ACCELERATED COMMUNICATION

EP₄ Prostanoid Receptor Coupling to a Pertussis Toxin-Sensitive Inhibitory G Protein

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ABSTRACT

The EP₂ and EP₄ prostanoid receptor subtypes are G-protein-coupled receptors for prostaglandin E₂ (PGE₂). Both receptor subtypes are known to couple to the stimulatory guanine nucleotide binding protein (Gₛ) and, after stimulation with PGE₂, can increase the formation of intracellular cAMP. In addition, PGE₂ stimulation of the EP₄ receptor can activate phosphatidylinositol 3-kinase (PI3K) leading to phosphorylation of the extracellular signal-regulated kinases (ERKs) and induction of early growth response factor-1 (EGR-1) (J Biol Chem 278: 12151–12156, 2003). We now report that the PGE₂-mediated phosphorylation of the ERKs and induction of EGR-1 can be blocked by pretreatment of EP₄-expressing cells with pertussis toxin (PTX). Furthermore, pretreatment with PTX increased the amount of PGE₂-stimulated intracellular cAMP formation in EP₄-expressing cells but not in EP₂-expressing cells. These data indicate that the EP₄ prostanoid receptor subtype, but not the EP₂, couples to a PTX-sensitive inhibitory G-protein (Gᵢ) that can inhibit cAMP-dependent signaling and activate PI3K/ERK-dependent signaling.

Prostaglandin E₂ (PGE₂) is an endogenous signaling molecule that is produced from arachidonic acid by the sequential actions of cyclooxygenase (COX) and PGE₂ synthase. PGE₂ is also referred to as a prostanoid, which is a term that encompasses the other prostaglandins (e.g., PGD₂ and PGF₂α) and thromboxanes. PGE₂ can bind to and stimulate four major prostanoid receptor subtypes that have been named EP₁, EP₂, EP₃, and EP₄ (Coleman et al., 1994). These receptors are all seven transmembrane-spanning receptors that activate intracellular second messenger signaling pathways by interacting with heterotrimeric G-proteins. There are four major subfamilies of G-proteins that are defined by their α subunits (Gα) and by the nature of the signaling pathways they activate (Hepler and Gilman, 1992). Perhaps the most well known are members of the Gαₛ and Gαᵢ subfamilies, whose activation affects the formation of intracellular cAMP by either stimulating or inhibiting the activity of adenyl cyclase, respectively. Members of the Gαₛ subfamily are also known as pertussis toxin (PTX) sensitive G-proteins because they can be inhibited by the actions of this toxin, which is the causative agent of whooping cough. Members of the Gαᵢ subfamily activate phospholipase C to stimulate inositol phosphate and Ca²⁺ signaling, whereas members of the Gα₁₂ subfamily affect signaling pathways that involve the activation of Rho, a member of the family of small monomeric G-proteins.

The EP receptor subtypes interact with several of the subfamilies of G-proteins to activate their respective signaling pathways. PGE₂ stimulation of the human EP₁ receptor increases the concentration of free intracellular Ca²⁺ (Funk et al., 1993) and stimulates inositol phosphate formation (J. W. Regan, unpublished observations), suggesting coupling to members of the Gαₛ subfamily. The EP₃ receptors are traditionally thought to couple to Gαᵢ to inhibit adenyl cyclase. However, the EP₃ receptors actually consist of multiple isoforms that are generated by alternative mRNA splicing, and their coupling to G-proteins is complex (Kotani et al., 1995). For example, in humans, there are eight isoforms, and at...
least two of these isoforms, the EP$_{3.1}$ and EP$_{3.1V}$, seem to couple to $G_q$ to stimulate adenylyl cyclase. The human EP$_{3.1}$ and EP$_{3.1V}$ can also couple to $G_q$ to stimulate inositol phosphate formation.

