Epac1, known as Rap guanine nucleotide exchange factor 3 (RAPGEF3), and Epac2, Rap guanine nucleotide exchange factor 4 (RAPGEF4). They only differ in that Epac2 has an extra cAMP binding site and a Ras-association domain for subcellular localization [6]. In response to cAMP binding, Epac activates the downstream effectors Rap1/2 to mediate a wide range of biological processes. In the central nervous system (CNS), Epac can regulate learning and memory [7], axon growth, guidance and regeneration [8], neuronal differentiation [9], neuronal excitability [10], learning and social interactions [11], brain oxidative stress [12], neuronal apoptosis [13], and inflammatory hyperalgesia [14,15]. PKA and Epac are often involved in the same biological process, in which they function either synergistically or oppositely [6]. For example, like PKA, Epac can also activate CREB directly [9]. Interestingly, PKA signaling is often related to neuronal

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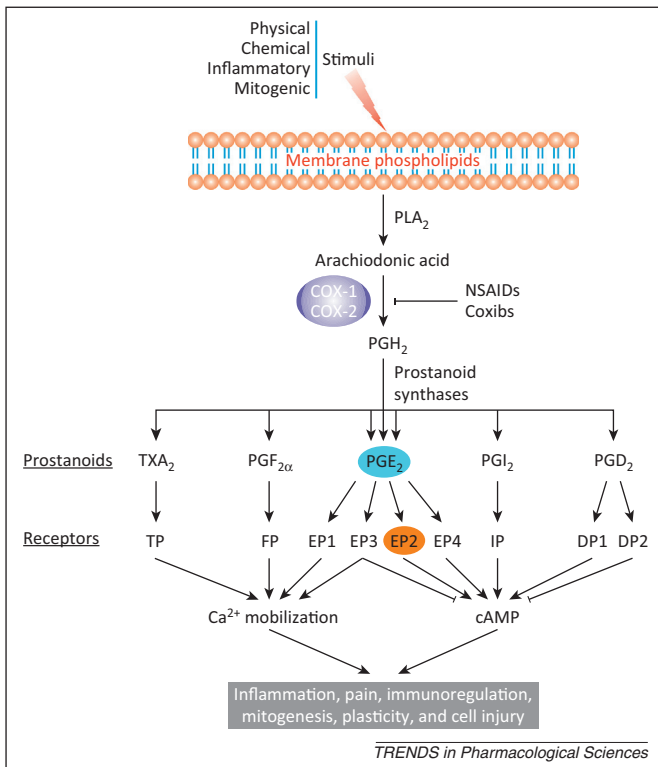


Figure 1. The cyclooxygenase (COX) signaling cascade regulates multiple physiological and pathological events. In response to a variety of stimuli, arachidonic acid (AA), a 20-carbon fatty acid, is freed from membrane phospholipids by phospholipase A2 (PLA₂) and then converted in a dual enzymatic reaction to unstable intermediate prostaglandin H₂ (PGH₂) by COX, which has two forms, COX-1 and COX-2. The COX-1 isozyme is constitutively expressed in most mammalian cells to maintain normal homeostasis, whereas COX-2 is usually undetectable in most normal tissues but strongly induced by excessive neuronal activity, growth factors, or pro-inflammatory stimuli in activated macrophages and other cells at sites of inflammation. Most nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, and naproxen, act as nonselective COX inhibitors, whereas the coxibs selectively inhibit the COX-2 isoform. Short-lived PGH₂ is then quickly converted to five prostanoids (PGD₂, PGE₂, PGF_{2α}, PGI₂, and TXA₂) by tissue-specific prostanooid synthases. Prostanoids exert their functions by activating a suite of G-protein-coupled receptors (GPCRs). Two GPCRs (DP1 and DP2) are activated by PGD₂ and four by PGE₂ (EP1, EP2, EP3, and EP4), whereas each of the other three prostanoids activates a single receptor (FP, IP, and TP). Prostanoids mediate multiple physiological and pathological effects including inflammation, pain, immunoregulation, mitogenesis, plasticity, and cell injury. Only the major pathways are shown.

survival [5,12,16], whereas Epac activation can lead to oxidative stress and neuronal injury (Figure 2) [12,13]. The differential regulation of PKA and Epac by cAMP could be related to the gradient of cytoplasmic cAMP because cAMP has a lower affinity for Epac than for PKA [17]: cAMP initially stimulates PKA signaling at the beginning of EP2 receptor activation, whereas for sustained EP2 activation the Epac pathway dominates as cytoplasmic cAMP levels continue to rise.

