Phosphorylation of Glycogen Synthase Kinase-3 and Stimulation of T-cell Factor Signaling following Activation of EP₂ and EP₄ Prostanoid Receptors by Prostaglandin E₂*

Hiromichi Fujino, Kimberly A. West, and John W. Regan‡

From the Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona 85721-0207

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Recently we have shown that the FP₁ prostanoid receptor, a G-protein-coupled receptor that couples to Goαq, activates T-cell factor (Tcf)/lymphoid enhancer factor (Lef)-mediated transcriptional activation (Fujino, H., and Regan, J. W. (2001) J. Biol. Chem. 276, 12489–12492). We now report that the EP₂ and EP₄ prostanoid receptors, which couple to Goαq, also activate Tcf/Lef signaling. By using a Tcf/Lef-responsive luciferase reporter gene, transcriptional activity was stimulated ~10-fold over basal by 1 h of treatment with progaglandin E₂ (PGE₂) in HEK cells that were stably transfected with the human EP₂ and EP₄ receptors. This stimulation of reporter gene activity was accompanied by a PGE₂-dependent increase in the phosphorylation of both glycogen synthase kinase-3 (GSK-3) and Akt kinase. H-89, an inhibitor of protein kinase A (PKA), completely blocked the agonist-dependent phosphorylation of GSK-3 in both EP₂- and EP₄-expressing cells. However, H-89 pretreatment only blocked PGE₂-stimulated Lef/Tcf reporter gene activity by 20% in EP₄-expressing cells compared with 65% inhibition in EP₂-expressing cells. On the other hand wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3 kinase), had the opposite effect and inhibited PGE₂-stimulated reporter gene activity by a similar degree in both EP₂- and EP₄-expressing cells. These findings indicate that the activation of Tcf/Lef signaling by EP₂ receptors occurs primarily through a PKA-dependent pathway, whereas EP₄ receptors activate Tcf/Lef signaling mainly through a phosphatidylinositol 3-kinase-dependent pathway. This is the first indication of a fundamental difference in the signaling potential of EP₂ and EP₄ prostanoid receptors.

An exciting connection is starting to emerge between the T-cell factor (Tcf)/β-catenin signaling pathway and G-protein-coupled receptors (GPCR). The potential for G-proteins to mediate this connection was suggested recently when it was shown that constitutively active Goα₁₂ and Goα₁₃ can interact with E-cadherin to cause the release of β-catenin and subsequent stimulation of Tcf/Lef lympoid enhancer factor (Lef) transcriptional activation (1). It has also been shown that a chimeric receptor constructed from the ligand binding and transmembrane domains of the β₂-adrenergic receptor and the cytoplasmic domains of rat Frizzled-1 can stimulate Tcf/Lef transcriptional activation through a mechanism that appears to involve signaling through Goαq and/or Goα₁ (2). The first example of the activation of this signaling pathway by a wild type GPCR and its cognate ligand was recently made when we demonstrated that progaglandin F₂α acting through the FP₁ prostanoid receptor could decrease the phosphorylation of cytoplastic β-catenin and stimulate Tcf/Lef-mediated transcriptional activation (3). Interestingly the FP₁ isoform, which only differs from the FP₁ by having an additional 46 amino acids in its carboxyl terminus, was nearly inactive with respect to activation of β-catenin/Tcf signaling even though both isoforms can stimulate inositol phosphate signaling to a similar degree (4, 5).

A key enzyme in the β-catenin/Tcf signaling pathway is glycogen synthase kinase-3 (GSK-3). This enzyme, which forms a complex with adenosomatous polyposis coli and axin, is responsible for the phosphorylation and subsequent degradation of cytosolic β-catenin. Direct inhibition of GSK-3 or disruption of the GSK-3-adenomatous polyposis coli-axin complex prevents the phosphorylation of cytoplastic β-catenin resulting in stabilization and translocation to the nucleus where it can alter gene expression through interactions with members of the Tcf/Lef family of transcriptional factors (6, 7). One well characterized mechanism for inhibiting the kinase activity of GSK-3 is through phosphorylation. For example stimulation of the frizzled receptor by the Wnt ligand leads to the phosphorylation and inhibition of GSK-3β and thereby promotes β-catenin/Tcf signaling (7). Similarly, activation of phosphatidylinositol 3-kinase (PI3 kinase) can result in the phosphorylation and activation of Akt kinase (also known as protein kinase B) which can then phosphorylate and inhibit GSK-3β. More recently, it has been found in vitro that cAMP-dependent protein kinase (PKA) can directly phosphorylate GSK-3β and inhibit its kinase activity (8, 9). In addition it is known that PKA can indirectly phosphorylate and activate Akt kinase, which could provide an indirect mechanism for the inhibition of GSK-3β by PKA (10).