Stimulation of the human EP$_2$ and EP$_4$ receptors with PGE$_2$ increases intracellular cAMP formation, indicating that both of these isoforms can couple to $G_s$ to stimulate adenylyl cyclase (Regan, 2003). However, functional coupling to the cAMP signaling pathway seems to be more efficient for the human EP$_2$ receptor subtype than for the EP$_4$ subtype. Thus, when stably expressed in HEK cells at similar levels of receptor expression, the maximal stimulation of intracellular cAMP formation by the EP$_4$ subtype is only 20 to 50% of that achieved by the EP$_2$ subtype (Fujino et al., 2002, 2005). It has also been found recently that the human EP$_4$ receptor subtype, but not the human EP$_2$ subtype, can activate a phosphatidylinositol 3-kinase (PI3K) signaling pathway by a mechanism that is independent of the activation of the cAMP/protein kinase A (PKA) pathway (Fujino et al., 2002, 2003, 2005). PGE$_2$-mediated activation of this PI3K signaling pathway by the human EP$_4$ receptor leads to the induction of functional expression of early growth response factor-1 (EGR-1) (Fujino et al., 2003) and to the inhibition of the activity of PKA (Fujino et al., 2005). We now report that activation of the PI3K signaling pathway by the human EP$_4$ receptor involves the coupling of this receptor to a PTX-sensitive, cAMP-inhibitory G-protein ($G_i$). Coupling of the EP$_4$ receptor to $G_i$ explains, in part, the less efficient coupling of the EP$_4$ receptor to the cAMP/PKA signaling pathway compared with the EP$_2$ receptor subtype.

**Materials and Methods**

**Cell Culture.** Cell lines stably expressing the EP$_2$ or EP$_4$ receptors were prepared using HEK-293–Epstein-Barr virus nuclear antigen cells and the mammalian expression vector pCEP (Invitrogen, Carlsbad, CA) as described previously (Fujino et al., 2002). Cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum, 250 $\mu$g/ml geneticin, 100 $\mu$g/ml gentamicin, and 200 $\mu$g/ml hygromycin B.

**cAMP Assay.** Cells were cultured in 12-well plates; 16 h before the immunoblotting experiments, cells were switched from their regular culture medium to Opti-MEM (Invitrogen) containing 250 $\mu$g/ml G-418 (Geneticin) and 100 $\mu$g/ml gentamicin. Cells were pretreated with either vehicle (water) or 100 ng/ml PTX (Calbiochem, San Diego, CA) for 16 h at 37°C. Cells were then treated with either vehicle (0.1% dimethyl sulfoxide), 1 $\mu$M PGE$_2$ for 10 min (phospho-ERKs), or 60 min (EGR-1) at 37°C. Cells were scraped into a lysis buffer (consisting of 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, pH 8.0, 1% Nonidet P-0.5, 0.5% sodium deoxycholate, 10 mM sodium fluoride, 10 mM disodium pyrophosphate, 0.1% SDS, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 10 $\mu$g/ml leupeptin, and 10 $\mu$g/ml aprotinin) and transferred to microcentrifuge tubes. The samples were rotated for 30 min at 4°C and were centrifuged at 16,000 g for 15 min. Aliquots of the supernatants containing 20–100 $\mu$g of protein were electrophoresed on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes as described previously (Fujino et al., 2003). Membranes were incubated in 5% nonfat milk for 1 h and were then washed and incubated for 16 h at 4°C with primary antibodies using the following conditions. For the ERKs, incubations were done in 3% nonfat milk containing either a 1:1000 dilution of anti-phospho-ERK1/2 antibody (Cell Signaling Technology Inc., Beverly, MA) or a mixture of a 1:500 dilution of anti-ERK1 antibody and a 1:10,000 dilution of anti-ERK2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). For EGR-1, incubations were done in 3% nonfat milk containing a 1:1000 dilution of anti-EGR-1 antibody (Santa Cruz Biotechnology). After incubating with the primary antibody, membranes were washed three times and incubated for 1 h at room temperature with a 1:10,000 dilution of the appropriate secondary antibodies conjugated with horseradish peroxidase using the same conditions as described above for each of the primary antibodies. After washing three times, immunoreactivity was detected by chemiluminescence as described previously (Fujino et al., 2003). To ensure equal loading of proteins, the membranes were stripped and re-probed with appropriate antibodies under the same conditions as described above.

