British Journal of Pharmacology (2006) 149, 611–623

0007–1188/06 $30.00
www.brjpharmacol.org

REVIEW

Prostaglandin E_2 receptor distribution and function in the gastrointestinal tract

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Prostaglandin E_2 (PGE_2) is one of the most important biologically active prostanoids found throughout the gastrointestinal tract. Despite the fact that PGE_2 regulates many physiological functions of the gut including mucosal protection, gastrointestinal secretion and motility, it is implicated in the pathophysiology of inflammatory bowel diseases (IBD) and colorectal neoplasia. The varied biological functions exerted by PGE_2 are through the pharmacologically distinct, G-protein coupled plasma membrane receptors termed EP receptors. Disruptions of various prostanoid receptor genes have helped in unravelling the physiological functions of these receptors. To date, all four subtypes of EP receptors have been individually knocked out in mice and various phenotypes have been reported for each subtype. Similarly, *in vitro* and *in vivo* studies using EP receptor agonists and antagonists have helped in uncoupling the diverse functions of PGE_2 signalling involving distinct EP receptors in the gut. In this review, we will summarize and conceptualize the salient features of EP receptor subtypes, their regional functions in the gut and how expressions of EP receptors are altered during disease states.
British Journal of Pharmacology (2006) **149,** 611–623. doi:10.1038/sj.bjp.0706923; published online 3 October 2006

Keywords: prostaglandin E_2; gastrointestinal tract; EP receptors; distribution; functions; inflammation; differential expression

Abbreviations: AA, arachidonic acid; Akt, protein kinase B; Ca^{2+}, calcium; cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; CREB, cAMP responsive element binding protein; DP, D prostanoid; EP, E prostanoid; EP_1, EP_2, EP_3 and EP_4, E prostanoid receptor subtypes 1, 2, 3 and 4; ERK, extracellular signal-regulated protein kinase; FP, F prostanoid; G ($_{p/q,\ \alpha s}$ and $_{\alpha i}$), G protein subunits; GI, gastrointestinal; GSK-3, glycogen synthase kinase-3; IBD, inflammatory bowel disease; IP, I prostanoid; K_D, dissociation constant; PG, prostaglandin; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostaglandin I_2; PI3K, phosphoinositide-3-kinase; PKA, protein kinase A; PLA_2, phospholipase A_2; TP, T prostanoid; TXA_2, thromboxane A_2

Introduction

The interests in understanding prostaglandin (PG)-mediated functions of the gastrointestinal (GI) tract have grown steadily since the discovery of PGs in 1957. In recent years, technological advances in the field of molecular biology have helped researchers make substantial progress towards unraveling the complex functions of PGs in the gut. It is only now that the functional roles of the various PGs in the GI tract are established. Apparently, each of the bioactive PGs exhibits versatility and diversity in functions in various segments of the gut. However, the mechanisms by which they exert their biological functions are not clearly known. To discern the variable roles of PGs, it is imperative to understand thoroughly the mode of action of these mediators, including the pharmacology, expression and distribution of their respective receptors in the gut. Nevertheless, insights into PG receptor biology in the GI tract will be of fundamental importance in developing strategies for pharmacological intervention in various GI ailments.

PG biosynthesis

PGs are 20-carbon fatty acid derivatives that are found ubiquitously in all tissues and organs and mediate variety of physiological and pathological functions. They are synthesized in the cell from different essential fatty acid precursors, including arachidonic acid (AA). PGs derived from AA are termed series-2 PGs, which consist of prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin I_2 (PGI_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TXA_2) (Calder, 2001). All of these PGs share a common initial biosynthetic pathway that begins with the hydrolysis of cell-membrane phospholipids, mediated by the enzyme phospholipase A_2 (PLA_2), which is found mostly in cellular membranes including the plasma membrane (Murakami *et al*., 1997). Diverse physiological and pathological stimuli can result in the activation of PLA_2 to liberate AA from

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Received 26 May 2006; revised 11 July 2006; accepted 29 August 2006; published online 3 October 2006

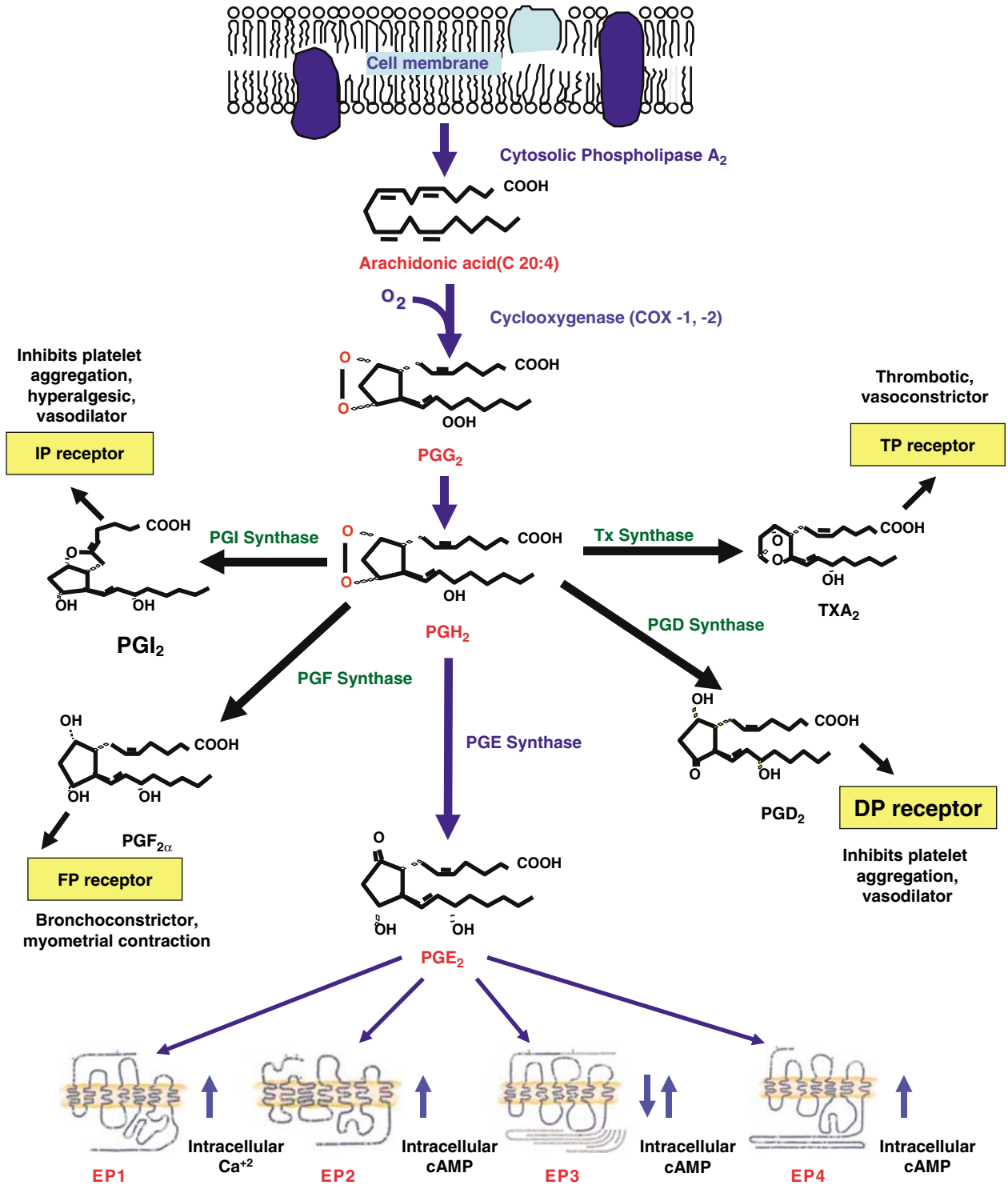

Figure 1 Biosynthesis of prostaglandins. Note that arachidonic acid liberated by the action of membrane bound phospholipase A2 and cyclooxygenase are the key rate-limiting steps for prostaglandin biosynthesis. Shown are the prostanoids, their respective receptors and their related pathophysiological functions.

membrane phospholipids into the cytoplasm (Murakami and Kudo, 2002). Upon release, AA is converted into unstable endoperoxide intermediates, PGG_2 and PGH_2 (Hamberg *et al.*, 1974) by the action of cyclooxygenase (COX) in a rate limiting enzymatic reaction. Three isoforms of COX have been identified to date. The constitutively expressed COX-1 and the inducible COX-2 are the most important isoforms (Smith *et al.*, 1994, 1996). COX-3 is a splice variant of COX-1 and is mostly expressed in the brain and the heart (Chandrasekharan *et al.*, 2002). The oxygenated intermediate PGH_2 is in turn metabolized by cell-specific synthases and isomerases into PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 (Vane

et al., 1998; Figure 1). Prostanoids are released outside the cell immediately after their synthesis where they exert their biological functions through their interaction with the cell surface prostanoid receptors in an autocrine or paracrine fashion (Narumiya, 1994). Alternatively, the action of prostanoids are terminated when they are transported across the cell membrane into the cytoplasmic compartment with the help of PG transporters (Kanai *et al*., 1995) where they are acted upon by oxidizing and reducing enzymes, 15 hydroxy PG dehydrogenases and delta 13–15-ketoprostaglandin reductase, respectively (Tai *et al*., 2002).