Given the ability of PKA to inhibit the activity of GSK-3, and the well known regulation of cAMP formation by GPCRs, we were interested in the potential activation of Tcf/Lef transcriptional activation by the EP₂ and EP₄ prostanoid receptors. The EP₂ and EP₄ receptors are two of the four subtypes of receptors for prostaglandin E₂ (PGE₂) (11, 12). Both the EP₂ and EP₄ receptors are coupled to Goαq and can activate adenylyl cyclase with E-cadherin...
and increase intracellular cAMP formation. Prior to the molecular cloning of these receptors, it was thought that the stimulation of adenyl cyclase by PGE₂ was mediated by a single EP receptor subtype. Molecular cloning revealed, however, two receptor subtypes that were the products of separate genes (13). The EP₂ and EP₄ receptors encoded by these genes only shared ~30% amino acid homology even though they shared the same endogenous ligand and apparent second messenger pathway. We now show that stimulation of EP₂ receptors by PGE₂ can activate a Tcf/Lef signaling pathway by a mechanism that mainly involves the phosphorylation of GSK-3 by PKA. Stimulation of EP₄ receptors by PGE₂ can also activate a Tcf/Lef signaling pathway, but the mechanism is more complex and involves the activation of both PI3 kinase and PKA.

**EXPERIMENTAL PROCEDURES**

**Stable Transfectants**—Cell lines stably expressing the EP₂ or EP₄ receptors were prepared using HEK-293 EBNA cells (Invitrogen) and the mammalian expression vector pCPE4 (Invitrogen). Briefly, DNA sequences corresponding to the encoding regions of the human EP₂ receptor (13) and human EP₄ receptor (14) were subcloned into pCPE4, and 20 μg of each purified plasmid was used to transfect one 10-cm plate of EBNA cells. Selecting with hygromycin B, clonal expansion was done as described previously in detail (15) for the preparation of FP receptor-expressing cell lines. Clones expressing the human EP₂ and EP₄ receptor isoforms were identified based on immunofluorescence microscopy using EP₂ and EP₄ receptor-specific antibodies (16) and PGE₂-stimulated cAMP formation. Cell lines were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) containing 10% fetal bovine serum, 250 μg/ml geneticin, 100 μg/ml gentamicin, and 200 μg/ml hygromycin B.

**Whole Cell Radioligand Binding Assay**—Cells were cultured in 10-cm plates and were incubated for 1 h at 37 °C with final concentrations of 0.1% dimethyl sulfoxide (Me₂SO, vehicle) or 1 μM PGE₂. They were then trypsinized, centrifuged at 500 × g for 2 min, and resuspended at a concentration of 10⁶ cells/ml in ice-cold MES buffer consisting of 10 mM MES (pH 6.0), 0.4 mM EDTA, and 10 mM MnCl₂. [³H]PGE₂ binding was performed using 100 μl of sample added to a final assay volume of 200 μl containing 2.5 nM [³H]PGE₂ (Amersham Biosciences) or 2.5 nM [³H]PGE₂ plus increasing concentrations of unlabeled PGE₂. Samples were incubated for 1 h at room temperature and were filtered through Whatman GF/C glass filters to terminate the [³H]PGE₂ binding was performed using 100 μl of sample added to a final assay volume of 200 μl containing 2.5 nM [³H]PGE₂ (Amersham Biosciences) or 2.5 nM [³H]PGE₂, plus increasing concentrations of unlabeled PGE₂. Samples were incubated for 1 h at room temperature and were filtered through Whatman GF/C glass filters to terminate the incubation. Filters were then washed five times with ice-cold MES buffer, and radioactivity was measured by liquid scintillation counting.

