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Two G_s-Coupled Prostaglandin E Receptor Subtypes, EP2 and EP4, Differ in Desensitization and Sensitivity to the Metabolic Inactivation of the Agonist

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SUMMARY

There are at least four subtypes of prostaglandin E (PGE) receptors. The EP1 and EP3 receptors are coupled to Ca^{2+} mobilization and the inhibition of adenylate cyclase, respectively, and the EP2 and EP4 receptors are coupled to the same signal transduction pathway, stimulation of adenylate cyclase. To identify the functional differences between EP2 and EP4 receptors, we examined agonist-induced desensitization of these two receptors using Chinese hamster ovary cells, which stably express these receptors. The EP4 receptor underwent short term agonist-induced desensitization, but no such desensitization was observed for the EP2 receptor. In contrast, the EP2 and EP4 receptors displayed similar patterns of down-regulation in response to prolonged exposure to PGE_2. On the other hand, PGE_2 is rapidly metabolized to 15-keto-PGE_2 and, subsequently, to 13,14-dihydro-15-keto-PGE_2. Thus, we compared the sensitivities of the two receptors to these two metabolites. The EP4 receptor markedly lost the response at the first metabolism, whereas the EP2 receptor gradually lost the response according to the degree of metabolism, having higher sensitivity to the first metabolite, 15-keto-PGE_2, than the EP4 receptor. Therefore, the physiological significance of EP2 and EP4 may lie in their different sensitivities to agonist-induced short term desensitization and their differential susceptibilities to the metabolic inactivation of the agonist.

PGE_2 produces a broad range of biological actions in a diverse range of tissues through its binding to specific receptors on cell membranes (1, 2). PGE receptors are pharmacologically divided into four subtypes (EP1, EP2, EP3, and the recently identified fourth subtype, EP4) on the basis of their responses to various agonists and antagonists (3, 4). They are presumed to be coupled to Ca^{2+} mobilization, stimulation, inhibition and stimulation of adenylate cyclase, respectively. They share the same endogenous agonists but exert their actions through different signal transduction pathways, implying the significance of multiple receptor subtypes. Among the four subtypes, however, EP2 and EP4 are coupled to the same signal transduction pathway, the stimulation of adenylate cyclase (3, 4). PGE_2 has been shown to increase cAMP levels in many tissues and cells (2, 5), suggesting that the EP2 and/or EP4 receptor is widely distributed and mediates the various actions of PGE_2. However, the functional heterogeneity between the two receptors is not known.

Recently, we cloned the cDNAs for the mouse EP2 (6) and EP4 (7, 8) receptors, which belong to the G protein-coupled rhodopsin-type receptor superfamily, and demonstrated that both of these receptor subtypes are coupled exclusively to the stimulation of adenylate cyclase. They exhibit similar efficiencies in the activation of adenylate cyclase and have a similar tissue distribution, as shown by Northern blotting, although the level of EP2 expression is lower than that of EP4 in most of the tissues (6). Thus, the physiological significance of the EP2 and EP4 receptors remains obscure.

Agonist-induced desensitization is a commonly observed phenomenon among various receptors and is defined as reduced responsiveness to repeated challenges by an agonist. Desensitization has been studied extensively in β-adrenergic receptors, and several mechanisms for desensitization have been delineated (9, 10). A short term (seconds to minutes) agonist exposure results in uncoupling of the receptor from its ability to activate the G protein through the phosphorylation of the receptor by several protein kinases, including the GRKs and PKA (11, 12). GRKs phosphorylate serine and

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ABBREVIATIONS: PGE_2, prostaglandin E_2; CHO, Chinese hamster ovary; GRK, G protein-coupled receptor kinase; PKA, cAMP-dependent kinase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PKI, protein kinase A inhibitor peptide; 15-keto-PGE_2, 11α-hydroxy-9,15-diketo-prosta-5,13-dienoic acid; 13,14-dihydro-15-keto-PGE_2, 11α-hydroxy-9,15-diketo-prost-5-enoic acid; PBS, phosphate-buffered saline.

threonine residues in the carboxyl-terminal domain of β_2-adrenergic receptors (11), whereas PKA phosphorylates the receptors on one or both of the two putative PKA sites located in the third intracellular loop and the carboxyl-terminal domain (12). A prolonged (>1 hr) agonist exposure results in the progressive loss of total cellular receptors in a process termed down-regulation. Comparison of the cloned mouse EP2 (6) and EP4 (7) sequences suggests that they have different patterns of agonist-induced desensitization. The EP4 receptor possesses a long carboxyl-terminal tail that contains ~3.5-fold more serine and threonine residues than that of the EP2. Furthermore, there are four potential PKA phosphorylation sites in EP4, but no site was found in EP2.