Prostanoid receptors

Prostanoid receptors belong to the family of Rhodopsin-type receptors characterized by their seven transmembrane domains that are intracellularly coupled to different subunits of G proteins (Breyer *et al*., 2001). Five major types of prostanoid receptors, namely; D prostanoid (DP), E prostanoid (EP), F prostanoid (FP), I prostanoid (IP) and T prostanoid (TP), which include six subtypes namely; DP_1, DP_2, EP_1, EP_2, EP_3 and EP_4, have been described for the prostanoids PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2. The structures, properties and functions of most of these receptors have previously been reviewed (Narumiya *et al*., 1999). Investigation of the functional role of these receptors in gut physiology as well as pathophysiology is presently an important pursuit. For example, the role of DP, IP, EP and TP receptors in the propulsive peristaltic movement of the guinea-pig small intestine has been studied and the receptors responsible for differential peristaltic motor effect identified (Shahbazian *et al*., 2002). Similarly, the role of DP, FP, IP, TP and the EP receptor subtypes in the development of carcinogen-induced aberrant crypt foci in colon has been studied extensively (Watanabe *et al*., 1999; Mutoh *et al*., 2002).

It is clear that the different prostanoids synthesized and their interactions with respective cellular receptors are responsible for the varied biological actions of the host. As the cellular components of the GI tract are known to possess the machinery for the biosynthesis of PG, it is not surprising that PGs are produced throughout the gut. Among the different PGs that are produced, PGE_2 is considered to be the most important for normal physiological functions of the GI tract including gastric mucosal protection and motility and is also implicated in the pathology of various disease conditions such as inflammatory bowel disease (IBD) (Ahrenstedt *et al*., 1994), entero-invasive bacterial diseases (Reseta and Barrett, 2002) and colorectal cancers (Eberhart *et al*., 1994). Diversity in the cellular functions of PGE_2 is attributed to its binding to four different subtypes of EP receptors that in turn propagates signals through alteration in the intracellular calcium (Ca^{2+}) or cyclic adenosine monophosphate (cAMP) levels. This results in the activation of an array of kinases modulating diverse cellular functions (Figure 2). Although, signalling through different EP receptor determines the various effects of PGE_2, regional and differential expression of EP receptors in the GI tract is critical for determining its biological functions. Based on this premise, we will summarize in this review the salient features of EP receptor subtypes, their regional functions in the gut and their differential expression during normal and disease states.

EP receptors

The cellular membrane receptors for PGE_2 are termed the EP receptors that consist of four different subtypes namely EP_1, EP_2, EP_3 and EP_4. They are encoded by different genes and are well conserved throughout the mammalian system from mouse to human. Phylogenetic analysis of the amino-acid sequence of all the EP receptors indicates that they were all sequentially (EP_2, EP_4, EP_3 and EP_1) derived from a primitive PGE receptor by gene duplication (Toh *et al*., 1995). Isoforms of EP receptor subtypes generated by alternative splicing of their mRNA have been reported for EP_3 and EP_1 (Irie *et al*., 1993; Namba *et al*., 1993; An *et al*., 1994; Breyer *et al*., 1994; Regan *et al*., 1994b; Schmid *et al*., 1995; Okuda *et al*., 1996; Pierce and Regan, 1998; Oldfield *et al*., 2001). All EP receptor subtypes are expressed on the plasma membrane; additionally EP_3 and EP_4 also exhibit nuclear membrane localization (Bhattacharya *et al*., 1998, 1999).

EP_1 receptor

EP_1 is a 42-kDa protein that has been cloned and expressed from humans and rodents (Funk *et al*., 1993; Watabe *et al*., 1993; Okuda *et al*., 1996). Two splice variants of EP_1 receptor have been reported (Okuda *et al*., 1996). Among the four subtypes of EP receptors, EP_1 has the least affinity for PGE_2 (dissociation constant (K_D) of 16–25 nM, (Table 2)). It binds PGs in the rank order PGE_2 > sulprostone, iloprost > PGE_1 > misoprostol, M&B-28767 > $PGF_{2\alpha}$ > PGD_2 (Abramovitz *et al*., 2000; Table 3). A few selective agonists for EP_1 like ONO-DI-004, 17-phenyl trinor PGE_2 are available, 17-phenyl trinor PGE_2 being the most well known of them. Other agonists including sulprostone, carbacyclin and enprostil exhibit the highest affinity for EP_1, but are also very potent EP_3 agonists. ONO-AE-829, ONO-8711, ONO-8713, SC-19220 and AH6809 are used as antagonists for the EP_1 receptor but AH6809 is a weak antagonist (Woodward *et al*., 1995, 2005).

EP_2 receptor

Before the discovery of the EP_2 receptor in 1994, the cloned EP_4 receptor was referred to as EP_2 (Bastien *et al*., 1994; Regan *et al*., 1994a). The EP_2 receptor is a 53 kDa protein that has been cloned and expressed from humans, bovine, rabbits, rodents and several other species (An *et al*., 1993; Katsuyama *et al*., 1995; Guan *et al*., 1996; Nemoto *et al*., 1997; Arosh *et al*., 2003). The affinity of PGE_2 for EP_2 receptor differs greatly across species. The rat EP_2 receptor has a significantly higher affinity for PGE_2 than human or mouse. The mouse EP_2 receptor shows the least affinity (Table 2). The relative affinity of binding of various EP ligands for the mouse EP_2 receptor was PGE_1, PGE_2 > 16, 16-dimethyl-PGE_2 > 11-deoxy-PGE_1 > butaprost > AH13205, misoprostol > AH-6809. Similarly, the human EP_2 receptor binds PGE_2 and PGE analogues with a rank order of PGE_2 > PGE_1 > 16, 16-dimethyl-

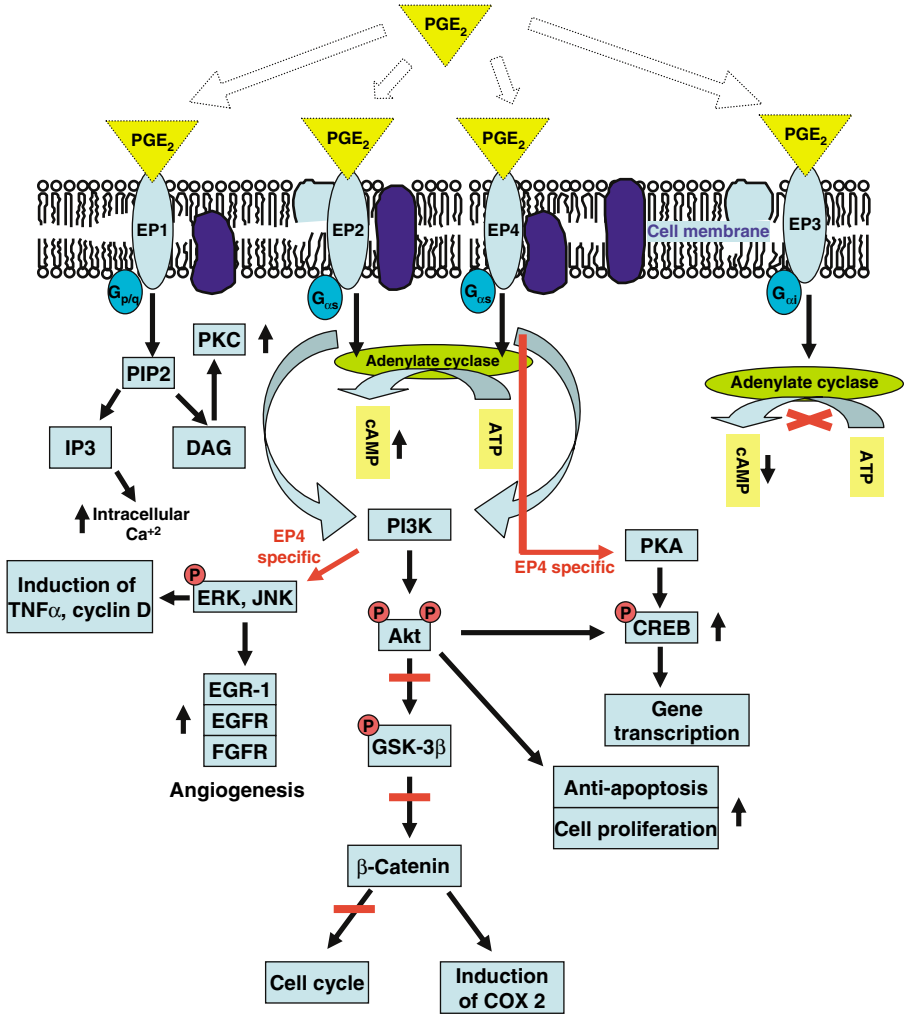

Figure 2 PGE_2-EP receptor signaling pathways. Four major types of EP receptors are involved in the signaling pathway mediated via different G proteins ($_{p/q,}$ $_{\alpha s}$ and $_{\alpha i}$) using different second messenger. PGE_2 induces intracellular Ca^{2+} or cAMP when it couples and signals through EP_1 or $EP_{2/4}$ receptors respectively. However, it reduces intracellular cAMP when it signals through EP_3 receptors. Several different kinases are involved in PGE_2 induced signaling pathways. PGE_2-EP_4 receptor coupling induces TNFα, cyclin D and angiogenesis using a specific pathway that is mediated via different MAP kinases. PGE_2 also promotes cancer cell proliferation and inhibits apoptosis. It also induces COX-2 gene transcription and induces cell proliferation. On the other hand, it could also inhibit cell cycle through phosphorylation of Akt, GSK-3β and β-catenin. Abbreviations: AC, adenylate cyclase; DAG, diacylglycerol; IP3, inositol triphosphate; PIP2, phosphatidylinositol diphosphate; PKA, protein kinase A; PKC, protein kinase C; PI3K, phosphoinositide-3-kinase; Akt, protein kinase B; ERK, extracellular signal-regulated protein kinase; JNK, c-Jun N-terminal kinase; EGR-1, early growth response 1; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GSK-3, glycogen synthase kinase-3; Ⓟ, phosphorylation; ↑, up regulated; ↓, down regulated; X, inhibition.