Activated GPCRs can be phosphorylated by G-protein-coupled receptor kinases (GRKs) and recruit β-arrestin to modify subsequent G-protein-dependent signaling by initiating receptor desensitization, internalization, and resensitization. β-Arrestin also serves as an adaptor and scaffold to switch signaling to G-protein-independent pathways. An EP4 receptor–β-arrestin signaling complex has been well characterized, whereas it was recently recognized that the EP2 receptor regulates β-arrestin signaling to initiate phosphoinositide 3-kinase (PI3K)–Akt, Ras–extracellular-signal-regulated kinase (ERK), and c-Jun

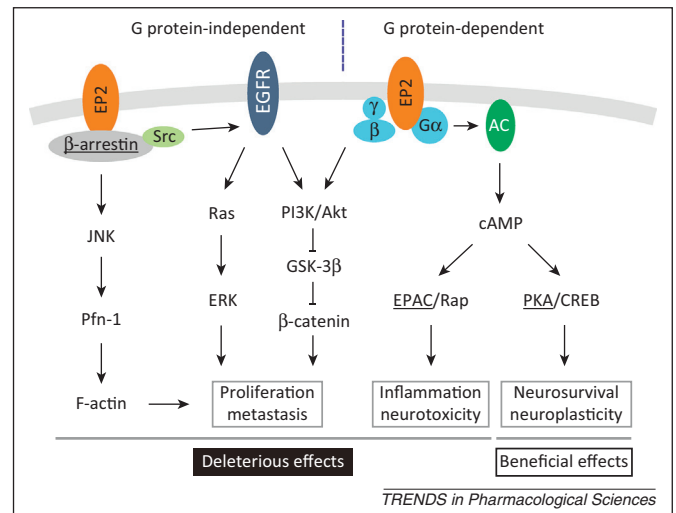


Figure 2. Signal transduction by the prostaglandin receptor EP2. In response to prostaglandin E₂ (PGE₂) the EP2 receptor mediates both G-protein-dependent and -independent signaling pathways with multiple beneficial and deleterious actions. We hypothesize that the EP2 receptor mediates cellular survival and neuroplasticity mainly via the cAMP–protein kinase A (PKA)–cAMP-responsive element binding protein (CREB) pathway, but inflammation and neurotoxicity via cAMP–exchange protein activated by cAMP (Epac)–Rap signaling, and cell proliferation and migration via β-arrestin. Signaling crosstalk occurs among these three EP2 downstream pathways, but only the major pathways and effects are indicated.

N-terminal kinase (JNK) pathways, which are particularly important for cell proliferation and migration (Figure 2) [18–20]. Like EP4, EP2 promotes T helper (Th1) cell differentiation through the PI3K–Akt pathway rather than its conventional cAMP signaling [21].

It has been shown that the EP2 receptor regulates synaptic transmission and cognitive function. RNAi for the EP2 receptor can decrease long-term potentiation (LTP) in rat visual cortex [22]. In response to theta-burst stimulation, the G_s-coupled EP2 receptor translocates from the cytosol to postsynaptic membranes, whereas the G_i-coupled EP3 receptor moves oppositely, resulting in enhanced postsynaptic cAMP–PKA signaling [22], which in turn activates CREB, a well-documented transcription factor for the late stage of LTP and memory (Figure 2) [5]. Thus, EP2 receptor trafficking mimics that of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type glutamate receptor during LTP. AMPA receptor trafficking to and away from postsynaptic surfaces modulates synaptic strength [23–25]. Therefore, it would be very interesting to examine whether EP2 signaling regulates synaptic transmission by regulating AMPA receptor trafficking in postsynaptic sites. However, presynaptic EP2 receptors might also be involved in synaptic transmission if postsynaptic PGE₂ acts as a retrograde messenger [26]. A contribution of the EP2 receptor to synaptic plasticity and cognitive functions is further indicated by findings of impaired hippocampal LTP, long-term depression, and cognitive functions in mice lacking EP2 receptors (EP2^{-/-}) [27,28]. Application of PGE₂ or buta-prost enhances synaptic transmission in wild type mice, which can be attenuated by the PKA inhibitor H-89, suggesting that PGE₂ modulates long-term synaptic plasticity and cognitive functions mainly through an EP2–G_s–cAMP–PKA–CREB signaling cascade (Figure 2), although ERK and IP3 pathways might also be involved [28].

Advances in chemical biology

EP2^{-/-} mice have been independently developed by at least three groups [29–31]. Genetic ablation of prostanoid receptors has been useful but is complicated by developmental and other homeostatic adjustments that result in reduced litter size and hypertension [29–31]. As a complement to the genetic strategy, a number of small-molecule

ligands that target the EP2 receptor have been developed. EP2 is activated by its natural agonist PGE₂ and by a number of PGE₂ analogs (butaprost, CAY10399, and ONO-AE1-259) and compounds with non-prostanoid structures such as CP-533536 and compound 9 (Figure 3). These agonists and the nonselective EP receptor antagonist AH6809 have been widely used to explore the roles of