PGE_2 is a short-lived and locally acting hormone. Once produced and released, PGE_2 is rapidly taken up by cells through the specific PG transporter (13) and metabolized within minutes, first into 15-keto-PGE_2 and subsequently into 13,14-dihydro-15-keto-PGE_2, by 15-hydroxy PG dehydrogenase and PG-Δ^{13}-reductase, respectively (14), which exist in most tissues and are particularly abundant in the liver, kidney, and lung (15). This metabolism is believed to be responsible for the reduced biological activity of prostanoids (16). In PGE_2 signaling, therefore, the metabolic clearance of the endogenous agonist, PGE_2, as well as desensitization of its receptors may contribute to rapid attenuation of the stimulated response.

We therefore examined the ability of the EP2 and EP4 receptors to undergo short or long term desensitization and the sensitivity of these receptors to the major metabolites of PGE_2 in CHO cells stably expressing the EP2 or EP4 receptor. We report here that the difference between EP2 and EP4 receptors may lie not only in their agonist-induced short term desensitization but also in their sensitivity to the metabolic inactivation of the agonist.

Experimental Procedures

Materials. PGE_2, 15-keto-PGE_2, and 13,14-dihydro-15-keto-PGE_2 were purchased from Cayman Chemical (Ann Arbor, MI). [5,6,8,11,12,14,15-^{3}H]PGE_2 (181 Ci/mmol) and a ^{125}I-labeled cAMP assay system was obtained from Amersham (Arlington Heights, IL). Heparin and PKI were from Sigma Chemical (St. Louis, MO) and Promega (Madison, WI), respectively.

Cell culture. CHO cells stably expressing the EP2 (6) or EP4 (8) receptor were cultured in the α modification of Eagle's medium lacking ribonucleosides and deoxyribonucleosides with 10% dialyzed fetal bovine serum in humidified air containing 5% CO_2 at 37°.

Adenylate cyclase assay. Adenylate cyclase activity was determined as described previously (17, 18). Briefly, CHO cells expressing the EP2 or EP4 receptor were washed twice with PBS and scraped into 3 ml of 10 mM Tris·HCl, pH 7.5, 5 mM EDTA, 100 nM okadaic acid, and 20 μM indomethacin, rendering >95% of the cells permeable to Trypan blue. The lysed cells were centrifuged at 40,000 × g for 25 min at 4°, washed once, and assayed for adenylate cyclase activity. The standard assay mixture contained 15 μg of protein in 30 mM Tris·HCl, pH 7.5, 0.8 mM EDTA, 5 mM $MgCl_2$, 10 μM GTP, 1 mM ATP, and 100 μM Ro-20-1724. Reactions were started by the addition of ATP, conducted at 37° for 10 min, and terminated by the addition of 5% trichloroacetic acid. The cAMP formed was determined by radioimmunoassay using an Amersham cAMP assay system. Data analysis was conducted as described previously (12). Briefly, after subtracting the basal activity, the adenylate cyclase activity in each experiment was normalized to the activity in the presence of 10 mM NaF and 10 μM $AlCl_3$. The maximum activity elicited by 10 μM PGE_2 in the lysed control (untreated) cells was considered to be 100%, and all the other activities were expressed as a percentage of this value.

Desensitization condition in the permeabilized cells. CHO cells expressing the EP2 or EP4 receptor were permeabilized with digitonin and loaded with protein kinase inhibitors essentially as described previously (19, 20). Briefly, cells at ~90% confluence were detached with calcium-free PBS in the presence of 1 mM EDTA, washed once with PBS and twice with KG buffer (150 mM potassium glutamate, 10 mM HEPES, 5 mM EGTA, 5.2 mM $MgCl_2$, pH 7.1), and then resuspended in KG buffer supplemented with 5 mM glucose and 3 mM ATP at a density of 4 × 10^7 cells/ml. The cells were permeabilized with digitonin (0.015–0.02%). Digitonin was added stepwise until >95% of the cells were positive for Trypan blue. The permeabilized cells were preincubated with or without 30 μM heparin or 10 μM PKI for 5 min and then incubated with or without 10 μM PGE_2 for 30 min at 37°. The incubation was terminated by the addition of 10 volumes of ice-cold KG buffer followed by centrifugation at 800 × g for 3 min. After two repetitive washes, the cells were lysed, and adenylate cyclase activity was assessed as described above.