PGE_2 > 11-deoxy-PGE_1 > butaprost, 1-OH-PGE_1, M&B-28767 > sulprostone (Table 3). Butaprost and ONO-AE1-259-01 are highly selective EP_2 receptor agonists, whereas AH-6809 is a well-known EP2 antagonist.

EP_3 receptor

The EP_3 receptor of humans and rodents has been successfully cloned and expressed (Sugimoto *et al.*, 1992; Yang *et al.*, 1994). The EP_3 is unique among the prostanoid family of receptors in the sense that multiple alternatively spliced variants of EP_3 exist that can activate contrasting second messenger signalling (Pierce and Regan, 1998). To date, eight different isoforms for human EP_3 have been reported. In general, the EP_3 receptor has relatively higher affinity for PGE_2 reflected by a very low K_D value of 0.33–2.9 (Table 2). The rank order of affinity of PGE_2 to the mouse EP_3 receptor is sulprostone, M&B-28767, PGE_2, PGE_1, 11-deoxy-PGE_1, 16,16-dimethyl-PGE_2, misoprostol > 1-OH-PGE_1. Different PGE analogs, SC-46275, ONO-AE-248, sulprostone, GR 63799X and 11-deoxy-PGE_1 are used as agonists for EP_3 with SC-46275 and ONO-AE-248 being highly selective. L826266 is an EP_3 antagonist.

EP_4 receptor

The EP_4 receptor cDNA encodes a 487–513 amino-acid polypeptide that has been cloned and expressed *in vitro* from human, rodents, rabbit, bovine and several other species (An *et al.*, 1993; Honda *et al.*, 1993; Breyer *et al.*,

Table 1 K_i values of PGE_2 for heterologously expressed human and mouse EP receptors

Species	*Heterologous system*	*EP_1*	*EP_2*	*EP_3*	*EP_4*	*References*
Humans	Human embryonic kidney cells	9.1	4.9	0.33	0.79	Abramovitz *et al*. (2000)
Mouse	Chinese hamster ovary cells	20	12	0.85	1.9	Kiriyama *et al*. (1997)

Abbreviations: EP, E prostanoid; PGE_2, prostaglandin.
Inhibitory constant (K_i) values are expressed in nM.

Table 2 Dissociation constants (K_d) of PGE_2 for EP receptor subtypes in various species

Receptor	*K_d (nM) (human)*	*K_d (nM) (rat)*	*K_d(nM) (mouse)*
EP_1	25 (Abramovitz *et al*., 2000) 16 (Sharif and Davis 2002)	24 (Boie *et al*., 1997)	21 (Watabe *et al*., 1993)
EP_2	13 (Abramovitz *et al*., 2000) 12.9 (Stillman *et al*., 1998)	5 (Boie *et al*., 1997)	116 (Nishigaki *et al*., 1996)
EP_3	0.33 (Abramovitz *et al*., 2000)	1 (Boie *et al*., 1997)	2.9 (Sugimoto *et al*., 1992)
EP_4	0.59 (Abramovitz *et al*., 2000) 0.72 (Davis and Sharif, 2000) 1.12 (Marshall *et al*., 1997)	1 (Boie *et al*., 1997)	1.27 (Nishigaki *et al*., 1996)

Abbreviations: EP, E prostanoid; PGE_2, prostaglandin E_2.

1996; Boie *et al*., 1997). They have very high affinity for PGE_2 with a K_D value of 0.59–1.27 (Table 2). The rank order of affinity of PGE ligands for the mouse EP_4 receptor was PGE_2, $PGE_1 > 11$-deoxy-PGE_1, 16, 16-dimethyl-PGE_2, misoprostol > 1-OH-PGE_1 and M&B-28767 (Table 3). At present, there are few selective agonists or antagonists available for EP_4. ONO-AE1-329 is a selective EP_4 agonist whereas L161982 and ONO-AE3-208 are selective antagonists.

Diversity in EP receptor signalling and functions

Diverse factors govern the outcome of EP receptor signalling. Basically, structural, pharmacological and functional differences that exist between the subtypes of EP receptors determine the biological effects of PGE_2. Structurally, differences observed in the length and composition of amino acids of the N and the C-termini, the second and the third extracellular as well as the intracellular domains are associated with variations in receptor signalling. In fact, differences in the length of C-termini of EP_3 isoforms are attributed for their differential activation of varied second messenger pathways (Pierce and Regan, 1998). Likewise, the extracellular sequence of the EP_2 receptor is a critical determinant of its structure and function (Stillman *et al*., 1999). Similarly, a cluster of hydrophobic aromatic amino acids in the second intracellular loop of EP_2 but not EP_3 isoform β is absolutely essential for activation of $G_{\alpha s}$ subunit (Sugimoto *et al*., 2003, 2004). Pharmacologically, EP receptors exhibit differences in binding to various ligands (Table 1) and surprisingly, the affinity of these receptors to its principal ligand, PGE_2, greatly varies between receptor subtypes (Tables 2 and 3). In fact, affinity of PGE_2 to its receptor may depend on the state of coupling of G protein subunits. A G-protein coupled receptor displays more affinity for PGE_2 than a free uncoupled receptor. Apart from differences in the ligand affinity to EP receptors, the phenomena of receptor desensitization and internalization may regulate preferential signalling through certain EP receptor subtypes. Agonist-induced desensitization is a common phenomenon in G-protein coupled receptor that is characterized by the loss of receptor signalling. The EP receptors have been shown to undergo PGE_2-induced desensitization. Interestingly, a differential desensitization of EP receptor subtypes has been observed wherein, the EP_4 receptor is highly desensitized in contrast to the EP_2 receptor which is not (Nishigaki *et al*., 1996). Similarly, differences in ligand-induced internalization of EP receptors have been reported. EP_4 and EP_3 isoform-I are readily internalized when activated by PGE_2 whereas EP_2 as well as EP_3 isoform-III and

Table 3 Competition for radioligand binding to HEK 293(EBNA) cell membranes expressing recombinant prostanoid receptors

Ligands	*EP_1*	*EP_2*	*EP_3*	*EP_4*
PGE_2	9.1	4.9	0.33	0.79
Butaprost	27 721	91	1643	19 104
AH-6809	1217	1150	1597	100 000
Sulprostone	107	100 000	0.35	7740
M&B-28767	419	988	0.14	10
Iloprost	11	1870	56	284
Misoprostol	11 935	34	7.9	23
PGD_2	5820	2973	421	1483
$PGF_{2\alpha}$	547	964	38	288
SC-51322	13.8	>100 000	698	14 032
Enprostil	82	>10 000	12	>10 000
Carbacyclin	23	942	14	352
Cicaprost	>1340	>1340	255	44

Abbreviations: EP, E prostanoid; PGD_2, prostaglandin D_2; PGE_2, prostaglandin; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.
Inhibitory constant (K_i) values are expressed in nM.
Adapted from Abramovitz *et al*. (2000).

IV are not. Interestingly, the C-terminus of EP_4 has been implicated in its ligand-induced sequestration (Desai *et al*., 2000; Bilson *et al*., 2004). Functionally, differences among EP receptors directly correlate with the type of signal that it transduces. For example, receptor activation leading to intracellular Ca^{2+} mobilization is associated with the contraction of smooth muscle cells, whereas increase in cytoplasmic cAMP levels is associated with its relaxation. Evidently, signalling through EP_1 receptors increases intracellular Ca^{2+} levels whereas, EP_2 and EP_4 have been shown to increase cytoplasmic cAMP. Signalling via EP_3 receptors is unique wherein cAMP levels are decreased (Narumiya *et al*., 1999). Clearly, diverse factors govern the fate of signalling through EP receptors and all these may play a critical role in determining the differential biological effect of PGE_2 in the GI tract.

Downstream signalling pathways affected by EP receptors

Coupling of PGE_2 or the specific receptor agonists to EP receptors results in their activation and induces signalling cascade inside the cell (Figure 2). Interestingly, signalling through different subtypes of EP receptors seems to alternate and sometimes overlap; yet they are unique in terms of their signalling outcomes. EP_1 receptors mediate signalling events by activation of phospholipase C and elevation of cytoplasmic signalling intermediates including inositol triphosphate, diacylglycerol and Ca^{2+}. Coupling of PGE_2 to EP_1 activates protein kinase Cα and c-Src. In addition to directly activating downstream kinases, signalling through EP_1 can also transactivate HER's-2/Neu tyrosine kinase receptor, which is mediated by c-Src resulting in upregulation of vegetative endothelial growth factor-C (Su *et al*., 2004). Recently, it was reported (Han and Wu, 2005) that c-Src mediated the transactivation of epidermal growth factor receptor by the EP_1 receptors through activation of protein kinase B (Akt) that promotes cell proliferation and invasion. These data corroborate with those obtained from studies in EP_1 receptor knockout mice, thus implicating a role of EP_1 receptors in colon carcinogenesis (Watanabe *et al*., 1999).