**Results**

**Pertussis Toxin Potentiates PGE$_2$-Stimulated cAMP Formation in HEK Cells Stably Expressing the Human EP$_2$ Prostanoid Receptor.** We have reported previously that the maximal level of PGE$_2$-stimulated cAMP formation is significantly lower in HEK cells stably expressing the human EP$_2$ prostanoid receptor compared with HEK cells stably expressing the human EP$_2$ receptor, even though the levels of receptor expression were very similar (Fujino et al., 2002). We have also found that the EP$_2$ receptor can activate a PI3K/ERKs signaling pathway to induce the expression of EGR-1, whereas the EP$_2$ receptor subtype does not (Fujino et al., 2003). We had hypothesized previously that the EP$_4$ receptor was less efficiently coupled to $G_q$, but recently we considered the possibility that the EP$_4$ receptor might be additionally coupled to $G_o$, as has been shown for cardiac $\beta_2$-adrenergic receptors (Xiao et al., 1999a,b). To test this hypothesis we pretreated cells with PTX, which catalyzes the transfer of ADP-ribose from NAD to $G_o$ and thereby blocks the ability of $G_o$ to inhibit the activity of adenylyl cyclase (U1, 1984). Thus, untransfected HEK cells and HEK cells stably expressing either the human EP$_2$ or EP$_4$ receptors were pretreated for 16 h with PTX and were then treated for 10 min with various concentrations of PGE$_2$. As shown in Fig.
there was no appreciable accumulation of cAMP in untransfected HEK cells with or without PTX pretreatment. In the absence of PTX pretreatment, the maximal stimulation of cAMP formation in HEK cells expressing the EP2 receptor was approximately twice that obtained in HEK cells expressing the EP4 receptor (36 pmol versus 19 pmol, respectively). Pretreatment with PTX resulted in a significant 33% increase in maximal PGE2-stimulated cAMP formation in HEK cells expressing the EP4 receptor, whereas, in EP2-expressing cells, pretreatment with PTX essentially had no effect. The EC50 for PGE2 stimulation of cAMP formation was approximately 4-fold lower for EP4-expressing cells compared with EP2-expressing cells (0.4 nM versus 1.7 nM, respectively), and it was not affected by pretreatment with PTX. These data clearly support the hypothesis that the human EP4 prostanoid receptor, but not the EP2 receptor, can functionally couple to Goq in addition to coupling to Goi.

Pertussis Toxin Potentiation of PGE2-Stimulated cAMP Formation in EP4 Cells Is Not Due to Activation of Endogenous EP3 Receptors. The evidence that PTX treatment of EP2-expressing cells did not potentiate PGE2-stimulated cAMP formation suggests that the potentiation of PGE2-stimulated cAMP formation after PTX pretreatment of EP4-expressing cells is not a consequence of the activation of endogenous Goi-coupled EP3 receptors. Nevertheless, this possibility was further examined using the EP4-selective agonist PGE1-OH in cells that were treated with forskolin, which stimulates intracellular cAMP formation by the direct activation of adenyl cyclase. As shown in Fig. 2, PTX pretreatment of untransfected HEK cells and HEK cells stably expressing EP2 receptors had no effect on forskolin-stimulated cAMP formation in the presence of PGE1-OH. On the other hand, in HEK cells stably expressing EP4 receptors, pretreatment with PTX resulted in a 37% increase in forskolin-stimulated cAMP formation in the presence of PGE1-OH. If the activation of endogenous EP3 receptors coupled to Goq was responsible for this increase, similar increases in forskolin-stimulated cAMP formation should have been observed after PTX pretreatment of the untransfected HEK cells and HEK cells expressing EP2 receptors.

Coupling of the Human EP4 Prostanoid Receptor to Goq Mediates PGE2-Stimulated ERK Phosphorylation and Induction of EGR-1 Expression. We have shown previously that PGE2 stimulation of the human EP4 receptor, but not the human EP2 receptor, can induce the functional expression of EGR-1 through the activation of the PI3K and ERK signaling pathways (Fujino et al., 2003). It has also been reported that the G beta-gamma subunits of the G protein can activate a PI3K signaling pathway by coupling with Goq (Jo et al., 2002). We therefore decided to examine whether the PGE2-mediated activation of PI3K/ERKs signaling and induction of EGR-1 expression occurs through a mechanism involving coupling of the EP4 receptor to Goq. For these experiments, cells were either untreated or pretreated with PTX for 16 h and were then incubated with either vehicle or 1 μM PGE2. The expression of the phospho-ERKs, total ERKs, and EGR-1 were then examined by immunoblot analysis. Figure 3A, top, shows that in the absence of PTX pretreatment, PGE2-stimulated ERK phosphorylation in EP4-expressing cells, but not in EP2-expressing cells, and that pretreatment with PTX completely abolished this effect. Likewise, Fig. 3B, top, shows that in the absence of PTX pretreatment, PGE2-stimulated expression of EGR-1 in EP4-expressing cells, but not in EP2-expressing cells, and that pretreatment with PTX also blocked this action. In addition, Fig. 3, A and B, bottom, show that nearly identical amounts of ERKs 1 and 2 were present in all cells, indicating that the amount of ERKs in the cells was not altered by PTX pretreatment.
under all conditions and in both cell lines. These data support the conclusion that the activation of ERK signaling and induction of EGR-1 by PGE$_2$ is mediated by coupling of the human EP$_4$ prostanoid receptor to a PTX-sensitive G-protein.