Membrane preparation and the [^{3}H]PGE_2 binding assay. [^{3}H]PGE_2 binding to the membranes of CHO cells expressing the EP2 or EP4 receptor was quantified as described previously (8). The harvested cells were homogenized using a Potter-Elvehjem homogenizer (Iuchi, Osaka, Japan) in an ice-cold solution containing 10 mM Tris·HCl, pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$, 0.1 mM phenylmethylsulfonyl fluoride, 20 μM indomethacin, and 0.25 M sucrose. The homogenate was centrifuged at 800 × g for 5 min, and the supernatant was further centrifuged at 300,000 × g for 20 min at 4°. The pellet was washed once and then resuspended in buffer A (20 mM HEPES-NaOH, pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$) and then used for the [^{3}H]PGE_2 binding assay. The membrane (40 μg of protein) was incubated with 4 nM [^{3}H]PGE_2 (170,000 dpm) at 30° for 1 hr in 100 μl of buffer A, and then [^{3}H]PGE_2 binding to the membrane was quantified as described previously (21). Nonspecific binding was determined using a 1,000-fold excess of unlabeled PGE_2 in the incubation mixture. The specific binding was calculated by subtracting the nonspecific binding value from the total binding value.

Results

Comparison of the cloned EP2 (6) and EP4 (7) receptor sequences suggests that they have different patterns of agonist-induced desensitization. Initially, we investigated the PGE_2-induced short term desensitization of the adenylate cyclase response in CHO cells stably expressing the EP2 or EP4 receptor. As shown in Fig. 1B, the EP4 receptor underwent a substantial desensitization after 10 min of exposure to PGE_2. Although the EC_{50} for PGE_2 was not affected, the maximum response in PGE_2-pretreated lysed cells was reduced to 77.8 ± 1.2% of the control value for the EP4 receptor. Exposure of the EP4 receptor-expressing cells to 10 μM PGE_2 time-dependently attenuated the PGE_2-induced cAMP formation, with attenuation reaching the maximum at 10 min (Fig. 2). In contrast, exposure of the EP2 receptor to PGE_2 for 10 min did not alter the EC_{50} value or the maximum response to subsequent stimulation by PGE_2 under the same condition used for desensitization of the EP4 receptor (Fig. 1A). A different sensitivity to agonist-induced short term desensitization was also observed with another pair of cell clones (data not shown).

GRKs and PKA have been shown to play a role in agonist-induced short term desensitization (11, 12). To assess whether GRKs and PKA are responsible for the desensitization of the EP4 receptor, we examined the effects of heparin, an inhibitor of GRKs, and PKI on the PGE_2-induced desen-

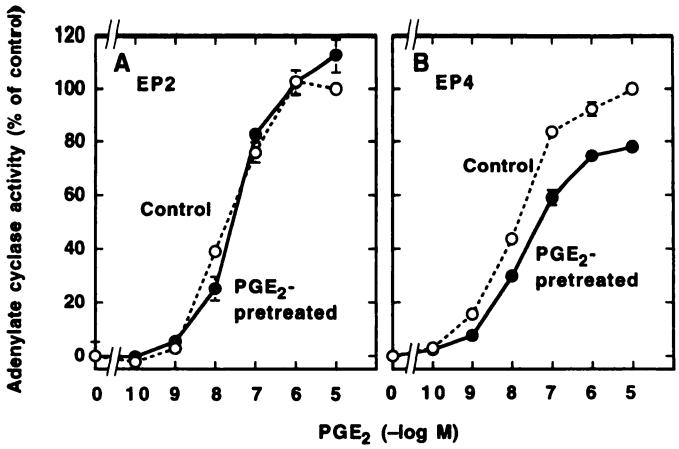

Fig. 1. Effects of short term PGE_2 exposure on EP2 and EP4 receptor function. After CHO cells stably expressing the EP2 (A) or EP4 (B) receptor had been exposed to 10 μM PGE_2 (●) or vehicle (○) for 10 min at 37°, the cells were washed, and adenylate cyclase activities were measured in the lysed cells in response to increasing concentrations of PGE_2 as described in Experimental Procedures. In each experiment, the maximum PGE_2 stimulation in the control cells was designated 100%, and all other cyclase activities in that experiment were expressed as percentages of that maximal response. Values are mean ± standard error of triplicate experiments. Basal adenylate cyclase activities were 121.4 ± 2.8 and 37.1 ± 2.0 pmol of cAMP/min/mg of protein; the maximal PGE_2-stimulated activities were 254.5 ± 3.6 and 128.9 ± 1.2 pmol of cAMP/min/mg of protein for the EP2 and EP4 receptors, respectively.