The EP_2 and EP_4 receptors are linked to the stimulation of cAMP/protein kinase A (PKA) signalling through the sequential activation of $G_{\alpha s}$ and adenylate cyclase. Contradictory reports associate this signalling pathway to growth and proliferation. Increased cAMP production, upon activation of EP_2 and EP_4 has been shown to have an antiproliferative effect in human gastric carcinoma cell lines (Okuyama *et al*., 2002). In contrast, activation of PKA is increasingly being linked to proliferation of various epithelial cell types. Phosphorylation of PKA is coupled to the regulation of glycogen synthase kinase-3 (GSK-3) and Akt (Filippa *et al*. 1999; Li *et al*., 2000). PKA phosphorylates and activates Akt kinase, which indirectly inhibits GSK-3. Inhibition of GSK-3 decreases the inhibitory phosphorylation of cytosolic β-catenin that promotes the translocation of β-catenin to the nucleus resulting in cellular proliferation (Cadigan and Nusse, 1997). A recent study has directly linked EP_2 receptor activation to cellular proliferation. Upon activation the $G_{\alpha s}$ subunit of EP_2 receptors can directly associate with the regulator of G protein signalling domain of Axin, which inactivates and releases GSK-3β from the Axin complex causing β-catenin activation and nuclear translocation (Castellone *et al*., 2005). Until recently, it was believed that the phosphoinositide-3-kinase (PI3K) signaling pathway was activated only by the EP_4 receptor. However, recent studies have shown that PGE_2 signalling through EP_2 receptors can activate both PI3K and Akt by using the free $\beta\gamma$ subunit of G-protein (Castellone *et al*., 2005).

Although EP_2 and EP_4 receptors are capable of stimulating both PKA and PI3K signalling pathway, a receptor-specific signalling outcome has always been observed. For example, a study showed that PGE_2 stimulation of EP_4, but not EP_2 receptor that are stably expressed in HEK cells lead to the phosphorylation of the extracellular signal-regulated kinases (ERKs) by a PI3K-dependent mechanism and induced the expression of early growth response factor-1 (Fujino *et al*., 2003). The differences in the signalling potential of both EP_2 and EP_4 receptors are corroborated by the fact that both receptors mediate the phosphorylation of cAMP responsive element binding protein (CREB) by different signalling pathways (Fujino *et al*., 2005). Signalling through EP_2 receptors can decrease the inhibitory tyrosine phosphorylation of PTEN, a phosphatase that can act as a PI3K pathway inhibitor (White *et al*., 2005). A novel EP_2 receptor-signalling pathway, which can transactivate the EGF receptor leading to increased migration and invasion of colon cancer cells, has also been reported (Pai *et al*., 2002; Buchanan *et al*., 2003).

The EP_3 receptors are unique in their ability to couple to multiple G proteins. They couple and activate the G_i subunits, which results in the inhibition of adenylyl cyclase. Apart from activation of G_i subunits, signalling through EP_3 receptors can also activate G_s resulting in cAMP production. Evidence also indicates that the EP_3 receptor can activate the small G protein Rho and its target p160 Rho-A binding kinase ROK α (Katoh *et al*., 1998; Tamma *et al*., 2003). EP_3 receptors have also been shown to activate the Ras signalling pathway leading to cancer (Yano *et al*., 2002).

Distribution of EP receptors in the gut

Although PGE_2 is produced by a variety of cells in the GI mucosa and is found throughout the gut, its cellular targets in the mucosa and the resulting physiological changes in the GI tract are predominantly determined by the presence and the distribution of EP receptors. Pharmacological as well as cellular localization studies have been aimed towards identifying the type and pattern of EP receptor distribution and their density in major cell types in the GI mucosa. However, very little is known of the regional distribution or the temporal changes in the pattern of EP receptor expression and function under normal and disease states in the gut.

EP_1 receptor distribution

EP_1 receptor expression has been reported in the GI tract of various species. In rat, EP_1 mRNA expression is detected at

the gastric, small intestinal and colonic muscle layers (Ding *et al*., 1997). A non-radioactive *in situ* hybridization technique used to study the pattern of localization of EP receptors in the rat GI tract revealed the presence of EP_1 receptors in gastric chief cells that secrete pepsinogen, parietal cells that secrete hydrochloric acid and in mucus secreting gastric epithelial cells. In the small intestine, EP_1 receptor expression was noticed only in goblet cells whereas in the large intestine, EP_1 receptor expression was seen in goblet cells and in other epithelial cell types. Expression of EP_1 receptors has been reported in enteric glial cells (Northey *et al*., 2000). In mice, EP_1 mRNA expression was noticed in the muscularis mucosa (Morimoto *et al*., 1997). In rabbits, EP_1 receptors are highly expressed on the intestinal brush border membranes of intestinal villi. Goblet cells of the jejunum and ileum also show considerable expression. In contrast, goblet cells of the duodenum show a lack of expression of EP_1 receptors. Interestingly, EP_1 receptors are also highly expressed in the neurons of the myenteric and submucosal ganglia throughout the rabbit intestinal tract (Grasa *et al*., 2006).

EP_2 receptor distribution

EP_2 receptors are normally expressed in rat gastric mucous cells, in the goblet cells of the small intestine and also in various epithelial cells of the large intestine (Northey *et al*., 2000). In mice, an abundant expression of EP_2 receptors in the stomach and ileum has been detected by Northern blot analysis (Katsuyama *et al*., 1995). A recent report suggested a relatively uniform expression of EP_2 receptors throughout the mouse GI tract except for the ileum and caecum, where it is weakly expressed. Interestingly, expression of EP_2 receptors depends on the state of differentiation of the epithelial cells. Undifferentiated crypt epithelial cells predominantly express EP_2 receptors on their nuclear membranes whereas the highly differentiated epithelial cells at the apex of the villi express these receptors on their plasma membrane (Houchen *et al*., 2003). In rabbits, EP_2 receptors are localized on the intestinal brush border membrane of the villi and are also strongly expressed in goblet cells throughout the small intestine. EP_2 receptors are weakly expressed in neurons but are completely absent in the smooth muscle cells of the small intestine (Grasa *et al*., 2006). In humans, EP_2 receptor expression is restricted to the luminal surface of the gastric epithelium (Takafuji *et al*., 2002) and at the apex of the colonic mucosa (Takafuji *et al*., 2000). In guinea-pigs, the presence of EP_2 receptors in the neurons of the enteric nerve plexus in the ileum was detected through pharmacological studies (Lawrence *et al*., 1992).

EP_3 receptor distribution

EP_3 receptor gene expression appears to be localized to the muscular parts of the rat intestine whereas in the stomach EP_3 mRNA expression is seen in the mucosal layer especially in parietal cells (Ding *et al*., 1997). In most rodents, EP_3 receptor expression is predominantly found in gastric parietal and small intestinal goblet cells. They are also highly expressed in enteric glial and myenteric neuronal cells (Morimoto *et al*., 1997; Takahashi *et al*., 1999; Northey *et al*., 2000). EP_3 receptors are highly expressed in rabbit intestinal circular and longitudinal smooth muscles of the duodenum and in the circular muscles of the ileum (Grasa *et al*., 2006). In humans, EP_3 receptor expressions are observed throughout the gastric epithelium (Takafuji *et al*., 2002) and in the apex of colonic mucosa (Takafuji *et al*., 2000).

EP_4 receptor distribution

EP_4 receptor mRNA are mainly expressed in the rodent intestinal mucosal layers and in the parietal as well as the mucosal epithelial cells of the gastric mucosa (Ding *et al*., 1997; Northey *et al*., 2000). In the ileum, EP_4 receptor expression was demonstrated on mature enterocytes of the villi (Morimoto *et al*., 1997). Normal EP_4 receptor mRNA expression was observed in rabbit gastric epithelial cells (Takahashi *et al*., 1999). However, EP_4 receptor proteins were not detected in any of the segments (Grasa *et al*., 2006). In humans, EP_4 expression was modest in the gastric epithelium but intense expression of EP_4 was detected in lamina propria mononuclear cells (Takafuji *et al*., 2002). In the colon EP_4 receptors are strongly expressed in the lateral crypt epithelia (Takafuji *et al*., 2000) and lamina propria mononuclear cells of CD + T lymphocytes (Cosme *et al*., 2000).

Physiological role of EP receptors in the GI tract

The role of PGE_2 in GI physiology has been distinctly proved in various animal models. Presently, agonist/antagonist-based approaches along with EP receptor knockout strategies are used to determine the role that each of the EP receptors plays in GI physiology. Using these approaches, the role that EP receptor subtype(s) play in gastric acid secretion, GI motility and mucosal barrier functions including bicarbonate secretion, mucus secretion and epithelial cytoprotection have been elucidated.

Gastric acid secretion

Gastric acid is the main secretion of the stomach. A dual action of PGE_2 on gastric acid secretion was observed in rats, wherein, lower concentrations inhibited and higher concentration stimulated secretion (Ding *et al*., 1997). The inhibitory action of PGE_2 on acid secretion in rats was mediated by the EP_3 receptor whereas the stimulatory effect was due to the EP_4 receptor. It is interesting to note that both EP_3 and EP_4 receptors are present in parietal cells and acid-producing chief cells in the gastric mucosa. Parietal cells can respond to histamine in releasing acid into the gastric lumen. An EP_4 agonist, ONO-AE1-329, was shown to stimulate gastric acid secretion through histamine released from entero chromaffin cells (Kato *et al*., 2005).

GI motility

The peristaltic movement of the GI tract is maintained through coordinated contraction and relaxation of GI smooth muscle and PGE_2 has been reported to play a major role in GI motility. In rabbits, a complex mechanism involving activation of both EP_3 and EP_1 receptors was shown to be responsible for the contraction of the small

intestine. A direct effect of EP_3-mediated activation of intestinal smooth muscles and an indirect effect of EP_1-mediated stimulation of myenteric neurons acts together during PGE_2-induced intestinal contractions (Grasa *et al*., 2006). In rats, inhibition of small intestinal hypermotility was mediated through EP_4 receptors (Kunikata *et al*., 2002). Studies conducted in EP receptor knockout mice clearly underscore the importance of EP_1 and EP_3 receptors in the contraction of longitudinal smooth muscles of gastric fundus and ileum and EP_4 receptors in the relaxation (Okada *et al*., 2000). In guinea-pig ileum, pharmacological investigation revealed that EP_3 receptor stimulation mediates contraction of circular smooth muscles and increases peristalisis, whereas EP_2 mediates relaxation and decreases peristalisis (Botella *et al*., 1993; Shahbazian *et al*., 2002).