**Results**

**EP₂ and EP₄ Receptor Expression and PGE₂-stimulated cAMP Formation**—HEK cells stably expressing the human EP₂ and EP₄ prostanoid receptors were prepared and used for the characterization of the signal transduction properties of these receptors. Fig. 1, panel A, shows the results for the competitive radioligand binding of [³H]PGE₂ to untransfected HEK cells or to HEK cells stably transfected with either the human EP₂ or human EP₄ receptors. In the absence of pretreatment with PGE₂ the EP₂ and EP₄ receptor-transfected cell lines showed similar maximal levels of specific [³H]PGE₂ binding that was more than 100 times the amount of specific [³H]PGE₂ binding to untransfected HEK cells (HEK, 0.86 ± 0.39 fmol/mg protein; EP₂, 122.60 ± 15.90 fmol/mg protein; EP₄, 112.37 ± 5.57 fmol/mg protein). Although the EP₂ and EP₄ receptor-transfected cells showed similar levels of maximal specific binding, their affinity for PGE₂ differed. Thus, EP₂ receptor-transfected cells had 7-fold greater affinity for PGE₂ (EC₅₀, 2.8 ± 1.6 nM) as compared with EP₄ receptor-transfected cells (EC₅₀, 19 ± 7.5 nM). Pretreatment of the cells with 1 μM PGE₂ for 1 h followed by wash-out decreased the whole cell specific binding of [³H]PGE₂ by 66% in EP₂ receptor-transfected cells but only decreased binding by 34% in EP₄ receptor-transfected cells. EP₂ and EP₄ receptors are Gα₃-coupled receptors and are known to activate adenyl cyclase; therefore, the ability of PGE₂ to stimulate cAMP formation was examined in these cells. As shown in Fig. 1, panel B, treatment of untransfected HEK cells with 1 μM PGE₂ for 1 h had negligible effects on cAMP formation as compared with vehicle; however, in EP₂ receptor-transfected cells there was a 71-fold stimulation and in EP₄ receptor-transfected cells a 10-fold stimulation of cAMP formation following treatment with PGE₂ as compared with the vehicle control. It is notable that the maximal levels of cAMP formation for the EP₂ receptor-transfected cells are so much lower as compared with the EP₂ receptor-transfected cells even though both receptors are expressed...
of GSK-3 and Akt have recently been shown to be regulated following in vitro phosphorylation by PKA (8–10). To explore the signaling potential between these kinases and the activation of adenyl cyclase stimulatory GPCRs, we examined the PGE₂-dependent phosphorylation of GSK-3 and Akt in untransfected HEK cells and in HEK cells transfected with the human EP₂ and EP₄ prostanoid receptors. For these experiments (Fig. 2) cells were treated with 1 μM PGE₂ for the times indicated and were then lysed, subjected to SDS-PAGE, and immunoblotted with antibodies that specifically recognized either GSK-3β or Akt, the phosphorylated forms of GSK-3 (pGSK-3α; pGSK-3β), or the phosphorylated form of Akt (pAkt). As shown in Fig. 2, panel A, in both EP₂ and EP₄ receptor-transfected cells GSK-3α was phosphorylated within 5 min following exposure to PGE₂ and remained phosphorylated for 60 min. The control HEK cells also showed some GSK-3α-dependent phosphorylation of GSK-3α, but it was considerably weaker and may reflect the small amount of specific [³H]PGE₂ binding that is present. Densitometric analysis of the phosphorylation of GSK-3α at 60 min, compared with time 0, showed a 7-fold increase in EP₂ receptor-transfected cells and a 4.5-fold increase in EP₄ receptor-transfected cells. To ensure equal loading of proteins, the blots shown in panel C were stripped and re-probed with antibodies to GSK-3β, and as shown in panel B nearly identical amounts of GSK-3β were present throughout the time course of treatment and among the three cell lines. Panel C shows the results obtained using antibodies directed against phospho-Akt. In all the cell lines there was a detectable level of phospho-Akt present at the zero time point, and in both the EP₂ and EP₄ receptor-transfected cell lines there was an increase in Akt phosphorylation after 60 min of treatment with PGE₂. Densitometric analysis showed this to be ~2-fold for both EP₂ and EP₄ receptor-transfected cells. To ensure equal loading of proteins, the blots shown in panel C were stripped and re-probed with antibodies to Akt, and as shown in panel D similar amounts of Akt were present throughout the time course.

