Prostaglandin E receptor EP3γ isoform, with mostly full constitutive Gi activity and agonist-dependent Gs activity

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Abstract We recently demonstrated that two exclusively Gi-coupled isoforms of the mouse EP3 receptor, EP3α and β, with different carboxyl-terminal tails, differed in agonist-independent constitutive Gi activity, and the carboxyl-terminal tail-truncated receptor showed full constitutive activity (Hasegawa, H., Negishi, M., and Ichikawa, A. (1996) J. Biol. Chem. 271, 1857–1860). Here we further examined Gi and Gs activities of the third isoform, EP3γ, coupled to both Gi and Gs. The EP3γ receptor showed mostly full constitutive Gi activity and agonist-dependent Gs activity. The truncated receptor also showed agonist-dependent Gs activity, but the level was lower than that of the EP3γ receptor. Thus, the carboxyl-terminal tail would differentially regulate Gi and Gs activities of the EP3 receptor.

Key words: Prostaglandin E_2; G protein; Constitutive activity

1. Introduction

Prostaglandin E_2 (PGE_2) produces a broad range of biological actions in diverse tissues through its binding to specific receptors on plasma membranes [1]. PGE receptors are pharmacologically subdivided into four subtypes, EP1, EP2, EP3, and EP4, on the basis of their responses to various agonists and antagonists [2,3]. Among these subtypes, the EP3 receptor has been best characterized; it has been suggested to be involved in such PGE_2 actions as contraction of the uterus [4], inhibition of gastric acid secretion [5], modulation of the neurotransmitter release [6], lipolysis in adipose tissue [7], and sodium and water reabsorption in the kidney tubules [8]. Various EP3 receptor-mediated actions are mediated through multiple signal transduction systems, and in addition, the dose-response curve and potency of PGE_2 vary with tissue, implying heterogeneity of EP3 receptors [9,10].

We have cloned the mouse EP3 receptor and demonstrated that this receptor is a G protein-coupled rhodopsin-type receptor that engages in inhibition of adenylate cyclase [11]. Furthermore, we identified two isoforms of the mouse EP3 receptor, EP3α and EP3β, with a different COOH-terminal tail, which were produced through alternative splicing and differed in agonist-independent constitutive Gi activity; the EP3α receptor showed marked agonist-independent Gi activity, while the EP3β receptor had no constitutive activity [12,13]. To assess the role of the COOH-terminal tails of the EP3 receptor in coupling to G proteins, we constructed a mutated EP3 receptor, T-335, in which the COOH-terminal tail was truncated at the alternative splicing site, and revealed that the truncated receptor showed only agonist-independent constitutive Gi activity, suggesting that the COOH-terminal tails after the alternative splicing site suppress activation of Gi by the EP3 receptor [13]. In addition to EP3α and β, which are exclusively coupled to Gi, we further identified a third isoform, EP3γ, which was also produced through alternative splicing and differed in the COOH-terminal tail from the other two isoforms [14]. However, the EP3γ receptor was coupled to both Gi and Gs, suggesting that the COOH-terminal tails of the EP3 receptor play an important role in the specificity of G protein coupling. To assess the role of the COOH-terminal tail of the EP3γ receptor in G protein coupling, we studied in more detail Gi and Gs activities of the EP3γ and truncated receptors. We report here that the EP3γ receptor showed mostly full constitutive Gi activity and the truncated receptor had agonist-dependent Gs activity.

2. Materials and methods

2.1. Materials

Sulprostone and TEI-3356 were generous gifts from Dr. K.-H. Thierauch of Schering and Dr. S. Kurozumi of Teijin Ltd., respectively. The ^{125}I-labeled cAMP assay system was obtained from Amersham Corp; pertussis toxin (PT) was from Seikagaku Kogyo (Tokyo, Japan); forskolin was from Sigma.

Chinese hamster ovary (CHO) cells stably expressing the EP3α, EP3β [12], EP3γ [14], or the truncated receptor, T-335 [15], were cultured in the α-modification of Eagle's medium lacking ribonucleosides and deoxyribonucleosides, with 10% dialyzed fetal bovine serum under humidified air containing 5% CO_2 at 37°C.