Bicarbonate secretion

GI bicarbonate secretion is one of the first lines of defense of the host epithelial cells against the harsh acidic environment of the stomach and the duodenum. Bicarbonates help in maintaining a narrow zone of neutral pH just above the mucosal lining that offers a protective barrier against diffusing acid (Takeuchi *et al*., 1997). PGE_2 has a stimulatory effect on the GI bicarbonate secretion. In fact, low physiological concentrations of PGE_2 in the duodenum would induce an acid-provoked bicarbonate secretion (Hirokawa *et al*., 2004). An agonist–antagonist-based approach in rats, as well as studies involving EP receptor knockout mice have proved the involvement of different EP receptor subtypes in bicarbonate secretion in different segments of the gut. In the stomach of rats and mice, EP_1 receptors are responsible for bicarbonate secretion whereas, the same effect was mediated through EP_3 receptors in the duodenum (Takeuchi *et al*., 1999a). In fact, the presence of EP_3 receptors in the duodenum is essential for duodenal bicarbonate secretion to counter luminal acid-induced mucosal damage (Takeuchi *et al*., 1999b). In addition to EP_3 receptors, EP_4 receptors have also been proved to be involved in duodenal bicarbonate secretion (Aoi *et al*., 2004). In humans, EP_4 receptors are solely responsible for duodenal bicarbonate secretion, which is in contrast to that seen in rats and mice, where EP_3 receptors mediate duodenal bicarbonate secretion (Larsen *et al*., 2005). This study suggests that species differences occur with regard to EP receptor function(s) and therefore caution should be taken in ascribing a particular function to a particular EP receptor.

Mucus secretion

Mucins are polymers made of glycoproteins that are secreted by the mucous cells of the stomach and the goblet cells of the intestine that form a protective covering over the lining of the mucosa. They form the major component of the innate immune response of the GI mucosa. PGE_2 is reported to be a potent mucin secretagogue. It strongly stimulates mucin secretion from rat gastric epithelial cells (Tani *et al*., 1997). More recently, the molecular mechanism of PGE_2-induced-mucin gene transcription via the ERK MAPK/RSK1/CREB pathway has been elucidated (Cho *et al*., 2005). PGE_2-induced mucus secretion from the gastric epithelial cells of rabbit are mainly mediated through EP_4 receptors in a cAMP/PKA-dependent manner (Takahashi *et al*., 1999). Mucin exocytosis from the antral mucous cells of guinea-pigs is also mediated through EP_4 but activated EP_1 receptors are shown to potentiate the effect of mucus secretion mediated by EP_4 (Ohnishi *et al*., 2001). An agonist-based approach in identifying the EP receptors responsible for mucin exocytosis in the rat colon and in a human colonic epithelial cell line LS174T revealed that coupling of PGE_2 with EP_4 receptors was essential for mucin secretion (Belley and Chadee, 1999).

Cytoprotection

PGE_2 plays an important role in protecting the GI mucosa from injuries caused by the harsh environment of the lumen. The mechanism by which PGE_2 exerts its protective effects on GI mucosa from noxious agents is termed cytoprotection. For example, PGE_2 exerts a potent cytoprotective effect on gastric glandular cells against indomethacin-induced injury, which is independent of neural, vascular and hormonal factors (Brzozowski *et al*., 2005). Current studies are underway to identify EP receptor subtype that mediates this protective action. It has been reported that, in an acute rat esophagitis model, low doses of PGE_2 confer a protective effect that is mediated through EP_1 receptors (Yamato *et al*., 2005). Similarly, in the stomach of rat, EP_1 receptors are essential for offering protection against indomethacin or ethanol-induced injury (Araki *et al*., 2000; Suzuki *et al*., 2001). In rodents, EP_1 receptors also mediate adaptive gastric cytoprotection, under conditions in which initial exposure to mild irritants prevents subsequent damage by more severe irritants (Takeuchi *et al*., 2001). Activation of the EP_1 receptors helps in maintaining gastric mucosal integrity (Takeuchi *et al*., 2002). In the small intestine of rats, EP_3 and EP_4 receptors offer cytoprotection against indomethacin-induced injury possibly through increased mucus secretion, enteropooling and through inhibition of hypermotility mediated by EP_4 alone (Kunikata *et al*., 2002). Activation of both EP_2 and EP_4 receptors in the guinea-pig gastric mucosal cells offers cytoprotection against ethanol-induced apoptosis (Hoshino *et al*., 2003). In mice, EP_2 receptors are essential for preventing radiation-induced apoptosis of crypt epithelial cells at the jejunum (Houchen *et al*., 2003).

Differential expression of EP receptors during pathological conditions in the gut

This area is perhaps the least studied aspect of EP receptor biology in the gut. To complicate the issue, the limited data that are available does not always discriminate whether EP receptor expressions are altered in individual cells, mucosa or submucosa or the full thickness of the gut. Nevertheless, these data on differential expression of EP receptors during various pathological conditions such as radiation injury, tumorigenesis and inflammation may explain the modus operandi of PGE_2 in exerting its pathological functions as against its well-known physiological roles.

Radiation-induced injury

A regional and temporal difference in the expression of EP receptors following radiation-induced injury in mice has been observed. Significant changes in EP_2 and EP_4 mRNA and protein levels were noticed in the jejunum and colon after radiation-induced injury. EP_2 receptor expression increased steeply whereas a decrease in EP_4 expression was observed. Interestingly, EP_2 receptor expression corresponded with epithelial restitution and early crypt morphogenesis of the injured tissues (Houchen *et al*., 2003).

GI tumorigenesis

A differential expression of EP receptors in the colon during early stages of tumorigenesis is observed in rodents and humans. The most notable changes reflect an increase in the expression of EP_1 and EP_2 receptors and a marked decrease in EP_3 receptors. However, EP_4 receptor levels remained constant (Shoji *et al*., 2004). Upregulation of EP_1 receptors is clearly observed during early tumorigenesis that helps in cellular proliferation and in the antiapoptotic effects observed in cancer cells, indicating a major role played by EP_1 receptors in cancer (Kawamori *et al*., 2005). A few studies involving RT–PCR analysis have revealed that as well as EP_1, EP_2 and EP_4 receptor mRNAs expression are increased in azoxymethane-induced colorectal cancer tissues (Mutoh *et al*., 2002; Kawamori *et al*., 2003). Similarly, EP_2 and EP_4 mRNA levels are also increased in ApcΔ716 mouse small intestinal and colonic polyps (Sonoshita *et al*., 2001). These studies implicate EP_2 and EP_4 receptors in tumorigenesis. The signalling mediated by $EP_{1/2/4}$ receptors that lead to cellular proliferation and tumorigenesis was briefly discussed under the previous section 'Downstream signalling pathways affected by EP receptors.'' Apart from the upregulation and activation of certain EP receptors during cancer, it is interesting to note that EP_3 receptor is downregulated. Downregulation of the EP_3 receptor is linked to the hypermethylation of the EP_3 receptor gene, thus inhibiting the suppressive role of EP_3 in colon cancer development. Increased expression of EP_4 receptors on $CD3^+$ lymphocytes adjacent to the tumour was also evident in human gastric mucosal cancers but the role of T lymphocyte EP_4 signalling in cancer is not clear (Takafuji *et al*., 2002). An increased expression of EP_2, EP_3 and EP_4 receptor mRNA levels was observed in rat oesophageal squamous cell dysplasia and Barrett's metaplasia induced by duodenal contents reflux; however, no changes in the mRNA levels of the EP_1 receptor was observed (Jang *et al*., 2004). This study suggests that regional differences may exist for the role of EP receptor subtypes in GI tumorigenesis.

GI inflammation

A differential expression of EP receptors on the GI mucosa may occur during inflammation. One interesting study showed defined patterns and identified striking differences of mucosal expression of the EP_4 receptors between cells in normal human colonic lamina propria and those from the inflamed human colon (Cosme *et al*., 2000). In particular, mucosal EP_4 receptor expression was upregulated in T lymphocytes. In ulcerative colitis, apart from an increase in EP_4 receptor expression in lamina propria T lymphocytes, an increase in the levels of EP_2 and EP_3 receptors was apparent in epithelial cells (Takafuji *et al*., 2000). At present, there are no reports on EP receptor expression levels in the gut during microbial or parasitic infections.