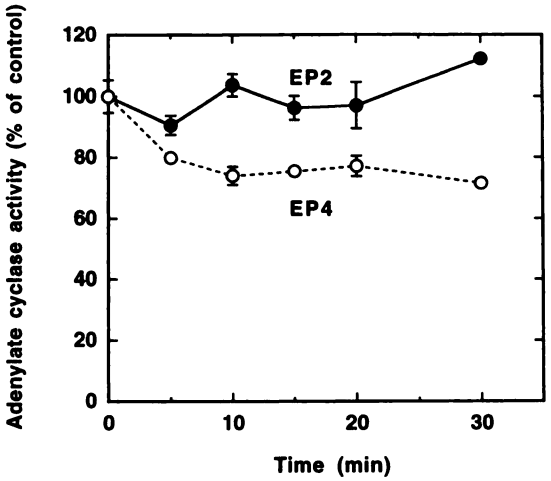

Fig. 2. Time course of PGE_2-mediated desensitization of the EP2 and EP4 receptors. CHO cells expressing the EP2 (●) and EP4 (○) receptor were incubated with 10 μM PGE_2 for the indicated times. The cells were washed, and the adenylate cyclase activities in response to 10 μM PGE_2 were measured in the lysed cells as described in Experimental Procedures. In each experiment, the adenylate cyclase activity was expressed as a percentage of the response in the untreated cells. Values are mean ± standard error of four experiments. Basal adenylate cyclase activities were 83.7 ± 9.9 and 21.2 ± 4.8 pmol of cAMP/min/mg of protein; the PGE_2-stimulated activities were 119.2 ± 1.1 and 81.9 ± 3.6 pmol of cAMP/min/mg of protein for the EP2 and EP4 receptors, respectively.

sitization of the EP4 receptor in the cells permeabilized with digitonin. As shown in Fig. 3B, heparin but not PKI inhibited the desensitization of the EP4 receptor. Inhibition of the EP4 receptor-mediated desensitization by heparin began at 10 μM and was complete at 30 μM, but the inhibition was not observed at 1 μM (data not shown). On the other hand, heparin

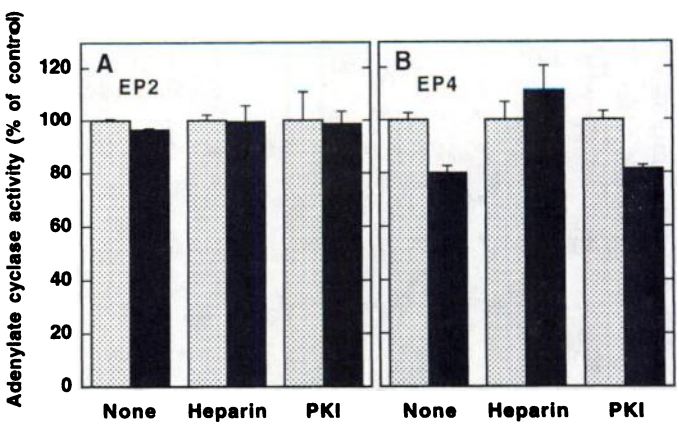

Fig. 3. Effects of protein kinase inhibitors on PGE_2-induced short term desensitization of the EP4 receptor. After CHO cells expressing the EP2 (A) or EP4 (B) receptor had been permeabilized with digitonin and loaded with 30 μM heparin or 10 μM PKI, they were incubated with (*closed bar*) or without (*hatched bar*) 10 μM PGE_2 for 30 min. The cells were then washed, and adenylate cyclase activities in response to 10 μM PGE_2 were measured in the lysed cells as described in Experimental Procedures. In each experiment, the PGE_2-stimulated adenylate cyclase activity in the PGE_2-pretreated cells was expressed as a percentage of that activity in the untreated cells. Values are mean ± standard error of nine experiments. Basal adenylate cyclase activities were 21.1 ± 0.5, 15.8 ± 1.8, and 23.3 ± 1.4 (for EP2) and 3.63 ± 0.25, 2.26 ± 0.14, and 3.44 ± 0.19 (for EP4) pmol of cAMP/min/mg of protein; the PGE_2-stimulated activities were 57.7 ± 0.2, 54.5 ± 0.8, and 68.5 ± 5.1 (for EP2) and 31.1 ± 1.6, 22.7 ± 1.1, and 32.9 ± 1.2 (for EP4) pmol of cAMP/min/mg of protein for the cells loaded with vehicle, heparin, and PKI, respectively. The adenylate cyclase activity was normalized to the activity in the presence of 10 mM NaF and 10 μM $AlCl_3$.

did not affect the EP2 receptor-mediated activation of adenylate cyclase (Fig. 3A). The agonist binding characteristics of G protein-coupled receptors are thought to reflect the state of physical coupling between receptors and G proteins (22). The G protein-coupled form of receptors shows high affinity for agonist, whereas the uncoupled form shows low affinity. Then, we examined the effect of short term PGE_2 exposure on PGE_2 binding affinity states of the EP4 receptor. As shown in Fig. 4, the PGE_2 binding affinity showed high affinity and low affinity states. The PGE_2 exposure for 10 min markedly decreased the number of the EP4 receptor in the high affinity state.