2.2. Measurement of cAMP formation

Cyclic AMP levels in CHO cells were determined as reported previously [16]. The receptor-expressing CHO cells cultured in 24-well plates (5×10^5 cells/well) were washed with HEPES-buffered saline containing 140 mM NaCl, 4.7 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 11 mM glucose, and 15 mM HEPES, pH 7.4, and preincubated for 5 min. Reactions were started by the addition of test agents along with 100 μM Ro-20-1724. After incubation for 10 min at 37°C, reactions were terminated by the addition of 10% trichloroacetic acid. The content of cAMP in the cells was measured by radioimmunoassay with an Amersham cAMP assay system.

3. Results and discussion

The EP3γ receptor is coupled to both Gi and Gs. We have recently demonstrated that the interaction of the carboxylic acid of PGE_2 and arginine residue of the seventh transmembrane domain of EP3 receptor was not essential for Gi coupling but was necessary for Gs coupling, and sulprostone, an EP3 agonist having the methanesulfonamide-modified carboxylic acid, induced preferential coupling of the EP3 recep-

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Abbreviations: PG, prostaglandin; G protein, heterotrimeric GTP-binding protein; CHO, Chinese hamster ovary; PT, pertussis toxin; COOH, carboxyl

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tor to Gi [17]. To determine the Gi activity of the EP3γ receptor, we used sulprostone as an agonist. We tested the ability of the EP3γ receptor to inhibit the forskolin-activated adenylate cyclase in the presence or absence of sulprostone, and compared the ability with those of the EP3α, EP3β, and the truncated receptor, T-335 (Fig. 1). In the absence of the agonist, forskolin increased the intracellular cAMP levels in the order of EP3β>EP3α>EP3γ>T-335. The EP3γ receptor showed the lowest level among the three isoforms, the level being close to that in the T-335 receptor. Sulprostone concentration-dependently inhibited the forskolin-stimulated cAMP formation in the three EP3 receptor isoforms. The IC_{50} value for the inhibition and the maximally reduced cAMP level decreased in the order of the EP3γ (0.03 nM)>EP3α (0.1 nM)>EP3β (30 nM). The EP3γ receptor had the lowest IC_{50} value and the lowest cAMP level among the three isoforms.

To assess the constitutive activity of the EP3γ receptor, we examined the effect of PT on the Gi activity of the receptor. As shown in Fig. 2A, PT treatment concentration-dependently increased the forskolin-stimulated cAMP formation in the absence of the agonist and maximally elevated at the level about 4-fold over the level without treatment at 100 ng/ml. PT treatment attenuated the sulprostone-induced inhibition of the forskolin-stimulated cAMP formation, the cAMP level reaching the same value as that in the absence of the agonist. This indicates that most of the EP3γ receptor is constitutively active receptor. We further compared the effect of PT on the agonist-dependent or independent Gi activity of the EP3γ receptor and the other receptors. As shown in Fig. 2B, PT treatment increased the forskolin-stimulated cAMP formation in the absence of the agonist in the EP3α, EP3γ and T-335 receptors to similar levels, and attenuated the sulprostone-induced inhibition in the EP3α and EP3γ receptors. On the other hand, no PT treatment-induced increase in forskolin-stimulated cAMP formation in the absence of the agonist was observed with the EP3β receptor, and this treatment rather suppressed the level, as reported previously [13]. This suppression by PT was also observed in the mock-transfected cells, indicating that the suppression is not due to the effect on the receptor function [13]. Thus, the constitutive Gi activity increased in the order of T-335>EP3γ>EP3α>EP3β, and the EP3γ receptor showed the highest constitutive Gi activity among the three isoforms.

