Cloning and Expression of a cDNA for Mouse Prostaglandin E Receptor EP₂ Subtype*

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A functional cDNA clone encoding mouse EP₂ subtype of prostaglandin (PG) E receptor was isolated from a mouse cDNA library by cross-hybridization with the mouse EP₁ subtype PGF₂α receptor cDNA. The mouse EP₂ receptor consists of 513 amino acid residues with putative seven-transmembrane domains. In contrast to EP₁ receptor, this receptor possesses long third intracellular loop and carboxyl-terminal tail. [3H]PGE₂ specifically bound to the membrane of mammalian COS cells transfected with the cDNA. The binding to the membrane was displaced with unlabeled PG in the order of PGE₂ > PGF₂α > iloprost ≧ PGF₂β > PGD₂.

The binding was also inhibited by misoprostol, an EP₂ and EP₃ antagonist, but not by sulprostone, an EP₂ and EP₃ agonist, and SC-19220, an EP₁ antagonist. PGE₂ markedly increased cAMP level in COS cells transfected with the cDNA. These results suggest that this receptor is EP₂ subtype. Northern blot analysis demonstrated that the EP₂ mRNA is widely expressed in various tissues, the abundant expression being observed in ileum, thymus, and mastocytoma P-815 cells.

Prostaglandin (PG)E₂ produces a broad range of biological actions in diverse tissues through its binding to specific receptors on plasma membranes (1, 2). PGE₂ receptors are pharmacologically subdivided into three subtypes, EP₁, EP₂, and EP₃ (3, 4), and these subtypes are suggested to be different in their signal transduction; they are presumed coupled to stimulation of phospholipase C and stimulation and inhibition of adenylate cyclase, respectively (4, 5). PGE₂ has been shown to increase the cAMP level in many tissues and cells (2, 6), suggesting that the EP₂ subtype is ubiquitously distributed and mediates various PGE₂ actions in many tissues and cells. The EP₂ receptor has been suggested to be involved in relaxation in trachea (7) and ileum circular muscle (8), vasodilation in various blood vessels (4), and stimulation of sodium and water reabsorption in kidney tubulus (9, 10). One of the most important functions of PGE₂ through EP₂ receptor has been proposed to be negative regulation of immune system (11) and inflammation (4). PGE₂ inhibits the function and the proliferation of T cells (12) and the histamine release from mast cells (13, 14) by increasing the intracellular level of cAMP. Recently, we cloned a cDNA for the mouse EP₂ receptor (15). However, other subtypes of PGE receptors have not been yet isolated, and their molecular characterization has been carried out only poorly. Previously, we demonstrated that high level of PGE₂ binding is seen in mouse mastocytoma P-815 cells, neoplastic mast cells, and PGF₁α strongly stimulates adeylate cyclase (16, 17), suggesting that the mastocytoma cells express EP₂ receptor. In order to isolate the cDNA for EP₂ receptor, we performed homology screening using the mouse EP₂ cDNA. We report here the complete nucleotide and deduced amino acid sequences of EP₂ receptor, and its ligand binding and biochemical properties.

EXPERIMENTAL PROCEDURES

Molecular Cloning by Cross-hybridization—Mouse mastocytoma P-815 cell-cDNA library carrying cDNAs larger than 2.0 kilobase pairs was prepared in λ Zap II vector (Stratagene) as previously described (15). The probe DNA was prepared by polymerase chain reaction using mouse EP₂ cDNA as a template; this 482-base pair fragment covers the transmembrane segments 1–4 region of the EP₂ receptor (15). The 2.0 × 10⁶ clones derived from the cDNA library were screened under either high (Sambrook) or low stringency conditions (15). The resultant positive clones were subjected to polymerase chain reaction, restriction, and sequence analyses and classified into two major groups; one group (six clones) belonged to EP₂ receptor cDNA, and the other (five clones) showed a sequence homologous but not identical to EP₂ cDNA. One representative clone (MP412) of the latter group, which contains a 1539-base pair open reading frame, was obtained. In order to make in vitro transcript for the EP₂ receptor, the plasmid DNA was subcloned into pcDNAI (Invitrogen), a modified eukaryotic vector. For the PGE₂ binding assay, the plasmid DNA was transfected into COS-1 cells by the DEAE-dextran method (18) and cultured for 72 h. [3H]PGE₂ binding to the membranes prepared from the harvested cells was determined as described previously (19). For cAMP assay, the plasmid DNA was transfected into COS-1 cells by the lipofection (20) and cultured for 72 h in a 24-well plate. Cyclic AMP levels in the cells were determined as described previously (21).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D13458.

