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Enhancement of Adenylate Cyclase Stimulation by Prostaglandin E Receptor EP3 Subtype Isoforms with Different Efficiencies

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Summary: We recently cloned the mouse prostaglandin (PG) E receptor EP3 subtype that is coupled to adenylate cyclase inhibition through Gi and identified two isoforms of EP3, EP3α and EP3β, which are produced through alternative splicing and differ only in the carboxyl-terminal domain. Preincubation of Chinese hamster ovary cells expressing each isoform with PGE_2 concentration-dependently enhanced both the basal and forskolin-stimulated cAMP formation, but two orders higher concentrations of PGE_2 were required for EP3β than EP3α for 50% enhancement of both formations. This enhancement by EP3 isoforms was completely blocked by pertussis toxin treatment, indicating that it is mediated through Gi activation. Thus, the two EP3 isoforms with different carboxyl-terminal tails induce enhancement of adenylate cyclase stimulation with different efficiencies.

Prostaglandin (PG) E_2 exerts a broad range of biological actions in diverse tissues through its binding to specific receptors on plasma membranes (1, 2). PGE receptors are pharmacologically divided into three subtypes: EP1, EP2 and EP3 (3, 4). Among these subtypes, EP3 mediates the diverse physiological actions of PGE_2, such as contraction of the uterus (5), inhibition of gastric acid secretion (6), modulation of neurotransmitter release (7), and inhibition of water reabsorption in kidney tubules (8). We have recently cloned mouse EP3 and demonstrated that this receptor is a G protein-coupled rhodopsin-type receptor that engages in inhibition of adenylate cyclase (9). Furthermore, we identified two isoforms of EP3 with different carboxyl-terminal domains, EP3α and EP3β, which are produced through alternative splicing (10).

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Abbreviations: PG, prostaglandin; CHO, Chinese hamster ovary; G protein, heterotrimeric GTP-binding protein; PT, pertussis toxin; IBMX, 3-isobutyl-1-methylxanthine.

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Repetitive exposure of receptors to agonists modulates the receptor-linked signal transduction pathways through several regulatory systems. One of the important regulatory systems is desensitization, which is a commonly observed phenomenon among various receptors and defined as reduced responsiveness to subsequent challenges by an agonist (11). Another important regulatory system is sensitization; in the case of Gi-coupled receptors, prolonged agonist exposure induces potentiation of adenylate cyclase stimulation after removal of agonist (12, 13). This sensitization represents an attempt by cells to maintain cAMP homeostasis on prolonged inhibition of adenylate cyclase by Gi-coupled receptor agonists. We have recently demonstrated that two isoforms of EP3 differ in desensitization; EP3α undergoes agonist-induced desensitization, whereas EP3β does not undergo desensitization (14). In addition to desensitization, we compared the two isoforms as to sensitization, another important regulatory system. We report here that the two isoforms show different efficiencies as to enhancement of adenylate cyclase stimulation.

MATERIALS AND METHODS

Cell Culture and Sensitization Conditions Chinese hamster ovary cells (CHO-dhfr$^-$) stably expressing each EP3 isoform (10) in monolayers were cultured in the α-modification of Eagle's medium lacking ribonucleotides and deoxyribonucleotides, but containing 10% dialyzed fetal bovine serum (Cell Culture Laboratories). Cells cultured in 24-well plates (5 x 10^5 cells/well) to approximately 90% confluence were incubated at 37°C for the indicated times with the indicated concentrations of PGE_2 in the same medium without serum. The cells were then washed twice with prewarmed Hepes-buffered saline comprising 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 then further incubated for 10 min at 37°C with 1 mM 3-isobutyl-1-methylxanthine (IBMX) for basal cAMP formation, or with 3 μM forskolin and 1 mM IBMX, in the absence of PGE_2.

Measurement of cAMP Formation The cAMP level in CHO cells expressing each EP3 isoform was determined as reported previously (15). After the incubation for 10 min at 37°C, as described above, the reactions were terminated by the addition of 10% trichloroacetic acid. The cAMP contents of the cells were determined by radioimmunoassaying with an Amersham [^{125}I]cAMP assay system.