Discussion

The regulation of intracellular cAMP by E-type prostaglandins has been known for nearly forty years (Butcher and Baird, 1968). Thus, PGE$_1$ was found to lower intracellular cAMP in isolated fat pads but to increase it in several other cell types. Direct evidence for the existence of specific receptors for the E-type prostaglandins was initially obtained in radioligand binding studies with $[^3H]$PGE$_1$ (Kuehl and Humes, 1972), which were also used to show that the binding of $[^3H]$PGE$_1$ could be modulated by guanine nucleotides (Moore and Wolff, 1973). This was among the first evidence that E-type prostaglandin receptors, together with the glucagon and catecholamine receptors, interacted with G-proteins and that this interaction might constitute a general mechanism for signaling between cell surface receptors and adenyl cyclase (Rodbell, 1980). Extensive physiological, pharmacological and molecular biological studies later defined the receptors for the E-type prostaglandins as EP receptors and classified them into the EP$_1$, EP$_2$, EP$_3$, and EP$_4$ subtypes (Coleman et al., 1994; Regan, 2003; Hata and Breyer, 2004). As reviewed in the Introduction, the EP$_1$ and EP$_3$ receptors have been generally regarded as coupling to G$_\alpha$q and G$_\alpha$i, respectively, whereas the EP$_2$ and EP$_4$ receptors have been considered to be exclusively coupled to G$_\alpha$o.

The present findings now show for the first time that in addition to coupling to G$_\alpha$o, EP$_4$ receptors can also couple to a PTX-sensitive G-protein to inhibit intracellular cAMP formation and activate PI3K and ERKs signaling cascades. Furthermore, the inhibition of cAMP formation by the EP$_4$ receptor suggests specific coupling to G$_\alpha$i.

We have reported previously that PGE$_2$ stimulation of human EP$_2$ and EP$_4$ receptors can activate Tec/Elk signaling but that EP$_2$ receptors do this primarily through a cAMP/PKA pathway, whereas EP$_4$ receptors mainly use a PI3K pathway (Fujino et al., 2002). We have also reported that PGE$_2$ stimulation of human EP$_4$ receptors, but not EP$_2$ receptors, results in the functional expression of EGR-1 through the activation of PI3K and MAP kinase signaling (Fujino et al., 2003). As for the present study, these previous studies were conducted exclusively with a recombinant cell system consisting of HEK cells stably transfected with either the human EP$_2$ or EP$_4$ receptors. There is increasing evidence, however, that such observations will eventually be extended to endogenous EP$_2$ and EP$_4$ receptors in native cell systems. For example, Sheng et al. (2001) reported that PGE$_2$ stimulation of endogenous EP$_4$ receptors in human colorectal cancer cells increased cell growth and motility through the activation of PI3K and Akt. Likewise, Pozzi et al. (2004) found that PGE$_2$ stimulation of endogenous EP$_4$ receptors in mouse colon adenocarcinoma cells increased cellular proliferation by a mechanism that was independent of any measurable effect on cAMP and that involved the activation of the Akt and MAP kinases. Reno and Cannas (2005) have reported that PGE$_2$ stimulation of endogenous EP$_2$ or EP$_4$ receptors in human myeloid leukemia cells increased PMA-induced macrophage differentiation by a mechanism that was independent of the activation of a cAMP/PKA pathway and that involved the activation of PI3K and MAP kinase signaling. Similar findings were also obtained by Caristi et al. (2005), who found that endogenous EP$_4$ receptors in human T lymphocytes mediate interleukin-8 gene transcription by a mechanism that is PKA-independent and involves the activation of PI3K signaling. Thus, there are endogenous EP$_4$ receptors in native cell systems that can activate PI3K signaling by mechanisms that seem to be independent of coupling to G$_\alpha$o.