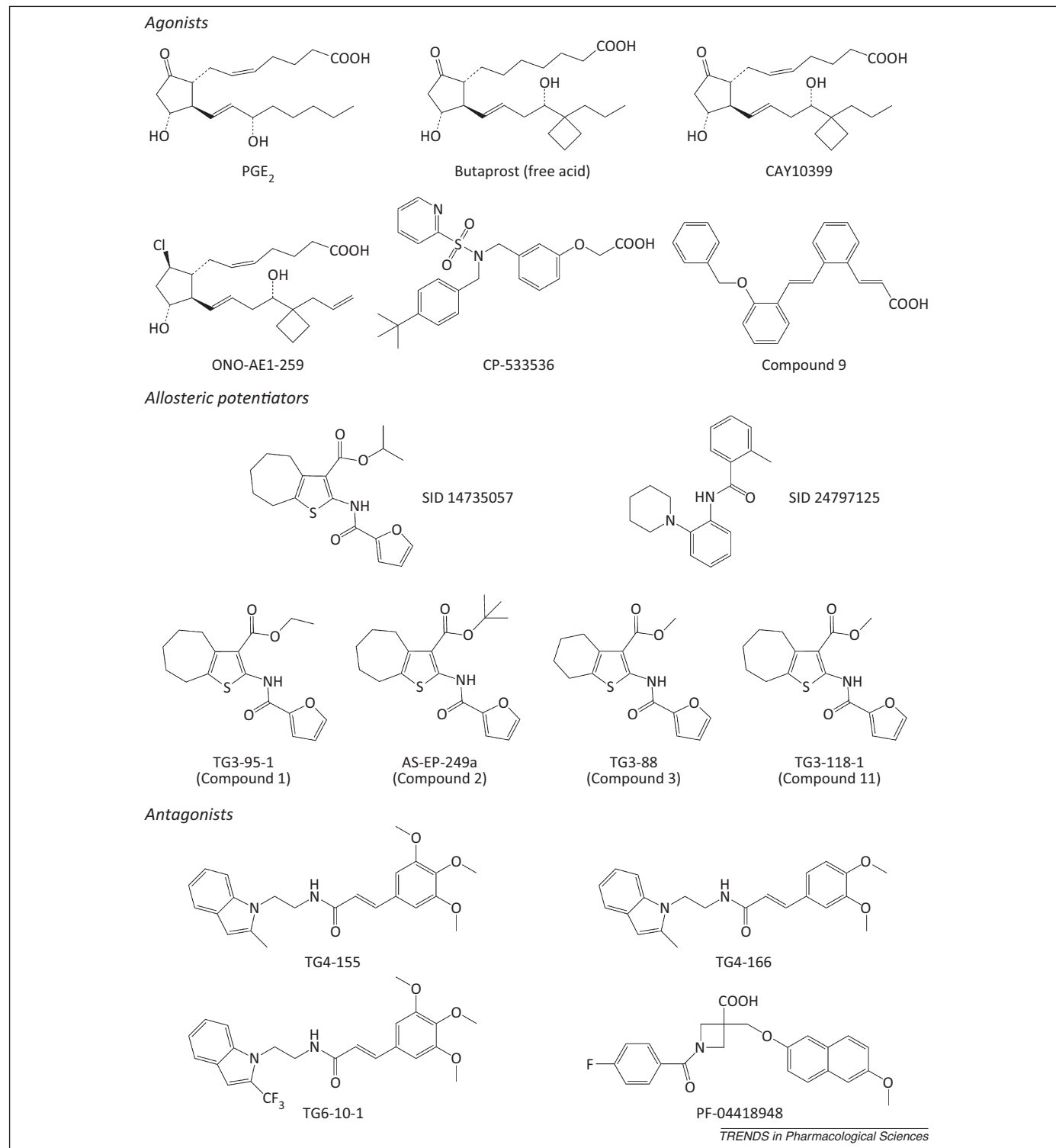


Figure 3. Chemical structure of selective small-molecule modulators of the EP2 receptor. Agonists: PGE₂, butaprost, CAY10399, ONO-AE1-259, CP-533536, and compound 9. Allosteric potentiators: substance identification number (SID) 14735057, SID 24797125, TG3-95-1 (referred to as compound 1 in [35]), AS-EP-249a (compound 2 in [35]), TG3-88 (compound 3 in [35]), and TG3-118-1 (compound 11 in [35]). Antagonists: TG4-155, TG4-166, TG6-10-1, and PF-04418948. These EP2 ligands are well characterized in terms of their potency and selectivity. Some of them have been evaluated for pharmacokinetics and tested in animal disease models.

PGE₂-EP2 signaling under normal and pathological conditions. However, butaprost is only approximately 18-fold selective for EP2 over EP3 in binding studies [32]; CAY10399 and ONO-AE1-259 are highly EP2-selective but have a prostanoid-like structure; CP-533536 is only approximately 64-fold selective over the EP4 receptor [33]; compound 9 is quite selective against other PGE₂ receptors but less than fourfold selective over the TP receptor [34]; AH6809 is neither selective nor potent and is unsuitable for *in vivo* study [32]. Recently reported allosteric potentiators and selective antagonists with non-prostanoid structures for the EP2 receptor provide alternative probes to elucidate the physiological functions of this key prostaglandin receptor (Figure 3) [35–37]. These small-molecule EP2 modulators make it possible to functionally differentiate EP2 from other prostanoid receptors, particularly EP4, in COX-2-mediated physiological and pathological events. As our tools for studying the EP2 receptor expand, so too will our understanding of its role in health and disease conditions.