RESULTS AND DISCUSSION

To determine whether or not the two EP3 isoforms enhance the stimulatory adenylate cyclase system, we examined the effect of prolonged exposure of isoform-expressing cells to PGE_2 on basal, or NaF- or forskolin-stimulated cAMP formation. As shown in Table I, although the cAMP levels in the cells did not change during PGE_2 preincubation, the basal and forskolin-stimulated cAMP formation as well as the NaF-stimulated formation, which is mediated through G protein activation, were enhanced after preincubation with PGE_2 in both isoforms. Since the cAMP phosphodiesterase inhibitor, IBMX, was included during the cAMP formation, the enhancement is the result of increased synthesis of cAMP rather than decreased degradation of cAMP.

Table I

Effects of PGE_2 preincubation on basal, NaF- and forskolin-stimulated cAMP formation in isoform-expressing cells. After EP3α- or EP3β-expressing CHO cells had been preincubated with or without 0.1 μM PGE_2 for 3 h at 37°C, they were washed and incubated for 10 min at 37°C with 1 mM IBMX (basal), 40 mM NaF + 10 μM $AlCl_3$ and 1 mM IBMX, or 3 μM forskolin and 1 mM IBMX, in the absence of PGE_2. The cAMP content was measured as described under "Materials and Methods". The results shown are the means ± S. E. for three independent experiments. The cAMP levels in EP3α- and β-expressing cells preincubated with PGE_2 were 0.603 ± 0.023 and 0.492 ± 0.034 pmol/10^5 cells, respectively, which are the same as those of the cells preincubated without PGE_2 (EP3α, 0.613 ± 0.035; EP3β, 0.514 ± 0.057 pmol/10^5 cells).

Stimulation	EP3α		EP3β	
	None	PGE_2	None	PGE_2
	cAMP content (pmol/10^5 cells)			
Basal	0.634 ± 0.065	0.843 ± 0.023	0.525 ± 0.020	0.773 ± 0.0092
NaF + $AlCl_3$	1.56 ± 0.12	2.96 ± 0.043	1.18 ± 0.026	2.29 ± 0.025
Forskolin	7.23 ± 0.56	20.4 ± 0.24	5.61 ± 0.20	14.6 ± 0.15

Thus, the two isoforms have the ability to enhance adenylate cyclase activity itself. We further examined the time courses of enhancement of both basal and forskolin-stimulated cAMP formation in two isoforms. As shown in Fig. 1, in EP3α-expressing cells both basal and forskolin-stimulated cAMP formation gradually increased as a function of the PGE_2 pretreatment time, reaching a maximum at 3 h, and the levels then decreased for over 6 h. Similar time courses were observed for the enhancement in the case of EP3β. Thus, PGE_2 preincubation of both isoforms induced long-term sensitization. We next compared the concentration dependencies of PGE_2-induced enhancement of both basal and forskolin-stimulated cAMP formation in these isoforms. As shown in Fig. 2, PGE_2 preincubation concentration-dependently increased both basal and forskolin-stimulated cAMP formation in EP3α-expressing cells, the half-maximal concentrations for the enhancement being 0.1 nM and 0.3 nM, respectively. PGE_2 preincubation also induced concentration-dependent enhancement of basal and forskolin-stimulated cAMP formation in EP3β-expressing cells, but the half-maximal concentrations for the enhancement were two orders of magnitude higher than those in the case of EP3α. Therefore, the two isoforms, with different carboxyl-terminal tails, show different efficiencies as to sensitization of adenylate cyclase stimulation. We have demonstrated that two EP3 isoforms are exclusively coupled to inhibition of adenylate cyclase through pertussis toxin (PT)-sensitive Gi, and that EP3α exerts activation of Gi

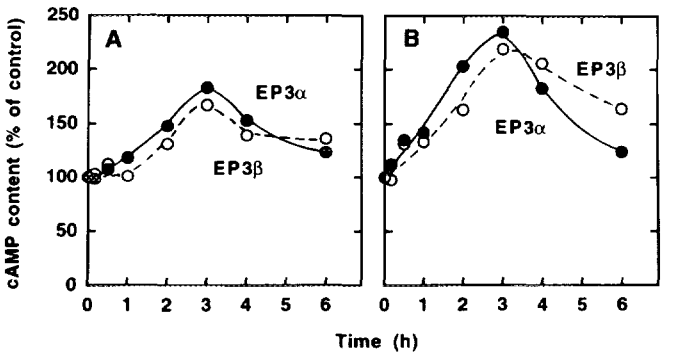

Fig. 1. Time course of PGE_2-induced enhancement of basal or forskolin-stimulated cAMP formation in isoform-expressing cells. After EP3α-(●) or EP3β-(○) expressing CHO cells had been preincubated at 37°C with 0.1 μM PGE_2 for the indicated times, they were washed and incubated for 10 min at 37°C with 1 mM IBMX (A), or 3 μM forskolin and 1 mM IBMX (B), in the absence of PGE_2. The cAMP content was measured as described under "Materials and Methods". The results shown are the means for three independent experiments, which varied by less than 5%, and are expressed as percentages of the cAMP contents of the respective preincubated cells at each time point. The cAMP levels in the cells preincubated with PGE_2 for the indicated times did not vary significantly.