It is well established that GPCRs can activate PI3K and Akt signaling through the interaction of G$\beta$y subunits with either the p110$\delta$ or p110y subunits of PI3K (Yart et al., 2002). In most cases in which it has been examined, the activation of PI3K and Akt signaling involves G$_\alpha$q-coupled receptors (Kim et al., 2004). Given the present findings, it is likely that the PTX-sensitive activation of PI3K and ERK signaling by the EP$_4$ receptor reflects specific coupling to G$_\alpha$i as opposed to G$_\alpha$o.

In many ways, the classification of the EP receptor subtypes and their pattern of G-protein coupling bears similarities to the adrenergic receptor subtypes. For example, the $\alpha_2$- and $\alpha_o$-adrenergic receptors are generally regarded as coupling to G$_\alpha$q and G$_\alpha$i, respectively, whereas the $\beta$-adrenergic receptor subtypes were long considered to be exclusively coupled to G$_\alpha$i. It has become apparent, however, that the $\beta_2$-adrenergic receptor has additional coupling to G$_\alpha$o, which results, as in the EP$_4$ receptor, in the inhibition of cAMP formation and activation of PI3K and ERK signaling cas-
cades (Daaka et al., 1997; Chesley et al., 2000). This is of particular functional significance for the cardiac β-receptors because it profoundly alters the consequences of persistent activation of these receptors. Thus, transgenic overexpression of β₁-adrenergic receptors in mice leads to cardiac hypertrophy, heart failure, and early death, whereas, overexpression of the β₂-adrenergic receptor actually improves cardiac function and does not adversely affect life span (Xiao et al., 1999b). Although cardiac β₂-adrenergic receptors can couple to G_{s}, it has been found that the protective effects of β₂-adrenergic receptor over expression depend upon coupling to G_{α_{i}} and G_{α_{s}} (Foerster et al., 2003). At present, the physiological and pathophysiological consequences of the unique signaling properties of the EP₄ receptor are unknown. However, like the β₁- and β₂-adrenergic receptor subtypes, the EP₂ and EP₄ prostanoid receptor subtypes are frequently coexpressed in the same tissues, and it is likely that there is a functional basis for this coexpression.

One possibility as it concerns the coexpression of the EP₂ and EP₄ receptor subtypes might be related to a cell or tissue’s ability to respond to different concentrations of endogenous PGE₂. It has been clearly established that the binding affinity of PGE₂ is ~10- to 20-fold higher for the EP₄ receptor compared with the EP₂ receptor (Kiriyama et al., 1997; Abramovitz et al., 2000; Fujino et al., 2002). Furthermore, this difference in affinity is reflected in functional measures of the activation of these receptors. For example, in one detailed study of the functional pharmacology of the human EP₂ and EP₄ receptor subtypes, the EC₅₀ for the stimulation of cAMP formation in cells expressing the EP₄ receptor was ~0.05 nM, whereas for the EP₂ receptor, it was ~30 nM (Wilson et al., 2004). Thus, cells expressing the EP₄ receptor are able to respond to lower concentrations of endogenous PGE₂. In addition, the pattern of intracellular signaling in cells expressing the EP₄ receptor will include the activation of both the G_{s} and G_{α_{i}} pathways. The activation of a G_{α_{i}} signaling pathway by the EP₄ receptor provides an interesting potential mechanism for further amplification of the initial PGE₂ signal. As demonstrated in the present study, the EP₄ receptor-mediated activation of G_{α_{i}} signaling leads to the activation of the ERKs and induction of EGR-1 expression. It has been shown that EGR-1 can induce the expression of PGE₂ synthase (Naraba et al., 2002), which could be expected to increase the biosynthesis of PGE₂, perhaps to a level that would initiate the activation of EP₂ (and EP₁) receptors. This amplification of PGE₂ signaling would take place only in tissues or cells that express the EP₄ receptor subtype and would represent a mechanism for generating a differential response to low levels of endogenous PGE₂. PGE₂ is produced at low levels by a large number of cell types, and under various physiological and pathophysiological conditions, its biosynthesis is dramatically increased. This increase in PGE₂ biosynthesis is frequently correlated with the induction of COX-2, but the conditions and factors that regulate these events are unclear. Invasion of tissues by macrophages and up-regulation of their EP₂ receptors, which has been shown to occur in a mouse model of autoimmune inflammation (Akaogi et al., 2004), or up-regulation of EP₄ receptors by resident dendritic cells (Harizi et al., 2003), could provide a potential mechanism for inducing COX-2 and PGE₂ synthase expression and increasing the biosynthesis of PGE₂.