Next, we examined the effect of long term agonist exposure on the total cellular receptor binding. In contrast to the different sensitivity to short term desensitization, PGE_2 binding to both EP2 and EP4 receptor-expressing cell membranes was markedly reduced, with a similar time course (Fig. 5). After 12-hr exposure, 65.6 ± 0.5% and 39.5 ± 0.4% of the binding activity had been lost, respectively. To elucidate whether the decrease in the PGE_2 binding to the membranes was due to a change in the number of binding sites (B_{max}) or in the binding affinity (K_d), we carried out saturation experiments. Table 1 shows the results of Scatchard analyses of the specific binding to the membranes of the control cells or the cells exposed to 1 μM PGE_2 for 12 hr. PGE_2 binding affinity of the EP4 receptor showed high affinity and low affinity states, but the binding affinity of the EP2 receptor showed a single affinity state. The 12-hr exposure to PGE_2 markedly decreased the B_{max} of both high and low affinity states of the EP4 receptor without any change in K_d. This exposure also decreased B_{max} of the EP2 receptor. Therefore,

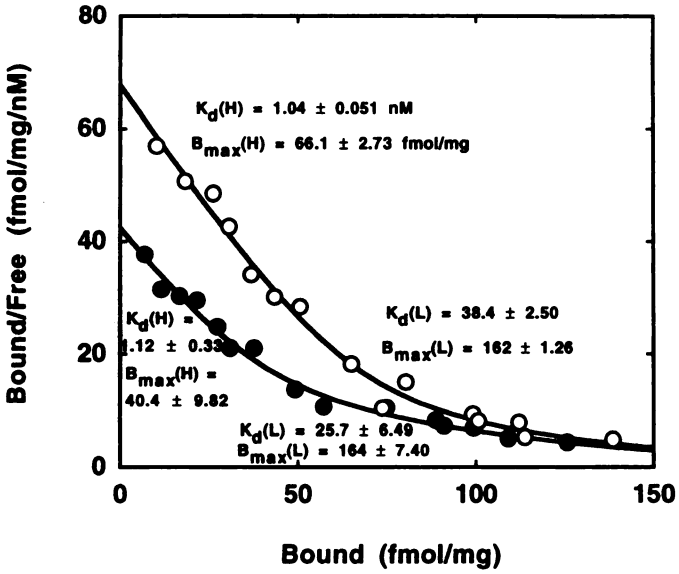

Fig. 4. Scatchard analysis of PGE_2 binding to the EP4 receptor after a 10-min exposure to PGE_2. CHO cells expressing the EP4 receptor were preincubated at 37° for 10 min with (●) or without (○) 10 μM PGE_2. The cells were then washed, and the specific [^{3}H]PGE_2 binding (0.25–30 nM) to the membranes prepared from the cells was determined as described in Experimental Procedures. The Scatchard plot was transformed from the values of specific [^{3}H]PGE_2 binding. *H*, high affinity binding state; *L*, low affinity binding state.

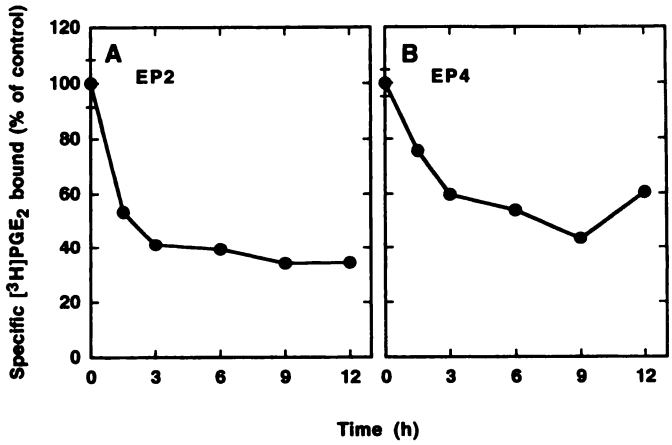

Fig. 5. PGE_2-induced down-regulation of the EP2 and EP4 receptors. After CHO cells stably expressing EP2 (A) or EP4 (B) receptor had been exposed to 1 μM PGE_2 for the indicated times, the cells were washed, and their membranes were prepared. Specific [^{3}H]PGE_2 binding to the cell membranes was determined as described in Experimental Procedures. Values are mean ± standard error of triplicate experiments and are expressed as percentage of the control values obtained from cells that had not been exposed to PGE_2. The control values for the EP2 and EP4 receptors were 495 ± 18.8 and 204 ± 1.0 fmol/mg of protein, respectively.

the reduction in PGE_2 binding to the membranes of both EP2 and EP4 receptor-expressing cells by the long term PGE_2 exposure is due to decrease in the receptor number.