PGE₂ Stimulation of Tcf/Lef Reporter Gene Activity in EP₂ and EP₄ Receptor-transfected Cells, Differential Effects of H-89 and Wortmannin on This and on PGE₂-stimulated Phosphorylation of GSK-3 and Akt—In Fig. 2 we showed that the stimulation of EP₂ and EP₄ receptors by PGE₂ resulted in increased phosphorylation of GSK-3α and Akt. Given that both of these receptors couple to Gαs and are known to activate cAMP/PKA signaling pathways, we decided to examine the effects of H-89, an inhibitor of PKA, on the PGE₂-stimulated phosphorylation of GSK-3 and Akt in EP₂ and EP₄ receptor-transfected cells. In addition, because phosphorylation of GSK-3 is known to stabilize β-catenin and promote Tcf/Lef-mediated transcriptional activation, we examined the potential of PGE₂ to stimulate the luciferase activity in EP₂ and EP₄ receptor-transfected cells using a Tcf/Lef-responsive luciferase reporter gene. For these experiments the cell lines were pretreated with either vehicle or 10 μM H-89 for 15 min followed by treatment with either vehicle or 1 μM PGE₂ for 1 h. The upper part of Fig. 3 shows the results of immunoblot analysis that was done in the same manner as described for Fig. 2. Fig. 3, panel A, shows that following pretreatment of EP₂ and EP₄ receptor-transfected cells with H-89 there was a complete block of PGE₂-stimulated phosphorylation of GSK-3α, suggesting the direct involvement of PKA in this process. There was also a notable decrease in the phosphorylation of GSK-3β following H-89 treatment in all the cell lines. On the other hand, Fig. 3, panel C, shows that H-89 pretreatment increased the phosphorylation of Akt and actually enhanced the PGE₂-stimulated phosphorylation of Akt in all the cell lines. The bottom part of Fig. 3 shows PGE₂-stimu-
cells over the vehicle-treated controls. After pretreatment with H-89, however, PGE2-stimulated luciferase activity was decreased by 65% in EP2 receptor-transfected cells but was only decreased by 20% in EP4 receptor-transfected cells. Therefore, H-89 inhibited PGE2-stimulated Tcf/Lef reporter gene activity much more effectively in EP2 receptor-transfected cells as compared with EP4 receptor-transfected cells, even though it was equally effective at blocking GSK-3α phosphorylation in both cell lines.

The results shown in Figs. 2 and 3 indicate that the phosphorylation of Akt following PGE2 treatment of EP2 and EP4 receptor-expressing cells is not a direct effect of PKA and suggest the involvement of additional kinases. One such candidate is P13 kinase because Akt is known to have roles both in the phosphorylation of GSK-3β and as a substrate for P13 kinase. Therefore, we examined the effects of wortmannin, an inhibitor of P13 kinase, on the PGE2-stimulated phosphorylation of GSK-3 and Akt and on the PGE2 stimulation of Tcf/Lef reporter gene activity in EP2 and EP4 receptor-transfected cells. For these experiments the cells were treated with either vehicle or 100 nM wortmannin for 15 min followed by treatment with either vehicle or 1 μM PGE2 for 1 h. The upper part of Fig. 4 shows the results of immunoblot analysis that was done in the same manner as described in Figs. 2 and 3. Fig. 4, panel A, shows that wortmannin pretreatment decreased the phosphorylation of GSK-3α in the vehicle-treated cells and produced a marked 62% inhibition of PGE2-stimulated GSK-3α phosphorylation in the EP2 receptor-transfected cells, but only a modest 14% inhibition in the EP4 receptor-transfected cells. In addition wortmannin pretreatment inhibited the phosphorylation of GSK-3β in all the cell lines and, most interestingly, revealed a clear PGE2-dependent stimulation of GSK-3β phosphorylation in both the EP2 and EP4 receptor-transfected cells. Fig. 4, panel C, shows that wortmannin pretreatment abolished both the basal and PGE2-stimulated phosphorylation of Akt in all the cell lines. The bottom part of Fig. 4 shows PGE2-stimulated Tcf/Lef luciferase reporter gene activity in untreated cells and in cells that were pretreated with 100 nM wortmannin as above. The data for untreated cells are the same as that shown in Fig. 3 and shows the robust PGE2 stimulation of luciferase activity in both the EP2 and EP4 receptor-transfected cells. In a clear distinction from the results obtained with H-89, however, pretreatment with wortmannin produced a significantly greater inhibition of PGE2-stimulated Tcf/Lef reporter luciferase activity in EP2 receptor-transfected cells (61%) as compared with the inhibition obtained in EP4 receptor-transfected cells (27%). These findings suggest a significant P13 kinase-mediated con-
Akt (7, 8). Immunoblotting results are presented in Fig. 2 and represent the immunoblotting of representative three experiments with each antibody and condition. Representative experiment that was repeated three times. Note, the reporter gene experiments shown in this figure and Fig. 3 were done simultaneously; therefore, the luciferase activity data for cells that were not pretreated with either H-89 or wortmannin are the same in both figures.