Inflammation and pain

Acute inflammation is initiated by tissue-resident immune cells such as macrophages and microglia, which undergo activation in response to signals released by injured tissue. Activated macrophages and microglia rapidly synthesize and release primary inflammatory mediators such as bradykinin, histamine, cytokines, and chemokines. These mediators dilate local blood vessels and increase their permeability, leading to leakage of plasma proteins into the tissue. They also increase pain sensitivity in tissues innervated by sensory nerve endings and attract leukocytes that migrate along a chemotactic path from blood into the tissue. Certain inflammatory cytokines induce COX-2 expression via an NF- κ B pathway [38], which in turn synthesizes PGE₂, a secondary mediator of inflammation that promotes local vasodilation and attraction and activation of neutrophils, macrophages, and mast cells during acute inflammation [39,40]. However, PGE₂ also induces anti-inflammatory cytokines including IL-10 and suppresses the production of pro-inflammatory cytokines. Thus, PGE₂ acts as an immune modulator rather than a simple pro-inflammatory molecule [41].

Inflammation often resolves rather quickly, but chronic inflammation appears to contribute to the pathophysiology of many chronic conditions including rheumatoid arthritis, pain, cancer, and neurological disorders [39,40,42]. Experiments with mice deficient in each of the four subtypes of the PGE₂ receptor demonstrate that PGE₂-EP2/EP4 and PGI₂-IP signaling play crucial roles in the development of collagen-induced arthritis (CIA) [43]. PGE₂ signaling through EP2 or EP4 exacerbates symptoms of inflammation by increasing IL-23 expression and reducing IL-12/IL-27, which together cause T cells to differentiate to Th17 effectors in inflammatory bowel disease (colitis) and CIA [42,44]. PGE₂, together with IL-1 β and IL-23, facilitates Th17 cell differentiation and cytokine expression mainly through EP2 and cAMP signaling; whereas PGE₂ acts on the EP4 receptor to downregulate IFN- γ and IL-10 produced in Th17 cells [45]. In addition, PGE₂ signaling via EP2 or EP4 receptors can regulate UV-induced acute skin

inflammation by increasing blood flow in the skin microenvironment [46]. Furthermore, EP2 activation by oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (OxPAPC), an oxidized phospholipid species that accumulates in atherosclerotic lesions and other sites of chronic inflammation, can activate β 1 integrin and stimulate monocyte binding to endothelial cells, accompanied by differential modification of TNF- α and IL-10 expression in monocytes and macrophages, which may contribute to vascular inflammation and thus accelerate atherosclerotic lesion formation [47]. This monocytic vascular inflammation via OxPAPC-mediated EP2 activation is independent of COX-2. EP2 signaling via cAMP also suppresses phagocytosis and antimicrobial function by pulmonary macrophages, and thus dampens innate antibacterial responses that would be expected to exacerbate inflammation [48].

As a prominent sign of acute inflammation, pain is triggered by stimulation of nerve endings by bradykinin and other inflammatory molecules. PGE₂ was initially identified as a pain modulator owing to its role in the generation of exaggerated pain sensations in inflamed tissues. In a model of peripheral inflammation, EP2^{-/-} mice show normal early peripheral hyperalgesia, but lack the chronic hyperalgesic phase of spinal origin that might be mediated by dorsal horn nociceptive neurons [49]. It appears that PGE₂ mediates inflammatory pain sensitization induced by spinal PGE₂ injection or peripheral inflammation by inhibiting the glycine receptor α 3 subtype and thus blocking inhibitory glycinergic neurotransmission [50]. However, this EP2 receptor-dependent inhibition of glycinergic neurotransmission does not contribute to pain sensitization in the chronic constriction injury model of neuropathic pain and chemical-induced pain [51]. This finding indicates that additional mechanisms of central sensitization are involved in inflammatory and neuropathic pain. For example, a recent study demonstrated that EP1-mediated NO production along with EP2 is involved in the maintenance of neuropathic pain by retaining activated microglia among the central terminals of primary afferent fibers [52]. Thus, blockade of EP2 and EP1 receptor signaling together is expected to afford better pain relief than either one individually. A challenge for the future is to uncover the signaling mechanisms involved.

Tumorigenesis and angiogenesis

Tumorigenesis, the process by which normal cells are converted to cancer cells, involves progressive disruption of the balance between cell division and apoptosis, leading to a state of uncontrolled proliferation. As a solid tumor grows it typically requires an augmented blood supply, which involves angiogenesis. COX-2 and its derived prostanoids have attracted substantial attention because of their possible roles in tumor progression and angiogenesis [53–57]. For example, genetic ablation of COX-2 reduces colorectal polyp formation by 86% in a mouse model of human familial adenomatous polyposis (FAP) and this can be recapitulated by administration of COX inhibitors [58]. Epidemiological and experimental data suggest a positive correlation between regular use of COX-2 inhibitor drugs and reduced rates of certain cancers and cancer-related deaths [59]. Multiple downstream prostanoid signaling