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The abbreviations used are: PG, prostaglandin; G protein, heterotrimeric GTP-binding protein; TX, thromboxane.
FIG. 1. Nucleotide and deduced amino acid sequences of MP412. The deduced amino acid sequence is shown below the nucleotide sequence using single-letter code. Positions of the putative transmembrane segments I-VI are underlined. The termini of each segment are tentatively assigned on the basis of a hydropathy profile and comparison with other G protein-coupled receptors. Asterisks, potential N-glycosylation sites in the extracellular regions; stars, potential phosphorylation sites by cAMP-dependent protein kinase.

Ugand concentration (-log U) of PGE2 (-log M)

FIG. 2. Binding of [3H]PGE2 to MP412-transfected COS-1 cell membrane. a, displacement of [3H]PGE2 binding by various PGs. Unlabeled PGs were added to the binding assay mixture at indicated concentrations, and specific [3H]PGE2 binding was determined as described under "Experimental Procedures." 0, PGE2; 0, PGE1; 0, iloprost; A, PGF2α; 0, PGD2.

b, displacement of [3H]PGE2 binding by agonist or antagonist for PGE receptor subtypes. 0, misoprostol; A, M&B 28,767; 0, sulprostone; A, butaprost; 0, SC-19220.

FIG. 3. Effect of PGE2 on cAMP level in MP412-transfected or untransfected COS-1 cells. MP412-transfected () or untransformed (○) COS-1 cells were incubated with the indicated concentrations of PGE2 in the presence of 1 mM 3-isobutyl-1-methylxanthine, and cAMP accumulation was determined as described (21). The results shown are the means ± S.E. for triplicate determinations.

Northern Blots—Total RNAs from various mouse tissues were isolated by the acid guanidinium thiocyanate-phenol-chloroform method (22), and poly(A)+ RNAs were purified using Oligotex dT30 (Takara Shuzo, Kyoto, Japan). Poly(A)+ RNAs (10 µg) from each tissue were separated by electrophoresis on a 1% agarose gel, transferred onto nylon membranes (Hybond-N, Amersham Corp.), and...
FIG. 4. Comparison of the amino acid sequences among the mouse EP₂, EP₃, and TXA₂ receptors. The amino acid sequences of the mouse EP₂ receptor (upper), EP₃ receptor (middle), and TXA₂ receptor (lower) are aligned to achieve the maximal homology using a computer program. The boxed amino acids represent residues that are identical in two or all of the sequences. Dashes show deletions of the amino acid residues when compared among the three sequences.

FIG. 5. Northern blot analysis of RNAs isolated from various mouse tissues and P-815 cells. Poly(A)⁺ RNAs were isolated from the tissues and a cell line, and 10 µg of RNA was applied in each lane except that 5 µg was used for P-815 cells, listed below. Hybridization analysis was carried out using the 1859-base pair EcoRI-XhoI fragment excised from clone MP412 as a probe, as described under "Experimental Procedures." Lane 1, brain; lane 2, thymus; lane 3, lung; lane 4, heart; lane 5, liver; lane 6, stomach; lane 7, spleen; lane 8, ileum; lane 9, kidney; lane 10, testis; lane 11, uterus; lane 12, P-815 cells.

RESULTS AND DISCUSSION

Cross-hybridization analysis of the mouse mastocytoma cell cDNA with the mouse EP₂ receptor cDNA gave five hybridizing clones showing significant homology to the EP₂ receptor. The restriction and sequence analyses of these clones displayed an identical sequence within their overlapping region. Fig. 1 shows nucleotide and deduced amino acid sequences of one clone (MP412). The amino acid sequence was assigned from the longest open reading frame (1539 base pairs) of the cDNA. The nucleotide sequence surrounding the initiation codon agrees reasonably well with the consensus sequence (23). The polypeptide consists of 513 amino acid residues with an estimated molecular weight of 56,157. The hydrophathy profile determined by the Kyte and Doolittle method (24) and the sequence homology analysis indicate that it possesses seven hydrophobic segments and shares a significant sequence similarity with other members of G protein-coupled receptors (25), especially with the mouse EP₃ receptor (15) (36.2% in transmembrane segments). This receptor possesses a longer third intracellular loop than the EP₃ receptor (15). This receptor also possesses a long carboxyl-terminal tail, as do the β₂-adrenergic (26) and dopamine-D₁ (27) receptors. Like the EP₃ receptor (15), two potential N-glycosylation sites are found at the amino-terminal and the second extracellular loop regions.