To determine whether EP2 and EP4 receptors have different sensitivities to metabolic inactivation of the endogenous agonist, we compared the agonist activities of PGE_2 and two of its major metabolites, 15-keto-PGE_2 and 13,14-dihydro-15-keto-PGE_2, for the receptors. As shown in Fig. 6, these agonists concentration-dependently stimulated adenylate cyclase activity in the EP4 receptor in the rank order of PGE_2 ≫ 15-keto-PGE_2 = 13,14-dihydro-15-keto-PGE_2; the rank order of activity in the EP2 receptor was PGE_2 > 15-keto-PGE_2 > 13,14-dihydro-15-keto-PGE_2. PGE_2 has similar potencies for both EP2 and EP4 receptors. However, the agonist potency of the first metabolite, 15-keto-PGE_2, for the EP2 receptor was 1 order of magnitude higher than that for the EP4 receptor, and the two receptors showed similarly low responses to the second metabolite, 13,14-dihydro-15-keto-PGE_2.

We further analyzed the binding affinities of the PGE_2 metabolites for EP2 and EP4 receptors by assessing the displacement of [^{3}H]PGE_2 binding. As shown in Fig. 7, the transformation of PGE_2 to two metabolites reduced the affinity of the two receptors. However, the degree of the reduction at the first metabolism in the EP2 receptor was much less than that in the EP4 receptor. The EC_{50} values for PGE_2 and two PGE_2 metabolites to stimulate adenylate cyclase activity and the IC_{50} values for inhibition of PGE_2 binding by these agents are shown in Table 2. These observations indicate that the EP2 and EP4 subtypes exhibit different patterns of susceptibility to the metabolic inactivation of PGE_2. Different susceptibility to the metabolic inactivation of agonist was also observed with another pair of cell clones (data not shown).

Discussion

The existence of two G_s-coupled PGE receptor subtypes, EP2 and EP4, has been defined pharmacologically (3, 4) and confirmed through molecular cloning (7, 23), but the physiological differences between the two subtypes have remained obscure. In this study, we revealed for the first time their differing properties, which may in part account for the significance of both receptor subtypes. We first demonstrated that the EP2 and EP4 receptors have different sensitivities to agonist-induced desensitization. The EP4 receptor underwent short term agonist-induced desensitization, but the EP2 receptor did not (Fig. 1). This observation suggests that the EP4 receptor could be involved in more rapidly waning signaling, whereas the responses mediated via the EP2 receptor could be long lasting.

As in β_2- and β_3-adrenergic receptors (24), different sensitivities to short term desensitization of the receptors could be explained by their ability to act as substrates for GRKs or PKA. EP4 has a long carboxyl-terminal tail that contains 42 serine and threonine residues, whereas there are 12 such residues in the same region of EP2. The β-adrenergic receptor kinase has been shown to have a substrate preference for peptides containing acidic residues on the amino-terminal side of a serine or threonine (25). Such sequences are found in the carboxyl-terminal region of the EP4 receptor but not in the EP2 receptor. In addition to the potential phosphorylation sites for GRKs, PKA consensus sequences, Arg-Arg-Xaa-Ser, appear four times in the third intracellular loop and carboxyl-terminal tail of the EP4 receptor, whereas there is no consensus sequence in the EP2 receptor (6, 7). Thus, the differences in their phosphorylation sites may result in differences in their sensitivity to short term agonist-induced desensitization. We demonstrated here that agonist-promoted short term desensitization of the EP4 receptor was completely inhibited by heparin, a GRK inhibitor, but not by PKI, indicating that GRKs play a major role in the EP4

TABLE 1

Characteristics of [^{3}H]PGE_2 binding sites in membranes from untreated and PGE_2-treated CHO cells expressing the EP4 and EP2 receptors

CHO cells expressing EP4 and EP2 receptors were treated with or without 1 μM PGE_2 for 12 hr. The cells were then washed, and the specific [^{3}H]PGE_2 binding (0.25–30 nM for EP4; 0.5–100 nM for EP2) to the membranes prepared from the cells was assessed as described in Experimental Procedures. The Scatchard plot was transformed from the values of specific [^{3}H]PGE_2 binding. Values are mean ± standard error of triplicate experiments.

Treatment	EP4				EP2	
	K_d (H)[a]	B_{max} (H)[a]	K_d (L)[a]	B_{max} (L)[a]	K_d	B_{max}
	nM	fmol/mg	nM	fmol/mg	nM	fmol/mg
None	0.89 ± 0.06	56.5 ± 0.82	26.1 ± 10.9	189 ± 26.2	116 ± 10.9	4430 ± 411
PGE_2	1.27 ± 0.22	16.6 ± 2.13	33.6 ± 4.71	62.5 ± 7.1	147 ± 15.9	3420 ± 346

[a] H and L, high and low affinity binding state, respectively.