The present study further emphasizes the differences in the signaling potential of the EP₂ and EP₄ receptors and clarifies the mechanism of the activation of the PI3K and ERK signaling pathways by the EP₄ receptor. Thus far, human EP₂ prostanoid receptors seem to be exclusively coupled to G_{α_{s}} and stimulation of these receptors by PGE₂ leads to a strong activation of the cAMP/PKA signaling pathway. On the other hand, PGE₂ stimulation of human EP₄ prostanoid receptors results in the activation of both G_{α_{i}} and G_{α_{s}}. Compared with the EP₂ receptor, the activation of the cAMP/PKA signaling pathway by the EP₄ receptor is significantly less, which is a consequence of two mechanisms. The first is that activation of G_{α_{s}} probably results in a direct inhibition of adenyl cyclase, which offsets the stimulation of adenyl cyclase through G_{s}. The second is that PGE₂-mediated activation of PI3K signaling by the EP₂ receptor inhibits the activity of PKA (Fujino et al., 2005). A similar inhibition of PKA activity has been reported after the activation of PI3K signaling by the β₂-adrenergic receptor (Jo et al., 2002). It is significant to note that even in the presence of PTX, the maximal cAMP response elicited by PGE₂ stimulation of the EP₂ receptor was less than that obtained with the EP₂ receptor (Fig. 1). This indicates that the efficiency of EP₂ receptor coupling to G_{α_{i}}-mediated signaling is less than that of the EP₂ receptor even in the absence of the activation of G_{α_{s}}-mediated signaling.

The G_{α_{s}}-mediated activation of PI3K signaling further differentiates the signaling properties of the EP₂ receptor compared with the EP₄ receptor. Thus, we have shown previously that PGE₂ stimulation of the EP₂ receptor leads to the PI3K-dependent activation of ERK signaling pathways, which is not observed after PGE₂ stimulation of the EP₂ receptor (Fujino et al., 2003). Despite these differences, some of the downstream signaling consequences after PGE₂ stimulation of the EP₂ or EP₄ receptors seem to be quite similar. For example, PGE₂ stimulation of either receptor leads to an increase Tcf transcriptional activation (Fujino et al., 2002) and in the phosphorylation of the cAMP response element binding protein (Fujino et al., 2005). However, the increase in Tcf transcription activation and cAMP response element binding protein phosphorylation by the EP₂ receptor is mainly through a PI3K-dependent mechanism, whereas for the EP₂ receptor, it is mainly through a cAMP/PKA-dependent pathway. This means that the regulation of EP₂ and EP₄ receptor signaling by cross-talk through the activation of other of types of receptors has the potential to be quite different. For example, receptors whose activation can modulate PI3K signaling will have greater potential to influence signaling mediated by the EP₄ receptor as opposed to that mediated by the EP₂ receptor.

In summary, we have shown that human EP₂ receptors, but not EP₄ receptors, can couple to PTX-sensitive G-proteins when expressed heterologously in HEK cells. Coupling of EP₂ receptors to PTX-sensitive G-proteins decreases PGE₂-mediated cAMP accumulation, suggesting specific coupling to G_{α_{i}} rather than G_{α_{s}}. The activation of PI3K signaling by the EP₂ receptor probably occurs through the release of G_{βγ} subunits after coupling of the receptor to G_{α_{s}}. We have discussed studies showing that PGE₂ stimulation of endogenous EP₂ receptors in native cell systems can activate PI3K and ERK signaling by mechanisms that seem to be independent of coupling to G_{α_{i}}. These findings suggest the coupling of en-
dogenous EP$_4$ receptors to Go$_q$, but clearly this will need to be further investigated. In fact, we do not believe that EP$_4$ receptors will be shown to have universal coupling to Go$_q$ and PI3K/ERK signaling. For example, in an elegant study of prostanoid receptor-mediated signaling in human airway smooth muscle cells, Clarke et al. (2005) found that the effects of EP$_4$ receptor stimulation could be explained solely by activation of a cAMP/PKA-dependent pathway. We speculate that the specific signaling pathways used by more "promiscuous" GPCRs, such as EP$_2$ and $\beta_3$-adrenergic receptors, will be very cell-type-dependent compared with more dedicated "monogamous" receptors, such as EP$_2$ and $\beta_1$-adrenergic receptors.

References


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