pathways appear to be involved in tumorigenesis. Upregulation of COX-2 in tumor tissues is usually accompanied by high levels of PGE₂ [59], and administration of PGE₂ can enhance colon carcinogenesis in an azoxymethane-induced colon tumor model [60]. Although the underlying mechanisms are unclear, a growing body of evidence supports PGE₂ as the predominant COX-2-derived prostaglandin that facilitates tumor activities, including tumor cell proliferation, migration, angiogenesis, and immunosuppression [55,57]. Intriguingly, genetic ablation of EP2, but not EP1 or EP3, reduces the number and size of intestinal polyps in the mouse FAP model [61], mimicking the effect of COX-2 gene disruption or COX inhibitors in the same model [58]. In addition, PGE₂ signaling through EP2 can in turn boost expression of COX-2 and vascular endothelial growth factor (VEGF) in polyp tissues [61]. Deletion of EP2 receptors also attenuates tumor growth and prolongs survival in syngeneic mouse tumor models, possibly because EP2 plays an essential role in PGE₂-induced inhibition of dendritic cell differentiation and function and cancer-associated immunodeficiency [62]. EP2 ablation also suppresses skin tumor development by limiting angiogenesis and promoting apoptosis [63]. By contrast, EP2 overexpression facilitates skin tumor development [54]. The EP2 agonist butaprost promotes growth and invasion of prostate tumor cells, and this effect is blocked by the EP2 antagonist TG4-155 [64]. PGE₂ signaling via EP2 in mammary epithelial cells triggers hyperplasia of mammary glands and regulates VEGF induction in mouse mammary tumor cells [65,66]. In addition, EP2 signaling directly regulates tumor angiogenesis in endothelium by enhancing endothelial cell motility and cell survival, mediates epidermal hypertrophy and tumor aggression in response to UV-irradiation, and induces skin carcinogenesis [54,67,68].

The EP2 receptor appears to regulate tumor development via multiple mechanisms. For example, EP2 receptor activation can promote squamous cell carcinoma growth by activating iNOS/guanylate cyclase (GC) and ERK1/2 via transactivation of the epidermal growth receptor (EGFR) [69]. In response to PGE₂ stimulation, the EP2 receptor recruits β -arrestin 1 to phosphorylate tyrosine-protein kinase Src, which in turn activates EGFR, leading to activation of PI3K–Akt and Ras–ERK pathways, which together promote tumor cell activities (Figure 2) [18,19]. Alternatively, the $\beta\gamma$ subunits liberated on G α subunit activation by EP2 receptor can directly stimulate PI3K–Akt signaling, leading to phosphorylation and inactivation of glycogen synthase kinase-3 β (GSK-3 β), which eventually causes nuclear translocation of β -catenin to initiate growth-promoting gene expression and thereby growth of colorectal cancer [70]. Furthermore, β -arrestin 1 also phosphorylates JNK, which upregulates profilin-1 (Pfn-1) to increase F-actin expression and organization, thus promoting tumor cell migration and proliferation (Figure 2) [20]. The involvement of G-protein-dependent signaling by EP2 in tumor progression cannot be excluded. For example, aromatase-dependent estrogen synthesis is associated with hormone-dependent breast carcinogenesis, and EP2 can regulate cytochrome P450 aromatase via the cAMP–PKA–CREB pathway [71]. In addition, PGE₂ facilitates

tube formation via EP2–PKA signaling in rat luteal endothelial cells, indicating the involvement of the EP2 receptor in luteal angiogenesis and progression of ovarian cancer [72]. EP2 activation promotes growth of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) by increasing β -catenin-mediated c-Myc and VEGF expression, in which both Epac and PKA pathways are involved [73].

Chronic inflammation promoted by EP2 signaling might underlie its role in tumor progression given that inflammation has long been associated with tumorigenesis [56,74]. Inflammatory events create a local microenvironment that fosters genomic alterations and tumor initiation. Some tumor cells release cytokines and chemokines to attract monocytes and macrophages. The infiltrating macrophages in turn secrete growth factors that promote tumor progression, and recruit secondary leukocytes to enhance and maintain this mutual promotion between inflammation and tumor. As a major inflammatory mediator derived from COX-2, PGE₂ via EP2 can induce many proinflammatory mediators including cytokines, chemokines, iNOS, and COX-2 itself, which then facilitate cell proliferation, cell survival, angiogenesis, invasion, and metastasis [64,74]. In addition, EP2 activation also downregulates IFN- γ and TNF- α expression in immune cells such as natural killer T cells, neutrophils, and macrophages [47,75–78], impairing the ability of these immune cells to induce apoptotic death and restrain tumorigenesis. EP2 activation converts TGF- β , often considered an anti-inflammatory cytokine, from a tumor suppressor to a tumor promoter by altering oncogenic TGF- β signaling, thus promoting breast tumor growth, angiogenesis, and pulmonary metastasis [79]. Therefore, attenuation of PGE₂–EP2 signaling by small-molecule antagonists might mitigate chronic inflammation in tumor tissues and thereby provide an alternative strategy for cancer treatment via an anti-inflammatory mechanism [64].