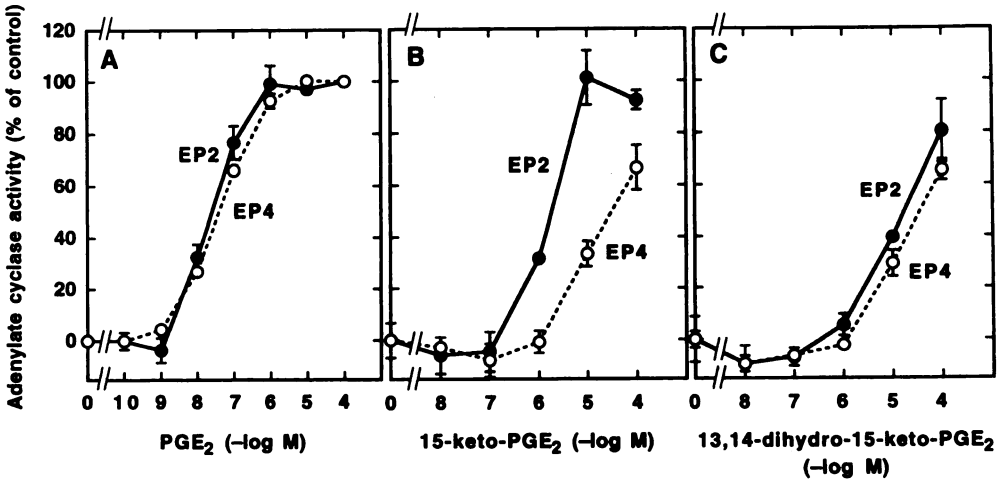

Fig. 6. Agonist activities of PGE_2 and its two metabolites for EP2 and EP4 receptors. PGE_2- (A), 15-keto-PGE_2- (B), and 13,14-dihydro-15-keto-PGE_2-(C) stimulated adenylate cyclase activities were measured in the particulate fractions of CHO cells stably expressing EP2 (●) or EP4 (○) receptor as described in Experimental Procedures. In each experiment, stimulation of each membrane with 10 μM PGE_2 was designated as 100%, and all other cyclase activities in that experiment were expressed as a percentage of that response. Values are mean ± standard error of triplicate experiments. Basal cyclase activities were 92.6 ± 2.4 and 23.9 ± 0.5 pmol of cAMP/min/mg of protein; the PGE_2-stimulated activities were 185.6 ± 24.3 and 103.1 ± 1.3 pmol of cAMP/min/mg of protein for the EP2 and EP4 membranes, respectively.

receptor desensitization (Fig. 3). To examine the phosphorylation state of the EP2 and EP4 receptors, preparation of specific antibodies against the EP2 and EP4 receptors for immunoprecipitation experiments is under way in our laboratory.

In contrast, both subtypes underwent down-regulation in a similar manner after prolonged exposure to the agonist (Fig. 5 and Table 1). The tyrosine residues in the carboxyl-terminal tail of the receptor were shown to be involved in the agonist-induced down-regulation of β_2-adrenergic receptors, but down-regulation still proceeded without these residues (26). The NPX_nY motif is thought to be involved in the internalization process of several types of G protein-coupled receptors (27). However, it was recently shown that the tyrosine residue in the NPX_nY motif of the human angiotensin II receptor type 1 was not essential for internalization of the receptor (28). Thus, there really are no clear determinants within receptor structure for down-regulation. Down-regulation of the EP2 and EP4 receptors was clearly observed, but structural determinants for the down-regulation is obscure.

PGE_2, an endogenous agonist for PGE receptors, is rapidly metabolized to 15-keto-PGE_2 and, subsequently, 13,14-dihydro-15-keto-PGE_2 (29), which have longer half-lives and thus accumulate to levels considerably higher than the PGE_2 levels (30). Hamberg and Samuelsson (31) showed that intravenously administrated PGE_2 is rapidly cleared from the blood

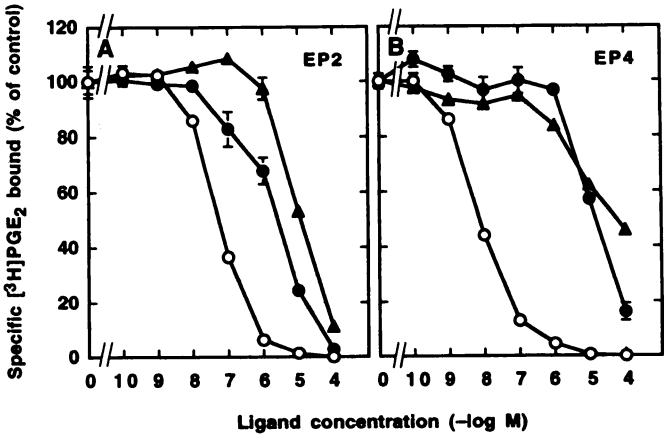

Fig. 7. The binding affinities of the PGE_2 metabolites. The membranes from cells expressing the EP2 (A) or EP4 (B) receptor were incubated with 4 nM of [^{3}H]PGE_2 and the indicated concentrations of PGE_2 (○), 15-keto-PGE_2 (●), and 13,14-dihydro-15-keto-PGE_2 (▲). Specific [^{3}H]PGE_2 binding was determined as described in Experimental Procedures. Values are expressed as percentage of the control membrane (495.7 ± 18.8 and 204.0 ± 1.0 fmol/mg protein for the EP2 and EP4 receptors, respectively). Values are mean for triplicate determination.