The effects of wortmannin on PGE2-stimulated phosphorylation of GSK-3 and Akt (immunoblots, panels A–D) and on stimulation of Tcf/Lef reporter gene activity (histograph) in untransfected HEK cells or HEK cells transfected with either the EP2 or EP4 prostanoid receptors. Cells were pretreated with either vehicle or 100 nM wortmannin for 15 min followed by either vehicle (v) or 1 μM PGE2 (P) for 1 h at 37 °C and were then immediately subjected to immunoblot analysis or were washed to remove PGE2 and incubated for 16 h after which luciferase activity was measured as described under “Experimental Procedures.” Panels A–D are exactly as described in Fig. 2 and represent the immunostaining of phospho-GSK-3α and -3β (panel A), total GSK-3β (panel B), phospho-Akt (panel C), and total Akt (panel D). Immunoblotting results are representative of three experiments with each antibody and condition. Luciferase data are the means ± S.E. of two measurements from a representative experiment that was repeated three times. Note, the reporter gene experiments shown in this figure and Fig. 3 were done simultaneously; therefore, the luciferase activity data for cells that were not pretreated with either H-89 or wortmannin are the same in both figures.

The EP2 and EP4 prostanoid receptors are GPCRs that are linked to the stimulation of cAMP/PKA signaling through the sequential activation of Goα and adenylyl cyclase. PGE2 is the endogenous ligand for both of these receptors and the fact that these receptors represented two unique subtypes were not fully appreciated until the molecular cloning of the EP2 receptor in 1994 (13). Comparison of the pharmacology of this receptor with a previously cloned adenyl cyclase stimulatory EP receptor led to recognition of the EP2 subtype (7, 8). Structurally, these receptors have less in common than one might think given their similarities with respect to ligand binding and second messenger coupling. The EP4 receptor is bigger, 488 amino acids versus 358, most of which is accounted for by a significantly longer carboxyl-terminal domain, 155 amino acids versus 34.

It is of considerable interest to understand the physiological and/or pathophysiological significance of the EP2 and EP4 prostanoid receptors. Nishigaki et al. (18) have found that these subtypes differ with respect to agonist-mediated desensitization. Thus, when transfected into Chinese hamster ovary cells the EP4 subtype underwent short term desensitization in response to treatment with PGE2, whereas the EP2 receptor did not. Related to this, Desai et al. (19) found that when transfected into HEK cells the EP4 receptor underwent rapid agonist-mediated internalization, and again, the EP2 did not. In the present study we have also found that the EP2 receptor subtype is much more sensitive to the regulatory effects of agonist exposure and that pretreatment with 1 μM PGE2 for 1 h decreased EP4 receptor number by ~70%, but only decreased EP2 receptor number by ~30%. Rapid desensitization may also partially account for the markedly lower amount of agonist-stimulated cAMP formation in EP4 receptor-transfected cells as compared with EP2 receptor-transfected cells. Thus, the maximal level of PGE2-stimulated cAMP formation in EP4 receptor-transfected cells was only ~20% that achieved in EP2 receptor-transfected cells, even though both receptors were expressed to nearly the same extent prior to agonist pretreatment. However, more rapid desensitization of the EP2 receptor is not the only explanation for its lower stimulation of cAMP formation. It is plausible that EP4 receptors are less efficiently coupled to adenylyl cyclase and/or they have additional pathways of signal transduction that do not involve the activation of cAMP/PKA signaling.

A major signaling pathway, which until recently was thought to be relatively unaffected by events in the cAMP/PKA pathway, is the Wnt signaling pathway. As reviewed in the Introduction, an important control point in this pathway involves the phosphorylation of GSK-3 which can serve to inhibit its kinase activity and promote β-catenin stabilization and Tcf/Lef transcriptional activation. There are two isoforms of GSK-3 designated as GSK-3α (51 kDa) and GSK-3β (47 kDa). At the amino acid level they share 85% homology, and both are phosphorylated by Akt as a consequence of Wnt signaling, at serine 9 in GSK-3β and at serine 21 in GSK-3α (20). PKA has been shown recently (8, 9) to phosphorylate GSK-3β and GSK-3α at these same positions leading to the possibility of cross-talk between the Wnt and cAMP/PKA signaling pathways. A second mechanism for such cross-talk has been described in which PKA can indirectly activate Akt resulting in the phosphorylation and inhibition of GSK-3 (10). In our studies we have shown that the activation of the adenylyl cyclase stimulatory EP2 and EP4 prostanoid receptors leads to a rapid (within 5 min) agonist-dependent phosphorylation of GSK-3α, but it actually enhanced the phosphorylation of Akt, suggesting that the phosphorylation of GSK-3α is mediated directly by PKA, whereas the phosphorylation of Akt is mediated by another kinase that is negatively regulated by PKA. This other kinase is likely to be PI3 kinase, which is corroborated by our finding that wortmannin, an inhibitor of PI3 kinase, completely blocked the agonist-dependent phosphorylation of Akt.