Innate immunity in the brain and neurotoxicity

The EP2 receptor can promote chronic neuroinflammation in both *in vitro* and *in vivo* models. EP2 expression is substantially induced during systemic inflammation in models of innate immunity produced by lipopolysaccharide (LPS), IL-1 β , or turpentine [80]. EP2 upregulation by LPS contributes to cerebral oxidative damage and secondary neurotoxicity, usually accompanied by induction of NOS and COX activities [81–83]. As the resident macrophages in the brain, microglia are the major executors of innate immunity in the CNS and their activities are highly regulated by PGE₂–EP2 signaling [52,84–86]. It is now clear that microglia are major cellular culprits of EP2-mediated chronic inflammation and neuronal damage [82] because only glia, particularly microglia, express TLR4 [87], which is activated by LPS or proteins released from nearby injured neurons to initiate an innate immune response via CD14 [88]. PGE₂ signaling via either EP1 or EP2 leads to TLR4-dependent degeneration of intermediate progenitor cells in the hippocampal subgranular zone [89]. TLR4 activates the NF- κ B pathway and all three mitogen-activated protein kinase (MAPK) pathways: ERK, stress-activated protein kinase (SAPK)/JNK, and p38 MAPK, which

in turn induce transcription of a series of proinflammatory genes such as COX-2, inducible nitric oxide synthase (iNOS), and NADPH oxidase (NOX). Other inflammatory mediators regulated by PGE₂-EP2 signaling include IL-6 [75,77,78,86], IL-10 [47,78,86], IFN- γ [76,86], TNF- α [47,75,77,78,86], CCL-2 (MCP-1) [86,90], and intercellular adhesion molecule-1 (ICAM-1) [91], although the EP4 receptor might also potentially contribute. EP2 activation in microglia induces inflammatory mediators such as COX-2 and iNOS and a host of inflammatory cytokines, which can be enhanced by the EP2 allosteric potentiator TG3-95-1 (referred to as compound 1 in [35]) and substantially blunted by the antagonist TG4-155 [37,86]. The inflammation regulated by microglial EP2 appears to be mediated largely via cAMP/Epac signaling (Figure 2) [86]. Interestingly, EP2 can also upregulate iNOS in activated astrocytes by potentiating the response to inflammatory cytokines such as TNF- α and IFN- γ [92].

Sustained inhibition of COX-2 can exert beneficial effects in neurodegenerative disease models such as Alzheimer's disease (AD) [93], Parkinson's disease (PD) [94], and amyotrophic lateral sclerosis (ALS) [95], and is accompanied by a reduced number of activated microglia [96], suggesting that downstream prostanoid signaling pathways in microglia are involved in disease progression. Given that EP2 has an immunomodulatory function [82,86], activation of EP2 in microglia could promote chronic neurodegeneration and neuroinflammation by regulating innate immunity. PGE₂ via its EP2 receptor increases the expression of amyloid precursor protein (APP) in cultured rat microglia [97]. Furthermore, EP2 is involved in PGE₂-stimulated production of amyloid- β (A β) peptides in both cell and mouse APP models, produced by β - and γ -secretases from APP and most commonly known in association with AD [98]. Interestingly, EP2 receptor ablation enhances microglia-mediated phagocytosis of A β and totally eliminates A β -triggered paracrine neurotoxicity mediated by microglia [82,99]. EP2 receptor activation by PGE₂ and butaprost can reduce A β -induced phagocytosis in cultured rat microglia [100], identifying microglial EP2 as a possible therapeutic target for AD. Consistently, EP2 activation suppresses phagocytosis of alveolar macrophages, the essential components of lung innate immunity [101]. Genetic ablation of EP2 reduces oxidative stress in the APPSwe-PS1 Δ E9 mouse model of familial AD, accompanied by reduced levels of A β peptides and APP C-terminal fragments, possibly by regulating β -secretase [102].

Deletion of EP2 also enhances microglia-mediated clearance of α -synuclein aggregates in the mesocortex of patients with Lewy body disease [84]. Conversely, EP2-regulated microglial activation contributes to neurotoxicity induced by aggregated α -synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD, in which NOX appears to play a critical role [84]. As a major regulator of inflammatory oxidative injury in innate immunity, the EP2 receptor is induced in microglia and astrocytes and regulates inflammatory neurodegeneration in the G93A superoxide dismutase (SOD) model of familial ALS by inducing proinflammatory effectors such as COX-2, iNOS, and NOX [103]. More recently, pharmacological inhibition of the EP2 receptor by the antagonists TG4-155 and TG6-10-1

(Figure 3), after pilocarpine-induced status epilepticus in mice, produced a number of beneficial effects: it reduced delayed mortality, accelerated weight regain, blunted the inflammatory reaction and gliosis in hippocampus, maintained blood-brain barrier integrity, and reduced delayed neurodegeneration in hippocampus [37,104]. These results strengthen the value of the EP2 receptor as a potential therapeutic target in the treatment of inflammation-related neurological disorders. Future studies using EP2 antagonists in AD and PD models will help to determine whether inflammatory EP2 signaling is a common pathogenic mechanism in other chronic neurologic conditions.