To identify a ligand for this receptor, MP412 was expressed in COS-1 cells, and the binding activities of various radioactive PGs to the membrane of the transfected cells were measured. Among the PGs tested, [³H]PGE₂ specifically bound to the membrane. Scatchard analysis of this binding yielded a dissociation constant of 11.2 nM and the maximal binding of 946 fmol/mg. Specific [³H]PGE₂ binding to the membrane of untransfected cells was almost negligible (data not shown). Fig. 2a shows the specificity of this binding. Specific [³H]PGE₂ binding was inhibited by unlabeled PG in the order of PGE₁ > PGE₂ > iloprost, a stable PGI₂ analogue ≥ PGF₂α ≥ PGD₂. Among these PGs, this receptor shows higher specificity for PGE₁ and PGE₂ than EP₃ receptor (15). We further characterized ligand binding specificity using several ligands that show characteristic agonist or antagonist activity for PGE receptor subtypes. As shown in Fig. 2b, the PGE₂ binding was inhibited by misoprostol, an EP₂ and EP₃ agonist, and more weakly by M&B 28,767, an EP₃ agonist. On the other hand, sulprostone, an EPI and EP₃ agonist, SC-19220, an EP₁ antagonist, and butaprost, an EP₂ agonist, did not inhibit it. The ability of misoprostol to inhibit PGE₂ binding and no ability of sulprostone suggest that MP412 encodes the PGE₂ subtype of PGE receptor, and this was also supported by weak cross-reaction of M&B 28,767 to EP₂ (8). The lack of binding activity of butaprost in mouse EP₂ might indicate that the action of butaprost is species-specific or there may be other forms of EP₂ receptor subtype. EP₂ receptor has been thought to be coupled to stimulation of adenylate cyclase (4). We tested whether PGE₂ induces cAMP accumulation in COS-1 cells expressing the receptor. As shown in Fig. 3, PGE₂ dose-dependently increased cAMP level in these...
cells, the maximal level being 4.05 pmol/10^6 cells, which is 2.8-fold higher than that accumulated by PGE_2 in untransfected cells. On the other hand, PGE_2 neither inhibited forskolin-induced cAMP formation and nor accumulated inositol phosphates (data not shown). These results demonstrate that this receptor is an EP_2 subtype coupled exclusively to stimulation of adenylate cyclase. Since it would be difficult to examine the signal transduction of EP_2 receptor in detail in transiently transfected cells, stable expression of the cloned EP_2 receptor in Chinese hamster ovary cells is now in progress in our laboratory.

Fig. 4 shows the comparison of the amino acid sequences of the mouse EP_3 (15) and thromboxane (TX) A_2 receptors (28). The sequence of the mouse EP_2 receptor is 36.2% identical to that of the mouse EP_3 receptor within the hydrophilic segments. The identity is 29.9% between the mouse EP_2 and TXA_2 receptors within the hydrophobic segments of the two. The most highly conserved region among these three receptors is segment VII. Within the segment VII, Arg-344 in the EP_2 receptor has the four to that of the mouse EP_3 receptor within the hydrophobic segments. The identity is 29.9% between the mouse EP_2 and TXA_2 receptors within the hydrophobic segments of the two.

In summary, we present here the complete amino acid sequence of the mouse EP_2 subtype coupled to stimulatory adenylate cyclase. This work will be useful for molecular studies on function of the EP_2 receptor and for understanding diverse physiological roles of PGE_2 through PGE receptor subtypes.

Note Added in Proof—While this manuscript was under review, existence of EP_2 subtype, another subtype of EP receptor coupled to stimulation of adenylate cyclase, was suggested (Louttit, J. B, Head, S. A, and Coleman, R. A (1992) The 8th International Conference on Prostaglandins and Related Compounds, July 26–31, 1992, Montreal, Canada, p. 68).

REFERENCES