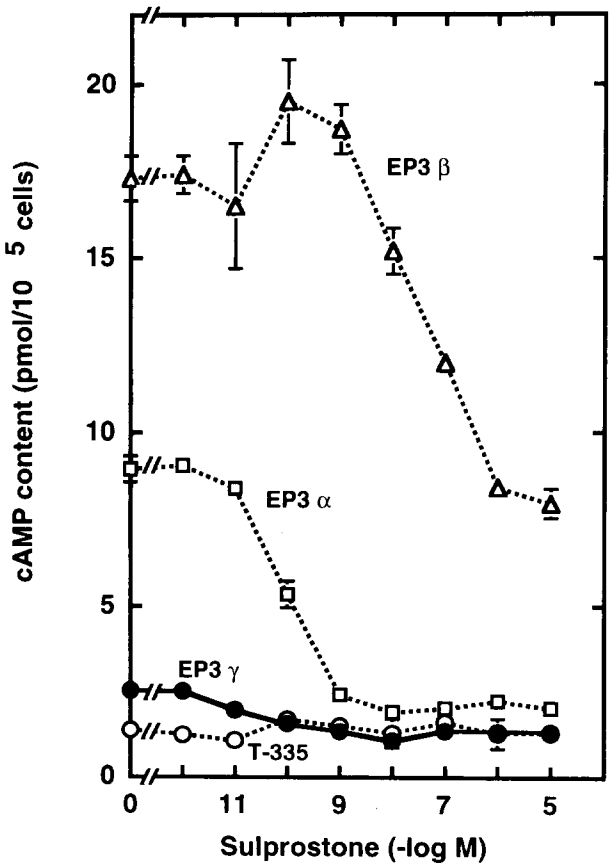

Fig. 1. Inhibition of adenylate cyclase by the three EP3 receptor isoforms and T-335 receptor. CHO cells expressing EP3α (□), EP3β (△), EP3γ (●), or T-335 receptor (○) were incubated at 37°C for 10 min with 10 μM forskolin in the presence or absence of the indicated concentrations of sulprostone, then cAMP contents were determined as described in section 2. The results shown are the means ± S.E.M. for triplicate determinations.

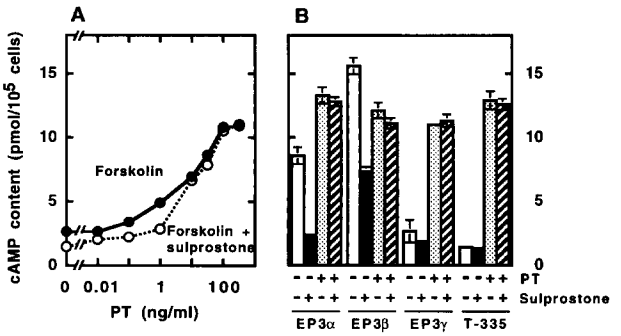

Fig. 2. Effects of PT treatment on agonist-dependent or -independent inhibition of adenylate cyclase by the EP3 receptor isoforms and T-335 receptor. A: After cells expressing the EP3γ receptor had been treated with the indicated concentrations of PT for 5 h, they were incubated at 37°C for 10 min with 10 μM forskolin in the absence (●) or presence (○) of 0.1 μM sulprostone, then cAMP contents were determined as described in Section 2. B: After cells expressing EP3α, EP3β, EP3γ, or T-335 receptor had been treated with (dotted bars, hatched bars) or without (open bars, black bars) 10 ng/ml PT for 5 h, they were incubated at 37°C for 10 min with 10 μM forskolin in the absence (open bars, dotted bars) or presence (black bars, hatched bars) of 0.1 μM sulprostone, then cAMP contents were determined as described in section 2. The results shown are the means ± S.E.M. for triplicate determinations.

Lefkowitz and co-workers have proposed a two-state model in which receptors are in equilibrium between the inactive conformation and the active conformation that can associate and activate G protein [18]. Receptors mostly have the inactive conformation in the absence of ligands and prevent their G protein activation domains from association with G proteins. Classical agonists release the constraint and increase the concentration of the active conformation of the receptors [18]. In this regard, the EP3γ receptor is a novel receptor, displacing the equilibrium towards the active conformation in the absence of ligands. The EP3γ receptor is the first example of native receptor with mostly full constitutive activity. We previously showed that the EP3γ receptor was expressed in a variety of tissues [14]. The expression of the receptor would induce sustained inhibition of adenylate cyclase, and the expression level would regulate the cAMP response. The three EP3 receptor isoforms differ only in the COOH-terminal tail, showing different constitutive activity, and the truncated receptor exhibits full agonist-independent constitutive activity.

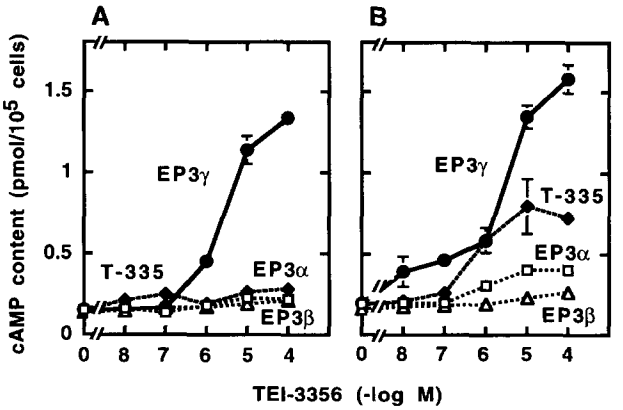

Fig. 3. Stimulation of adenylate cyclase by the three EP3 receptor isoforms and T-335 receptor. After cells expressing EP3α (□), EP3β (△), EP3γ (●), or T-335 receptor (◆) had been treated with (B) or without (A) 10 ng/ml PT for 5 h, they were incubated at 37°C for 10 min with the indicated concentrations of TEI-3356, then cAMP contents were determined as described in section 2. The results shown are the means ± S.E.M. for triplicate determinations.