and inhibition of adenylate cyclase more efficiently than EP3β (10, 16). Thus, it is assumed that the different efficiencies of the two isoforms as to the sensitization are due to a difference in the activation of Gi. To determine whether or not this isoform-induced enhancement is mediated by the isoform-induced activation of Gi, we examined the effect of PT treatment on the enhancement. As shown in Table II, PT

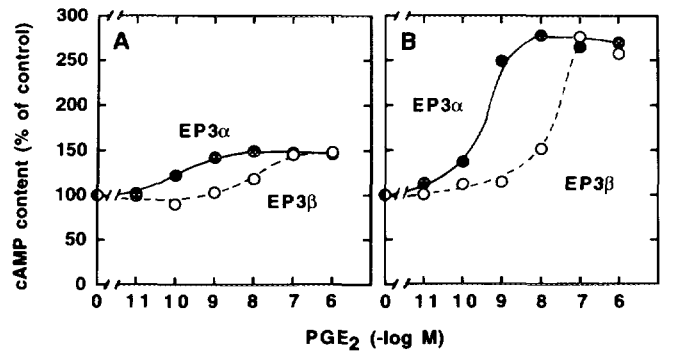

Fig. 2. Concentration dependency of PGE_2-induced enhancement of basal or forskolin-stimulated cAMP formation in isoform-expressing cells. After EP3α-(●) or EP3β-(○) expressing CHO cells had been preincubated at 37°C with indicated concentrations of PGE_2 for 3 h, they were washed and incubated for 10 min at 37°C with 1 mM IBMX (A), or 3 μM forskolin and 1 mM IBMX (B), in the absence of PGE_2. The cAMP content was measured as described under "Materials and Methods". The results shown are the means for three independent experiments, which varied by less than 5%, and are expressed as percentages of the cAMP contents of the respective preincubated cells. The cAMP levels in the cells preincubated with the indicated concentrations of PGE_2 did not vary significantly.

Table II

Effect of PT treatment of isoform-expressing cells on PGE_2-induced enhancement of basal or forskolin-stimulated cAMP formation. EP3α- or EP3β-expressing CHO cells were cultured in the presence or absence of 20 ng/ml PT for 12 h. After the cells had been preincubated with 0.1 μM PGE_2 for 3 h at 37°C, they were washed and incubated for 10 min at 37°C with 1 mM IBMX (basal), or 3 μM forskolin and 1 mM IBMX (forskolin), in the absence of PGE_2. The cAMP content was measured as described under "Materials and Methods". The results shown are the means ± S. E. for three independent experiments and are expressed as percentages of the cAMP contents of the respective preincubated cells, which did not vary significantly each other.

Isoform	Basal		Forskolin	
	-PT	+PT	-PT	+PT
	cAMP content (%)			
EP3α	152 ± 5.4	103 ± 6.8	286 ± 6.5	98.5 ± 3.5
EP3β	147 ± 1.8	91.2 ± 3.7	276 ± 3.0	110 ± 1.5

treatment completely inhibited the PGE_2 preincubation-induced enhancement of both basal and forskolin-stimulated cAMP formation in two isoforms. The sensitization of adenylate cyclase stimulation is mediated through Gi activation by these isoforms. Although the exact mechanism of EP3-mediated sensitization of adenylate cyclase stimulation is unknown, it is clear that the activation of Gi by these EP3 isoforms leads to the sensitization. Considering that the sensitization is mediated through Gi activation, the different efficiencies of the isoforms as to the sensitization are assumed to be due to different potencies of the receptors as to Gi activation.

The present results suggest that prolonged agonist exposure of EP3α attenuates its action due to its desensitization and in turn enhances the adenylate cyclase stimulation in a low PGE_2 concentration range, whereas EP3β exerts enhancement of the adenylate cyclase stimulation without its desensitization in a high PGE_2 concentration range. Thus, alternative splicing creates two EP3 isoforms with distinct properties at the level of sensitization as well as desensitization. These findings will be of help in understanding the diversity of cellular responses to PGE_2.

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