Neuroprotection and other beneficial effects

PGE₂ is a major COX-2 product in the brain, and the EP2 receptor is widely expressed in both neurons and glia [16,103]. PGE₂-EP2 signaling is involved in a variety of physiological and pathological events in the nervous system, as discussed above, but EP2 activation can also be beneficial in excitotoxicity models. EP2 activation by the selective agonist butaprost or EP2 allosteric potentiators can protect neurons from *N*-methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity and oxygen-glucose deprivation (OGD)-induced anoxia in cultured neurons and hippocampal organotypic slices [16,35,105,106]. Activation of EP2 by PGE₂ can rescue postnatal motor neurons from chronic glutamate toxicity induced by glutamate transport inhibitors in organotypic spinal cord slices [107]. In addition, butaprost can protect cultured dopaminergic neurons from 6-hydroxydopamine-induced neurotoxicity [108]. An *in vivo* study showed that the EP2 agonist ONO-AE1-259 protects the ganglion cell layer and the inner plexiform layer in rat retina from NMDA-induced neurotoxicity, which suggests that EP2 activation might provide a therapy for retinal injuries involving glutamate excitotoxicity, such as diabetic retinopathy and glaucoma [109]. EP2 receptor-mediated neuroprotection is probably mediated by cAMP-PKA signaling (Figure 2), because the PKA-specific inhibitors H-89 and KT-5720, can abolish, whereas the adenylate cyclase activator forskolin can mimic, these beneficial effects [16,107,108].

Neuroprotection by EP2 activation has been well investigated in models of ischemic stroke. Genetic ablation of EP2 increases cerebral infarction in cerebral cortex, subcortical structures, and stroke volume in both the transient [16] and permanent [105] middle cerebral artery occlusion (MCAO) models of forebrain ischemia. Administration of misoprostol, an anti-ulcer agent and agonist for EP2, EP3, and EP4 receptors, reduces infarct volume in the transient MCAO model. The EP3 receptor should be excluded from involvement of misoprostol-mediated neuroprotection because EP3 deficiency does not change the infarct volume and EP3^{+/+} and EP3^{-/-} mice treated with misoprostol exhibit similar levels of infarct rescue [110]. Moreover, the EP2 agonist ONO-AE1-259 reduces infarct volume and neurologic dysfunction in the transient MCAO model [111]. The findings that EP2 agonists and allosteric potentiators can be neuroprotective, and that ischemic damage is increased in EP2^{-/-} mice, raise the possibility that pharmacological activation or potentiation of EP2 could be beneficial in ischemic stroke therapy.

In the periphery, EP2 activation can improve survival of gastrointestinal epithelial cells after radiation injury via transactivation of EGFR and enhancement of the PI3K–Akt signaling cascade, which suppresses translocation of the proapoptotic protein Bax to the mitochondrial membrane and thus abrogates a prominent apoptotic pathway in these cells [112]. Among all four subtypes of PGE₂ receptor, EP2 dominantly promotes the biomechanical strength properties of bone. EP2 activation by the agonist CP-533536 stimulates local bone formation and enhances fracture healing, suggesting that EP2 activation restricted to the injured area could be a therapeutic alternative for the treatment of fractures and bone defects [113,114]. EP2 also can protect cystic epithelial cells from apoptosis and promote cystogenesis through cAMP signaling in autosomal-dominant polycystic kidney disease [115]. Interestingly, EP2 and EP4 receptors synergistically exert beneficial actions under some pathological circumstances, possibly because of their similarity in signal transduction profiles (i.e., they are both Gs-coupled and are activated by PGE₂). For example, both EP2 and EP4 receptors play important roles in slowing the progression of chronic kidney failure, although only EP4 provides protection in an acute kidney failure model [116]. In addition, both EP2 and EP4 receptors can improve survival of cardiac transplants in mice by inhibiting the alloimmune response, whereas EP4 activation appears to be more effective than EP2 in suppressing acute allograft rejection [117]. Similarly, PGE₂ dampens thromboxane-induced platelet aggregation via both EP2 and EP4 receptors [118], which demonstrates their value as targets for anti-platelet therapy. Both EP2 and EP4 receptors mediate the anabolic functions of PGE₂ in bone formation, but via p38- and ERK-dependent MAPK signaling pathways, respectively [119]. Finally, PGE₂ signaling via EP2 and EP4 receptors promotes survival of human endometriotic cells by transactivating cell survival pathways including ERK, Akt, NF- κ B, and β -catenin, suggesting that inhibition of EP2 and EP4 might represent a non-estrogen-targeted therapy for endometriosis [120].

Concluding remarks

The EP2 receptor exerts both beneficial and deleterious effects, depending on the type of injury and the responding components (Figure 2). This dichotomy of EP2 functions is particularly conspicuous in the CNS. For example, intracerebroventricular administration of the EP2 agonist butaprost immediately after termination of pilocarpine-induced status epilepticus affords moderate neuroprotection in a rat [2]. This finding is seemingly incongruent with the broad benefits from delayed systemic administration of EP2 antagonists in a similar model [37,104], but might reflect the complexity of inflammatory signaling in the brain and indicate a dual consequence of EP2 activation: early neuroprotection followed by later neurotoxicity involving chronic inflammation. It appears that neuronal EP2 activation promotes acute neuroprotection, neuronal survival, and neuronal plasticity, clearly through cAMP–PKA signaling. Conversely, glial – especially microglial – EP2 activation often leads to secondary neurotoxicity and neuronal injury via upregulation of inflammatory mediators such as COX-2, iNOS, and NOX in chronic brain