Role of EP_4 receptors in colitis

Even though PGE_2 levels are significantly increased during IBD, the functional role PGE_2 and individual EP receptors play in the pathogenesis of IBD remains undefined. PGE_2–EP receptor coupling may have a critical role in homeostasis or in the onset of GI inflammation and/or tissue repair. The limited data to date suggest that signalling via EP receptors can predetermine whether PGE_2 exerts a proinflammatory or anti-inflammatory effect in the gut. For example, studies *in vitro*, to address early responses of PGE_2 in a variety of colonic epithelial cell lines clearly demonstrate that PGE_2 couples via high-affinity EP_4 receptors to upregulate IL-8 mRNA expression and protein secretion confirming a proinflammatory role for PGE_2 (Yu and Chadee, 1999). IL-8 is a potent chemokine that can attract and activate neutrophils to cause nonspecific tissue damage important in the onset of colonic inflammation. In contrast, in EP_4 receptor knockout mice, a 7-day regime of dextran sodium sulphate-induced colitis was reported to be more severe than that in wild-type controls. This suggests that signalling via EP4 receptors may play a critical role in maintaining normal mucosal integrity and/or to promote healing (Kabashima *et al*., 2002). Unfortunately, the role of EP_4 signaling in either epithelial cells or the submucosal cells that induce an anti-inflammatory effect has not been elucidated. Moreover, it is not clear whether epithelial barrier functions or cytokine productions are altered in EP_4 knockout animals. Similarly, studies in rats using an EP_4 receptor agonist reported the suppression of colitis caused by extended DSS treatment through upregulation of an anti-inflammatory cytokine, IL-10 (Nitta *et al*., 2002). Suppression of Th1-type response, coupled with evidence of increased expression of EP_4 receptors on the mucosal T lymphocytes in inflamed colonic mucosa (Cosme *et al*., 2000), suggests a fundamental role for PGE_2-EP_4 signalling in T lymphocytes in repair and restitution of damaged tissues. It appears that EP_4 signalling outcomes differ between the early onset (pro-inflammatory on mucosal epithelial cells) and the late progressive stages of colitis (anti-inflammatory on immune cells in the lamina propria). Collectively, these studies emphasize the importance of PGE_2-EP_4 receptor signalling events in the cell types present in colonic mucosa as a fundamental determinant of colitis. More studies are needed to define the diverse functions of PGE_2-EP receptor coupling on various cell types before we can model their role in normal and disease states.

Concluding remarks

Recent evidence clearly suggests that PGE_2-EP receptor signalling plays an important role in the GI tract. Impor-

tantly, PGE_2-EP receptor coupling on different cell types may exert either a pro-inflammatory or anti-inflammatory response. In general, it appears that EP_1, EP_2 and EP_4 receptors are major determinants in the early stages of intestinal tumorigenesis and inflammation, whereas EP_4 receptors on immune cells may promote the restitution of colitis/inflammation. There maybe redundancy of EP receptor signalling and EP receptor functions may vary from onset to different stages of disease (initiation versus progression). However, it appears that all receptors are needed for various normal functions of the GI tract. As regional, temporal and species differences exist in EP receptor expression and distribution in the GI tract, future research should aim at identifying EP receptor expression in various cellular components of the mucosa across the length and the breath of the gut. As regional and species differences are apparent with regards to EP receptor functions, it is dangerous to ascribe specific functions for EP receptors in the gut. A priority is to define the distinct EP receptor signalling events in various cell types in the gut. Knowledge of receptor expression coupled with insights into specific signalling events in specific cell types may help the cause of understanding distinct roles that EP receptors play in the GI tract. More research is also needed to define the activity and the toxicity of single/combination EP receptor functions under normal and inflamed/diseased conditions. The long-term goal of future research in EP receptor biology should aim at identifying the Achilles' heel at the receptor level as well as at the signalling that it propagates. This would help in effective pharmacological intervention of various GI ailments whose pathologies are mediated by PGE_2.

Acknowledgements

Research in Dr Chadee's laboratory is supported by grants from the Crohn's and Colitis Foundation of Canada, the Canadian Institute for Health Research, the Canadian Foundation for Innovation, the Natural Sciences and Engineering Research Council of Canada and the Canadian Association of Gastroenterology-Industry-CIHR Research and Fellowship Awards. Dr Chadee holds a Canada Research Chair in Gastrointestinal Inflammation.

Conflict of interest

The authors state no conflict of interest.

References

Abramovitz M, Adam M, Boie Y, Carriere M, Denis D, Godbout C *et al*. (2000). The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochem Biophys Acta* **1483**: 285–293.

Ahrenstedt O, Hallgren R, Knutson L (1994). Jejunal release of prostaglandin E_2 in Crohn's disease: relation to disease activity and first-degree relatives. *J Gastroenterol Hepatol* **9**: 539–543.

An S, Yang J, So S, Zeng L, Goetzl E (1994). Isoforms of the EP3 subtype of human prostaglandin E_2 receptor transduce both intracellular calcium and cAMP signals. *Biochemistry* **33**: 14496–14502.

An S, Yang J, Xia M, Goetzl E (1993). Cloning and expression of the EP2 subtype of human receptors for prostaglandin E_2. *Biochem Biophys Res Commun* **197**: 263–270.

Aoi M, Aihara E, Nakashima M, Takeuchi K (2004). Participation of prostaglandin E_2 receptor EP4 subtype in duodenal bicarbonate secretion in rats. *Am J Physiol Gastrointest Liver Physiol* **287**: G96–G103.

Araki H, Ukawa H, Sugawa Y, Yagi K, Suzuki K, Takeuchi K (2000). The roles of prostaglandin E receptor subtypes in the cytoprotective action of prostaglandin E_2 in rat stomach. *Aliment Pharmacol Ther* **14**: 116–124.

Arosh JA, Banu SK, Chapdelaine P, Emond V, Kim JJ, Maclaren LA *et al*. (2003). Molecular cloning and characterization of bovine prostaglandin E_2 receptors EP2 and EP4: expression and regulation in endometrium and myometrium during the estrous cycle and early pregnancy. *Endocrinology* **144**: 3076–3091.

Bastien L, Sawyer N, Grygorczyk R, Metters K, Adam M (1994). Cloning, functional expression, and characterization of the human prostaglandin E_2 receptor EP2 subtype. *J Biol Chem* **269**: 11873–11877.

Belley A, Chadee K (1999). Prostaglandin E_2 stimulates rat and human colonic mucin exocytosis via the EP4 receptor. *Gastroenterology* **117**: 1352–1362.

Bhattacharya M, Peri K, Almazan G, Ribeiro-da-silva A, Shichi H, Durocher Y *et al*. (1998). Nuclear localization of prostaglandin E_2 receptors. *Proc Natl Acad Sci USA* **95**: 15792–15797.

Bhattacharya M, Peri K, Ribeiro-da-silva A, Almazan G, Shichi H, Hou X *et al*. (1999). Localization of functional prostaglandin E_2 receptors EP3 and EP4 in the nuclear envelope. *J Biol Chem* **274**: 15719–15724.

Bilson HA, Mitchell DL, Ashby B (2004). Human prostaglandin EP3 receptor isoforms show different agonist-induced internalization patterns. *FEBS Lett* **572**: 271–275.

Boie Y, Stocco R, Sawywe N, Slipetz DM, Ungrin MD, Neuschafer-rube F *et al*. (1997). Molecular cloning and characterization of the four rat prostaglandin E_2 prostanoid receptor subtypes. *Eur J Pharmacol* **340**: 227–241.

Botella A, Delvaux M, Fioramonti J, Frexinos J, Bueno L (1993). Stimulatory (EP1 and EP3) and inhibitory (EP2) prostaglandin E_2 receptors in isolated ileal smooth muscle cells. *Eur J Pharmacol* **237**: 131–137.

Breyer RM, Bagdassarian CK, Myers SA, Breyer MD (2001). Prostanoid receptors: subtypes and signaling. *Ann Rev Pharmacol Toxicol* **41**: 661–690.

Breyer R, Davis L, Nian C, Redha R, Stillman B, Jacobson HR *et al*. (1996). Cloning and expression of the rabbit prostaglandin EP4 receptor. *Am J Physiol Renal Physiol* **270**: F485–F493.

Breyer RM, Emeson RB, Breyer MD, Abromson RM, Davis LS, Ferrenbach SM (1994). Alternative splicing generates multiple isoforms of a rabbit prostaglandin E_2 receptor. *J Biol Chem* **269**: 6163–6169.

Brzozowski T, Tarnawski A, Hollander D, Sekhon S, Krause WJ, Gergely H (2005). Comparison of prostaglandin and cimetidine in protection of isolated gastric glands against indomethacin injury. *J Physiol Pharmacol* **56**: 75–88.

Buchanan FG, Wang D, Bargiacchi F, Dubois RN (2003). Prostaglandin E_2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. *J Biol Chem* **278**: 35451–35457.

Cadigan KM, Nusse R (1997). Wnt signaling: a common theme in animal development. *Genes Dev* **11**: 3286–3305.

Calder PC (2001). Polyunsaturated fatty acids, inflammation, and immunity. *Lipids* **36**: 1007–1023.

Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS (2005). Prostaglandin E_2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science* **310**: 1504–1510.

Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS *et al*. (2002). COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA* **99**: 13926–13931.