This suggests that the COOH-terminal tails after the alternative splicing site suppress activation of Gi by the EP3 receptor to different extents dependent on the structure of the COOH-terminal tail, and the suppression by the tail of the EP3γ receptor may be very low. In addition to constitutive Gi activity, the three isoforms differed in IC_{50} value for the agonist-mediated inhibition and the maximal inhibition response (Fig. 1). According to the two-state model of receptor activation, the two parameters, IC_{50} and maximal response, are dependent on the level of constitutive activity of receptor. As the percentage of constitutive activity increases, the value of IC_{50} decreases and the level of maximal response increases [19]. Variation of the two parameters of three EP3 receptor isoforms are consistent with the diversity of constitutive Gi activities of the receptors. The EP3 receptor isoforms with different constitutive activity produce diverse agonist sensitivity. The human EP3 receptor has been reported to have several isoforms, which were also produced through alternative splicing and differed only in the COOH-terminal tail, and they showed Gi activity but the levels of forskolin-stimulated cAMP formation in the absence of agonist varied among the isoforms [20]. These findings suggest that EP3 receptor isoforms differ in constitutive Gi activity, and the structures of the COOH-terminal tail add the receptor variation of constitutive Gi activity.

To assess the role of the COOH-terminal tails in the coupling of the EP3 receptor to Gs, we next examined the Gs activities of the three EP3 receptor isoforms and T-335 receptor. CHO cells slightly expressed the PGE receptor EP4 subtype, which is coupled to Gs, and the cells produced a significant amount of cAMP in response to PGE_2 or agonists cross-reactive to the EP4 subtype (data not shown). We recently found that TEI-3356 was a highly selective agonist for subtype EP3, and this agonist did not increase the cAMP level of mock-transfected CHO cells by 100 μM [21]. Thus, we chose TEI-3356 as an agonist for determination of EP3 receptor-mediated stimulation of cAMP formation. In cells not treated with PT, TEI-3356 did not affect the basal cAMP level in the EP3α-, EP3β-, or T-335-expressing cells, but the agonist concentration-dependently increased the cAMP level in the EP3γ-expressing cells (Fig. 3A). To ablate receptor coupling to Gi, the cells were treated with PT, and we examined the Gs activities of the receptors. As shown in Fig. 3B, TEI-3356 concentration-dependently increased the cAMP level in the cells expressing the three isoforms and the truncated receptor, the maximal levels increasing in the order of EP3γ> T-335>EP3α>EP3β. The cAMP levels in the absence of the agonist in the cells expressing the receptors were the same as that in the mock-transfected cells (0.204 pmol/10^5 cells), indicating that the three isoforms and T-335 receptor have no constitutive Gs activity. The EP3γ receptor showed marked Gs activity in the cells not treated with PT, even though the receptor had the agonist-independent constitutive Gi activity. On the other hand, the Gs activities of the EP3α and T-335 receptors were observed only in the condition of ablation of Gi coupling. The T-335 receptor has agonist-dependent Gs activity without the constitutive activity. Therefore, Gs activation is an intrinsic ability of the EP3 receptor, but the COOH-terminal tail is not the domain for Gs activation. On the other hand, the Gs activity of the EP3γ receptor was higher than that of the truncated receptor, T-335, while the activities of the EP3α and EP3β receptor were lower than that of the T-335 receptor. The COOH-terminal tail of the EP3γ receptor may enhance the Gs activity of the EP3 receptor, but the tails of the EP3α and EP3β receptors suppress the activity, suggesting that the COOH-terminal tails modulate the Gs activity of the EP3 receptor.

In summary, we present evidence here that the EP3γ receptor isoform shows mostly full constitutive Gi activity, suggesting that the COOH-terminal tail of the EP3γ receptor has low potency in the constraint of the EP3 receptor in its inactive conformation. We also show that the truncated receptor has agonist-dependent Gs activity and the COOH-terminal tails of the EP3 receptor modulate this activity. This study will contribute not only to an understanding of the heterogeneity of PGE_2 actions but will also help to elucidate the molecular mechanism of G protein activation induced by receptors.

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