Given the effects of EP2 and EP4 receptor activation on the phosphorylation of GSK-3α, it is not surprising that we observed a stimulation of Tcf/Lef reporter gene activity following the incubation of these receptors with PGE2. What is surprising, however, is that the maximal stimulation of reporter gene activity is the same, or even higher, for the EP1 receptor as compared with the EP2 receptor, even though the EP2 receptor only yielded ~20% of the maximal amount of cAMP formation as that obtained with the EP2 receptor. Furthermore, PGE2-stimulated phosphorylation of GSK-3α in EP2 receptor-transfected cells was approximately twice that obtained in EP4 re-
Receptor-transfected cells, suggesting that the stimulation of Tcf/Lef reporter gene activity should have been significantly greater for the EP2 receptor. It is very relevant, therefore, that H-89 only inhibited PGE2-stimulated reporter gene activity by ~20% in EP2 receptor-transfected cells in contrast to the 65% inhibition obtained in EP4 receptor-transfected cells. Given the similar maximal stimulation of reporter gene activity by these receptors, the 20% inhibition of activity obtained with H-89 for the EP2 receptor is exactly as one would predict based upon the relative ability of these receptors to stimulate cAMP formation and strongly suggests that stimulation of Tcf/Lef reporter gene activity by the EP4 receptor involves an additional signaling pathway.

As suggested above, based upon the inhibition of Akt phosphorylation by wortmannin, this additional pathway appears to involve PI3 kinase. This premise is also supported by the differential effects of wortmannin on PGE2-stimulated Tcf/Lef reporter gene activity. Thus, in contrast to the results obtained with H-89, wortmannin had nearly the opposite effect and inhibited agonist-stimulated reporter gene activity to a much greater extent in EP4 receptor-transfected cells than in EP2 receptor-transfected cells. The putative involvement of PI3 kinase with EP2 receptor signaling is further supported by the more obvious time course and extent of phosphorylation of Akt following the treatment of EP4 receptor-transfected cells with PGE2 (cf. Fig. 2). Therefore, although the phosphorylation of GSK-3α by activation of EP2 receptors is entirely dependent on cAMP/PKA, the stimulation of Tcf/Lef reporter gene activity primarily involves activation of PI3 kinase and Akt. In contrast, both the phosphorylation of GSK-3α and the stimulation of reporter gene activity by EP2 receptor activation are primarily dependent on cAMP/PKA.

One apparent discrepancy in our data, with respect to the putative involvement of PI3 kinase and Akt in the stimulation of Tcf/Lef reporter gene activity by the activation of EP2 receptors, is that we did not observe enhanced phosphorylation of either GSK-3α or GSK-3β following pretreatment of EP2-expressing cells with H-89 (cf. Fig. 3). Thus, it would be reasonable to expect that one of these isoforms would show increased phosphorylation given the observed increase in Tcf/Lef reporter gene activity. This apparent discrepancy may be explained, however, by the 16-h time differential between the measurement of GSK-3 phosphorylation and the assay of luciferase reporter gene activity. Thus, GSK-3 phosphorylation is measured immediately after the 1-h incubation with PGE2, whereas the reporter gene activity is measured 16 h after PGE2 treatment and washout. (Attempts to measure luciferase activity following the treatment of EP4 receptor-transfected cells with PGE2 (cf. Fig. 2). Therefore, although the phosphorylation of GSK-3α by activation of EP2 receptors is entirely dependent on cAMP/PKA, the stimulation of Tcf/Lef reporter gene activity primarily involves activation of PI3 kinase and Akt. In contrast, both the phosphorylation of GSK-3α and the stimulation of reporter gene activity by EP2 receptor activation are primarily dependent on cAMP/PKA.

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Phosphorylation of Glycogen Synthase Kinase-3 and Stimulation of T-cell Factor Signaling following Activation of EP$_2$ and EP$_4$ Prostanoid Receptors by Prostaglandin E$_2$

Hiromichi Fujino, Kimberly A. West and John W. Regan

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