and is followed by the appearance of its metabolites. Only 3% of the injected amount remained as PGE_2, and 40% existed as 13,14-dihydro-15-keto-PGE_2 after 1.5 min (31). The kinetics

TABLE 2

Comparison of binding affinities and agonist activities of PGE_2 metabolites for the EP4 and EP2 receptors

Experimental conditions were exactly as described in Figs. 6 and 7. Values are mean ± standard error of triplicate determination.

	EP4		EP2	
	K_i	EC_{50}	K_i	EC_{50}
	nM			
PGE_2	7.3 ± 0.40	3.9 ± 0.31	53 ± 1.8	25 ± 7.4
15-Keto-PGE_2	15,000 ± 1,800	>33,000 ± 23,000	2,600 ± 430	1,800 ± 380
13,14-Dihydro-15-keto-PGE_2	57,000 ± 13,000	>38,000 ± 10,000	12,000 ± 920	>18,000 ± 4,900

of PGE_2 metabolism is comparable to the PKA- and GRK-mediated desensitization of β_2-adrenergic receptor with a half-life of 3 min and 15 sec, respectively (32). Thus, in addition to agonist-induced short term desensitization, the metabolic inactivation of agonists contributes to termination or attenuation of receptor responses. In this study, we demonstrated that the EP4 receptor markedly loses the response at the first metabolism, whereas the EP2 receptor gradually loses the response according to the degree of PGE_2 metabolism. The EP2 receptor has a higher sensitivity to the first metabolite, 15-keto-PGE_2, than the EP4 receptor (Fig. 6 and Table 2). Thus, the EP2 and EP4 receptors show different sensitivities to the metabolic inactivation of PGE_2.

Recently, we demonstrated that the EP2 and EP4 receptors are expressed in a wide variety of tissues (6). The EP4 receptor is expressed most abundantly in uterus, followed by thymus, ileum, heart, lung, kidney, spleen, and stomach. The EP2 receptor is expressed most abundantly in uterus, followed by spleen, lung, thymus, ileum, and stomach; the expression pattern of the EP2 receptor is similar to that of the EP4 receptor. The two enzymes responsible for the metabolism of PGE_2, 15-hydroxy PG dehydrogenase and PG-Δ^{13}-reductase, have also been isolated from many tissues and have been well characterized. Both enzymes are widely distributed, but their expression levels are different among the tissues (15). The dehydrogenase is highly expressed in kidney, spleen, and lung, whereas the expression is low in intestine, ovary, and testicle. On the other hand, the reductase is expressed in intestine, ovary, testicle, and brain. Thus, the levels of dehydrogenase expression are much higher than those of reductase expression in the lung, spleen, and kidney, suggesting a longer half-life for 15-keto-PGE_2 in these organs. In contrast, the levels of dehydrogenase expression are much lower than those of reductase expression in the intestine, ovary, and testicle, suggesting a shorter half-life for 15-keto-PGE_2 in these organs. However, clear determination of the degree of colocalization of the receptors and the enzymes requires their distribution to be determined in detail in *in situ* hybridization experiments. The EP2 receptor exhibited higher sensitivity than the EP4 receptor to only 15-keto-PGE_2 (Fig. 6 and Table 2). Thus, the duration of PGE_2 action through the EP2 and EP4 receptors may be determined by the expression levels of its degradative enzymes.

Furthermore, our observations suggest that the hydroxyl group of PGE_2 at C15 plays an important role in the subtype specificity of EP2 and EP4 receptors. The EP4 receptor may more strictly recognize the 15-hydroxyl group of PGE_2 than the EP2 receptor. In this regard, it is interesting that butaprost, a selective EP2 agonist that has a cyclobutane structure at C16, can specifically bind to and activate the EP2 receptor but not the EP4 receptor (8).

In conclusion, we have shown for the first time that the two G_s-coupled PGE receptor subtypes, EP2 and EP4, differ in their sensitivity to agonist-induced short term desensitization and to the metabolic inactivation of the agonist. These findings will be helpful in understanding the physiological significance of two G_s-coupled PGE receptor subtypes and the diversity of cellular responses to PGE_2.

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