Cho KN, Choi JY, Kim CH, Baek SJ, Chung KC, Moon UY *et al*. (2005). Prostaglandin E_2 induces *MUC8* gene expression via a mechanism

involving ERK MAPK/RSK1/CREB activation in human airway epithelial cells. *J Biol Chem* **280**: 6676–6681.
Cosme R, Lublin D, Takafuji V, Lynch K, Roche JK (2000). Prostanoids in human colonic mucosa: effects of inflammation on PGE_2 receptor expression. *Hum Immunol* **61**: 684–696.
Davis TL, Sharif NA (2000). Pharmacological characterization of [(3) H]-prostaglandin E_2 binding to the cloned human EP4 prostanoid receptor. *Br J Pharmacol* **130**: 1919–1926.
Desai S, April H, Nwaneshiudu C, Ashby B (2000). Comparison of agonist-induced internalization of the human EP2 and EP4 prostaglandin receptors: role of the carboxyl terminus in EP4 receptor sequestration. *Mol Pharmacol* **58**: 1279–1286.
Ding M, Kinoshita Y, Kishi K, Nakata H, Hassan S, Kawanami C *et al.* (1997). Distribution of prostaglandin E receptors in the rat gastrointestinal tract. *Prostaglandins* **53**: 199–216.
Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, Dubois RN (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* **107**: 1183–1188.
Filippa N, Sable CL, Filloux C, Hemmings B, Van obberghen E (1999). Mechanism of protein kinase B activation by cyclic AMP-dependent protein kinase. *Mol Cell Biol* **19**: 4989–5000.
Fujino H, Salvi S, Regan JW (2005). Differential regulation of phosphorylation of the cAMP response element-binding protein after activation of EP2 and EP4 prostanoid receptors by prostaglandin E_2. *Mol Pharmacol* **68**: 251–259.
Fujino H, Xu W, Regan JW (2003). Prostaglandin E_2 induced functional expression of early growth response factor-1 by EP4, but not EP2, prostanoid receptors via the phosphatidylinositol 3-kinase and extracellular signal-regulated kinases. *J Biol Chem* **278**: 12151–12156.
Funk C, Furchi L, FitzGerald G, Grygorczyk R, Rochette C, Bayne MA *et al.* (1993). Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. *J Biol Chem* **268**: 26767–26772.
Grasa L, Arruebo MP, Plaza MA, Murillo MD (2006). PGE_2 receptors and their intracellular mechanisms in rabbit small intestine. *Prostaglandins Other Lipid Mediat* **79**: 206–217.
Guan Y, Zhang YH, Stillman BA, Schneider A, Saito O, Davis LS *et al.* (1996). Cloning and functional expression of the rabbit prostaglandin EP2 receptor. *J Am Soc Nephrol* **7**: 1646.
Hamberg M, Svensson J, Samuelsson B (1974). Prostaglandin endoperoxides. A new concept concerning the mode of action and release of prostaglandins. *Proc Natl Acad Sci USA* **71**: 3824–3828.
Han C, Wu T (2005). Cyclooxygenase-2-derived prostaglandin E_2 promotes human cholangiocarcinoma cell growth and invasion through EP1 receptor-mediated activation of the epidermal growth factor receptor and Akt. *J Biol Chem* **280**: 24053–24063.
Hirokawa M, Furukawa O, Guth PH, Engel E, Kaunitz JD (2004). Low-dose PGE_2 mimics the duodenal secretory response to luminal acid in mice. *Am J Physiol* **286**: G891–G898.
Honda A, Sugimoto Y, Namba T, Watabe A, Irie A, Negishi M *et al.* (1993). Cloning and expression of a cDNA for mouse prostaglandin E receptor EP2 subtype. *J Biol Chem* **268**: 7759–7762.
Hoshino T, Tsutsumi S, Tomisato W, Hwang HJ, Tsuchiya T, Mizushima T (2003). Prostaglandin E_2 protects gastric mucosal cells from apoptosis via EP2 and EP4 receptor activation. *J Biol Chem* **278**: 12752–12758.
Houchen CW, Sturmoski MA, Anant S, Breyer RM, Stenson WF (2003). Prosurvival and antiapoptotic effects of PGE_2 in radiation injury are mediated by EP2 receptor in intestine. *Am J Physiol Gastrointest Liver Physiol* **284**: 490–498.
Irie A, Sugimoto Y, Namba T, Harazono A, Honda A, Watabe A *et al.* (1993). Third isoform of the prostaglandin-E-receptor EP3 subtype with different C-terminal tail coupling to both stimulation and inhibition of adenylate cyclase. *Eur J Biochem* **217**: 313–318.
Jang TJ, Min SK, Bae JD, Jung KH, Lee JI, Kim JR *et al.* (2004). Expression of cyclooxygenase 2, microsomal prostaglandin E synthase 1, and EP receptors is increased in rat oesophageal squamous cell dysplasia and Barrett's metaplasia induced by duodenal contents reflux. *Gut* **53**: 27–33.
Kabashima K, Saji T, Murata T, Nagamachi M, Matsuoka T, Segi E *et al.* (2002). The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J Clin Invest* **109**: 883–893.
Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, Schuster VL (1995). Identification and characterization of a prostaglandin transporter. *Science (Washington DC)* **268**: 866–869.
Kato S, Aihara E, Yoshii K, Takeuchi K (2005). Dual action of prostaglandin E_2 on gastric acid secretion through different EP-receptor subtypes in the rat. *Am J Physiol Gastrointest Liver Physiol* **289**: G64–G69.
Katoh H, Aoki J, Ichikawa A, Negishi M (1998). P160 RhoA-binding kinase ROKalpha induces neurite retraction. *J Biol Chem* **273**: 2489–2492.
Katsuyama M, Nishigaki N, Sugimoto Y, Morimoto K, Negishi M, Narumiya S *et al.* (1995). The mouse prostaglandin E receptor EP2 subtype: cloning, expression and Northern blot analysis. *FEBS Lett* **372**: 151–156.
Kawamori T, Kitamura T, Watanabe K, Uchiya N, Maruyama T, Narumiya S *et al.* (2005). Prostaglandin E receptor subtype EP (1) deficiency inhibits colon cancer development. *Carcinogenesis* **26**: 353–357.
Kawamori T, Uchiya N, Sugimura T, Wakabayashi K (2003). Enhancement of colon carcinogenesis by prostaglandin E_2 administration. *Carcinogenesis* **24**: 985–990.
Kiriyama M, Ushikubi F, Kobayashi T, Hirata M, Sugimoto Y, Narumiya S (1997). Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br J Pharmacol* **122**: 217–224.
Kunikata T, Tanaka A, Miyazawa T, Kato S, Takeuchi K (2002). 16, 16-Dimethyl prostaglandin E_2 inhibits indomethacin-induced small intestinal lesions through EP3 and EP4 receptors. *Dig Dis Sci* **47**: 894–904.
Larsen R, Hansen MB, Bindslev N (2005). Duodenal secretion in humans mediated by the EP4 receptor subtype. *Acta Physiol Scand* **185**: 133–140.
Lawrence RA, Jones RL, Wilson NH (1992). Characterization of receptors involved in the direct and indirect actions of prostaglandins E and I on the guinea-pig ileum. *Br J Pharmacol* **105**: 271–278.
Li M, Wang X, Meintzer MK, Laessig T, Birnbaum MJ, Heidenreich KA (2000). Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. *Mol Cell Biol* **20**: 9356–9363.
Marshall FH, Patel K, Lundstrom K, Camacho J, Foord SM, Lee MG (1997). Characterization of [3H]-prostaglandin E_2 binding to prostaglandin EP4 receptors expressed with Semliki Forest virus. *Br J Pharmacol* **121**: 1673–1678.
Morimoto K, Sugimoto Y, Katsuyama M, Oida H, Tsuboi K, Kishi K *et al.* (1997). Cellular localization of mRNAs for prostaglandin E receptor subtypes in mouse gastrointestinal tract. *Am J Physiol* **272**: G681–G687.
Murakami M, Kudo I (2002). Phospholipase A2. *J Biochem (Tokyo)* **131**: 285–292.
Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I (1997). Regulatory functions of phospholipase A_2. *Crit Rev Immunol* **17**: 225.
Mutoh M, Watanabe K, Kitamura T, Shoji Y, Takahashi M, Kawamori T *et al.* (2002). Involvement of prostaglandin E receptor subtype EP (4) in colon carcinogenesis. *Cancer Res* **62**: 28–32.
Namba T, Sugimoto Y, Negishi M, Irie A, Ushikubi F, Kakizuka A *et al.* (1993). Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP3 determines G-protein specificity. *Nature* **365**: 166–170.
Narumiya S (1994). Prostanoid receptors. Structure, function and distribution. *Ann N Y Acad Sci* **744**: 126–138.
Narumiya S, Sugimoto Y, Ushikubi F (1999). Prostanoid receptors: structures, properties and functions. *Physiol Rev* **79**: 1193–1226.
Nemoto K, Pilbeam CC, Bilak S, Raisz L (1997). Molecular cloning and expression of the rat prostaglandin E_2 receptor of the EP2 subtype. *Prostaglandins* **54**: 713–725.
Nishigaki N, Negishi M, Ichikawa A (1996). Two Gs-coupled prostaglandin E receptor subtypes, EP2 and EP4, differ in

desensitization and sensitivity to the metabolic inactivation of the agonist. *Mol Pharmacol* **50**: 1031–1037.

Nitta M, Hirata I, Toshina K, Murano M, Maemura K, Hamamoto N *et al*. (2002). Expression of the EP4 prostaglandin E2 receptor subtype with rat dextran sodium sulphate colitis: colitis suppression by a selective agonist, ONO-AE1-329. *Scand J Immunol* **56**: 66–75.

Northey A, Denis D, Cirino M, Metters KM, Nantel F (2000). Cellular distribution of prostanoid EP receptors mRNA in the rat gastrointestinal tract. *Prostaglandins Other Lipid Mediat* **62**: 145–156.

Ohnishi A, Shimamoto C, Katsu K, Ito S, Imai Y, Nakahari T (2001). EP1 and EP4 receptors mediate exocytosis evoked by prostaglandin E_2 in guinea-pig antral mucous cells. *Exp Physiol* **86**: 451–460.

Okada Y, Hara A, Ma H, Xiao CY, Takahata O, Kohgo Y *et al*. (2000). Characterization of prostanoid receptors mediating contraction of the gastric fundus and ileum: studies using mice deficient in prostanoid receptors. *Br J Pharmacol* **131**: 745–755.

Okuda AE, Sakamoto K, Ezashi T, Miwa K, Ito S, Hayaishi O (1996). Suppression of prostaglandin E receptor signaling by the variant form of EP1 subtype. *J Biol Chem* **271**: 31255–31261.