inflammation [121]. More studies are needed to clarify whether the cAMP–Epac or β -arrestin signaling pathway is dominant in microglial EP2-mediated deleterious actions, although it has already been demonstrated that cAMP–Epac promotes oxidative stress [12], neuronal apoptosis [13], inflammatory hyperalgesia [14,15], and microglia-produced proinflammatory mediators [86]. Epac1 is upregulated in AD and after PGE₂-mediated inflammation [122,123]. Interestingly, the EP2 receptor mediates neuroprotection in rat pure neuronal cultures treated with NMDA through a PKA-dependent pathway [16], whereas EP2 activation exacerbates NMDA receptor-mediated neurotoxicity in rat cortical cultures with glia present through a cAMP- but not PKA-dependent pathway, suggesting the involvement of Epac in EP2-regulated neurotoxicity [124]. In addition, the EP2-regulated protein dedicator of cytokinesis 2 (DOCK2), another GEF family member, contributes to A β plaque burden via regulation of microglial innate immune function [125]. The net effect of EP2 receptor activation might be determined by a yin and yang balance of the receptor in neurons and glia, and possibly by the cytoplasmic cAMP level, which is spatiotemporally regulated by the receptor to favor either the PKA or Epac pathway (Figure 4). Future studies using neuron- or monocyte-specific conditional EP2^{-/-} mice might definitively distinguish the roles of the EP2 receptor in neurons or microglia.

The past decade has witnessed growing recognition of the adverse effects of selective COX-2 inhibitors [3], suggesting that the downstream prostanoid synthases or receptors should be explored as next-generation therapeutic targets. PGE₂–EP2 signaling plays multiple essential roles in inflammation, tumorigenesis, cytoprotection, and

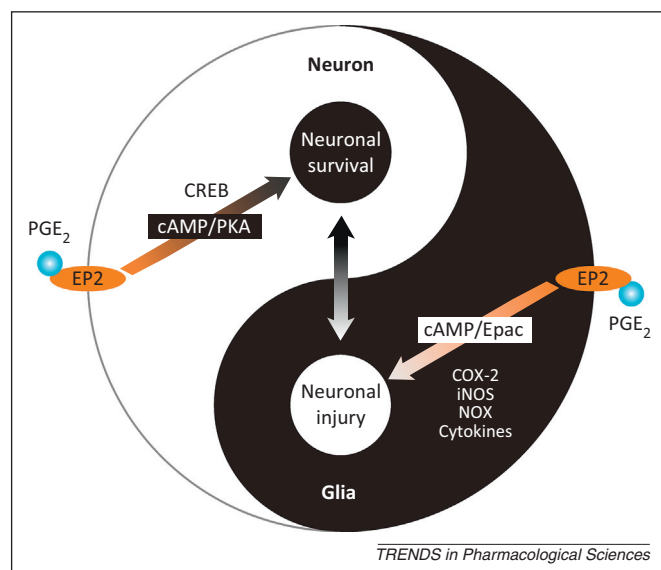


Figure 4. The yin and yang of prostaglandin receptor EP2 in the brain. It is hypothesized that EP2 receptors in neurons promote cAMP–protein kinase A (PKA)-dependent neuroprotection. By contrast, glial EP2 activation leads to neurotoxicity and neurodegeneration, in part via cAMP–exchange protein activated by cAMP (Epac) signaling-mediated upregulation of proinflammatory mediators including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), NADPH oxidase (NOX), and many cytokines. The net effect of EP2 activation is determined by the injury type and is spatiotemporally regulated by responding cells and molecules.

neurodegeneration, which renders EP2 a therapeutic target candidate for a broad range of peripheral and CNS diseases [126]. Emerging allosteric potentiators and antagonists have already proved valuable as tools to explore the roles of the EP2 receptor under normal and disease conditions [35–37,64,86,104]; however, the development of EP2-targeted drugs for therapeutic use will require careful attention to the temporal and probably spatial extent of drug actions to avoid widespread effects. For example, transient and early delivery of EP2 allosteric potentiators might provide neuroprotection in acute neuronal injuries from excitotoxic conditions such as ischemic and hemorrhagic strokes [127], whereas delayed inhibition of EP2 via selective antagonists would be expected to reduce brain inflammation and injury in chronic inflammation-associated neurological disorders such as epilepsy, AD, PD, and ALS. Targeted blockade of EP2 might also be useful in combating peripheral inflammation and pain or slowing tumor progression.

The multiplicity of signal transduction mechanisms engaged by EP2, involving both G-protein-dependent and -independent pathways, endow this receptor with diverse physiologic and pathological functions. Agonists biased towards G-protein-independent signaling pathways have recently been reported for β 1- and β 2-adrenoceptors [128,129]. These small molecules preferentially engage β -arrestin-dependent effects rather than the canonical G-protein-dependent signaling by changing the GRK-dependent phosphorylation pattern of the receptor cytoplasmic regions to regulate the conformational states of the receptor [129,130]. Small molecules that are biased toward EP2-coupled cAMP–PKA, cAMP–Epac, or β -arrestin signaling pathway could form the next generation of EP2 modulators with pharmacological effects targeting one signaling pathway. For example, an EP2 agonist biased towards cAMP–PKA might provide neuroprotection and other beneficial effects without triggering neurotoxicity and other deleterious effects that are mediated by cAMP–Epac or β -arrestin, and *vice versa*.

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