Okuyama T, Ishihara S, Sato H, Rumi MA, Kawashima K, Miyaoka Y *et al*. (2002). Activation of prostaglandin E_2-receptor EP2 and EP4 pathways induces growth inhibition in human gastric carcinoma cell lines. *J Lab Clin Med* **140**: 92–102.

Oldfield S, Grubb BD, Donaldson LF (2001). Identification of a prostaglandin E_2 receptor splice variant and its expression in rat tissues. *Prostaglandins Other Lipid Mediat* **63**: 165–173.

Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS (2002). Prostaglandin E_2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* **8**: 289–293.

Pierce KL, Regan JW (1998). Prostanoid receptor heterogeneity through alternative mRNA splicing. *Life Sci* **62**: 1479–1483.

Regan JW, Bailey TJ, Donello JE, Pierce KL, Pepperl DJ, Zhang D *et al*. (1994b). Molecular cloning and expression of human EP3 receptors: evidence of three variants with differing carboxyl termini. *Br J Pharmacol* **112**: 377–385.

Regan JW, Bailey TJ, Pepperl DJ, Pierce KL, Bogardus AM, Donello JE *et al*. (1994a). Cloning of a novel human prostaglandin receptor with characteristics of pharmacologically defined EP2 subtype. *Mol Pharmacol* **46**: 213–220.

Reseta LS, Barrett KE (2002). Enteroinvasive bacteria alter barrier and transport properties of human intestinal epithelium: role of iNOS and COX2. *Gastroenterology* **122**: 1070–1087.

Schmid A, Thierauch K, Schleuning W, Dinter H (1995). Splice variants of the human EP3 receptor for prostaglandin E_2. *Eur J Biochem* **15**: 23–30.

Shahbazian A, Heinemann A, Peskar BA, Holzer P (2002). Differential peristaltic motor effects of prostanoid (DP, EP, IP, TP) and leukotriene receptor agonists in the guinea-pig isolated small intestine. *Br J Pharmacol* **137**: 1047–1054.

Sharif NA, Davis TL (2002). Cloned human EP1 prostanoid receptor pharmacology characterized using radioligand binding techniques. *J Pharm Pharm* **54**: 539–547.

Shoji Y, Takahashi M, Kitamura T, Watanabe K, Kawamori T, Maruyama T *et al*. (2004). Down regulation of prostaglandin E receptor subtype EP3 during colon cancer development. *Gut* **53**: 1151–1158.

Smith WL, Garavito M, Dewitt D (1996). Prostaglandin endoperoxide H synthase (cyclooxygenases) -1 and -2. *J Biol Chem* **271**: 33157–33160.

Smith WL, Meade EA, Dewitt DL (1994). Pharmacology of prostaglandin endoperoxide synthase isozyme 1 and 2. *Ann N Y Acad Sci* **714**: 136–142.

Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S *et al*. (2001). Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc (Delta 716) knockout mice. *Nat Med* **7**: 1048–1051.

Stillman BA, Audoly L, Breyer RM (1998). A conserved threonine in the second extracellular loop of the human EP2 and EP4 receptors is required for ligand binding. *Eur J Pharmacol* **357**: 73–82.

Stillman BA, Breyer MD, Breyer RM (1999). Importance of the extracellular domain for prostaglandin EP (2) receptor function. *Mol Pharmacol* **56**: 545–551.

Su JL, Shih JY, Yen ML, Jeng YM, Chang CC, Hsieh CY *et al*. (2004). Cyclooxygenase-2 induces EP1- and HER-2/Neu-dependent vascular endothelial growth factor-C up-regulation: a novel mechanism of lymph angiogenesis in lung adenocarcinoma. *Cancer Res* **64**: 554–564.

Sugimoto Y, Nakato T, Kita A, Hatae N, Tabata H, Tanaka S *et al*. (2003). Functional domains essential for G_s activity in prostaglandin EP2 and EP3 receptors. *Life Sci* **74**: 135–141.

Sugimoto Y, Nakato T, Kita A, Takahashi Y, Hatae N, Tabata H *et al*. (2004). A cluster of aromatic amino acids in the i2 loop plays a key role for Gs coupling in prostaglandin EP2 and EP3 receptors. *J Biol Chem* **279**: 11016–11026.

Sugimoto Y, Namba T, Honda A, Hayashi Y, Negishi M, Ichikawa A *et al*. (1992). Cloning and expression of a cDNA for mouse prostaglandin E receptor EP3 subtype. *J Biol Chem* **267**: 6463–6466.

Suzuki K, Araki H, Mizoguchi H, Furukawa O, Takeuchi K (2001). Prostaglandin E inhibits indomethacin-induced gastric lesions through EP-1 receptors. *Digestion* **63**: 92–101.

Tai HH, Ensor CM, Tong M, Zhou H, Yan F (2002). Prostaglandin catabolizing enzymes. *Prostaglandins Other Lipid Mediat* **68–69**: 483–493.

Takafuji V, Cosme R, Lublin D, Lynch K, Roche JK (2000). Prostanoid receptors in intestinal epithelium: selective expression, function, and change with inflammation. *Prostaglandins Leukot Essent Fatty Acids* **63**: 223–235.

Takafuji VA, Evans A, Lynch KR, Roche JK (2002). PGE_2 receptors and synthesis in human gastric mucosa: perturbation in cancer. *Prostaglandins Leukot Essent Fatty Acids* **66**: 71–81.

Takahashi S, Takeuchi K, Okabe S (1999). EP4 receptor mediation of prostaglandin E_2-stimulated mucus secretion by rabbit gastric epithelial cells. *Biochem Pharmacol* **58**: 1997–2002.

Takeuchi K, Araki H, Umeda M, Komoike Y, Suzuki K (2001). Adaptive gastric cytoprotection is mediated by prostaglandin EP1 receptors: a study using rats and knockout mice. *J Pharmacol Exp Ther* **297**: 1160–1165.

Takeuchi K, Ogawa Y, Kagawa S, Ukawa H (2002). Gastric ulcerogenic responses following barrier disruption in knockout mice lacking prostaglandin EP1 receptors. *Aliment Pharmacol Ther* **2**: 74–82.

Takeuchi K, Ukawa H, Furukawa O, Kawauchi S, Araki H, Sugimoto Y *et al*. (1999a). Prostaglandin E receptor subtypes involved in stimulation of gastroduodenal bicarbonate secretion in rats and mice. *J Physiol Pharmacol* **50**: 155–167.

Takeuchi K, Ukawa H, Kato S, Furukawa O, Araki H, Sugimoto Y *et al*. (1999b). Impaired duodenal bicarbonate secretion and mucosal integrity in mice lacking prostaglandin E-receptor subtype EP3. *Gastroenterology* **117**: 1128–1135.

Takeuchi K, Yagi K, Shinichi K, Ukawa H (1997). Roles of prostaglandin E-receptor subtypes in gastric and duodenal bicarbonate secretion in rats. *Gastroenterology* **113**: 1553–1559.

Tamma G, Wiesner B, Furkert J, Hahm D, Oksche A, Schaefer M *et al*. (2003). The prostaglandin E_2 analogue sulprostone antagonizes vasopressin-induced antidiuresis through activation of Rho. *J Cell Sci* **116**: 285–294.

Tani S, Okuda M, Morishige R, Tanaka T (1997). Gastric mucin secretion from cultured rat epithelial cells. *Biol Pharm Bull* **20**: 482–485.

Toh H, Ichikawa A, Narumiya S (1995). Molecular evolution of receptors for eicosanoids. *FEBS Lett* **361**: 17–21.

Vane JR, Bakhle YS, Botting RM (1998). Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* **38**: 97.

Watabe A, Sugimoto Y, Honda A, Irie A, Namba T, Negishi M *et al*. (1993). Cloning and expression of cDNA for a mouse EP1 subtype of prostaglandin E receptor. *J Biol Chem* **268**: 20175–20178.

Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H *et al*. (1999). Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res* **59**: 5093–5096.

White ES, Atrasz RG, Dickie EG, Aronoff DM, Stambolic V, Mak TW *et al*. (2005). Prostaglandin E_2 inhibits fibroblast migration by E-prostanoid 2 receptor-mediated increase in PTEN activity. *Am J Respir Cell Mol Biol* **32**: 135–141.

Woodward DF, Pepperl DJ, Burkey TH, Regan JW (1995). 6- Isopropoxy-9-oxoanthene-2-carboxylic acid (AH6809), a human EP2 receptor antagonist. *Biochem Pharmacol* **50**: 1731–1733.

Woodward T, Inoue H, Nishihara T (2005). SC-19220, antagonist of prostaglandin E_2 receptor EP1, inhibits osteoclastogenesis by RANKL. *J Bone Miner Res* **1**: 15–22.

Yamato M, Nagahama K, Kotani T, Kato S, Takeuchi K (2005). Biphasic effect of prostaglandin E_2 in a rat model of esophagitis mediated by EP1 receptors: relation to pepsin secretion. *Digestion* **72**: 109–118.

Yang J, Xia M, Goetzl E, Songzhu A (1994). Cloning and expression of the EP3-subtype of human receptors for prostaglandin E_2. *Biochem Biophys Res Commun* **198**: 999–1006.

Yano T, Zissel G, Muller QJ, Jae SS, Satoh H, Ichikawa T (2002). Prostaglandin E_2 reinforces the activation of Ras signal pathway in lung adenocarcinoma cells via EP3. *FEBS Lett* **518**: 154–158.

Yu Y, Chadee K (1999). Prostaglandin E_2 stimulates IL-8 gene expression in human colonic epithelial cells by a posttranscriptional mechanism. *J Immunol* **161**: 3746–3752.