The mouse prostaglandin E receptor EP_2 subtype: cloning, expression, and Northern blot analysis

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Abstract **A functional cDNA clone for the mouse prostaglandin (PG) E receptor EP_2 subtype was isolated from a mouse cDNA library. The mouse EP_2 receptor consists of 362 amino acid residues with seven putative transmembrane domains. [^{3}H]PGE_2 bound specifically to the membrane of Chinese hamster ovary cells stably expressing the cloned receptor. This binding was displaced by unlabeled prostanoids in the order of PGE_2 = PGE_1 ≫ iloprost, a stable PGI_2 agonist > $PGF_{2\alpha}$ > PGD_2. Binding was also inhibited by butaprost (an EP_2 agonist) and to a lesser extent by M&B 28767 (an EP_3 agonist), but not by sulprostone (an EP_1 and EP_3 agonist) or SC-19220 (an EP_1 antagonist). PGE_2 and butaprost increased the cAMP level in the Chinese hamster ovary cells in a concentration-dependent manner. Northern blot analysis revealed that EP_2 mRNA is expressed most abundantly in the uterus, followed by the spleen, lung, thymus, ileum, liver, and stomach.**

Key words: Prostaglandin E_2; Prostanoid EP_2 receptor; Tissue distribution

1. Introduction

Prostaglandin (PG) E_2 produces a broad range of biological actions in diverse tissues through binding to specific receptors on plasma membranes [1,2]. The pharmacological actions of PGE_2 are diverse among tissues; PGE_2 causes contraction or relaxation of vascular and nonvascular smooth muscle, and stimulates or suppresses the secretion of neurotransmitters and hormones. Pharmacological studies suggested that there are four subtypes of PGE receptors, EP_1, EP_2, EP_3 and EP_4, which are thought to differ in their signal transduction mechanisms; they are presumed to be coupled to Ca^{2+} mobilization, and the stimulation, inhibition, and stimulation of adenylate cyclase, respectively [3–5]. Among the four subtypes, EP_2 and EP_4 are coupled to the same signal transduction pathway, stimulation of adenylate cyclase, but differ in their activities induced by some ligands including butaprost and AH23848B [5–7]. While EP_2 is sensitive to butaprost, EP_4 is insensitive to butaprost but sensitive to AH23848B. PGE_2 has been shown to increase the cAMP level in many tissues and cells [1], suggesting that the EP_2 and/or EP_4 receptor is widely distributed and mediates various actions of PGE_2. However, it remains unclear which EP receptor contributes to each action of PGE_2 in various tissues. We have isolated cDNAs for three subtypes of mouse PGE receptor, EP_1, EP_3, and EP_4. Using these mouse clones, we characterized their structural, binding and signaling properties and tissue distribution [6,8–10]. Recently, Regan et al. cloned a human PGE receptor, which is similar in its ligand binding properties to the pharmacologically defined EP_2 subtype [11]. However, tissue distribution study using the human clone has not been well established because of the restriction of available human tissues. Furthermore, the results of various pharmacological studies have been interpreted to indicate that there are differences among prostanoid receptors from different species [3]. It is important to examine the properties of the receptor molecules from identical species to understand the actions of prostanoids. For the purpose of comparative analyses in the mouse PGE receptors, we cloned a cDNA for the mouse EP_2 receptor by PCR and hybridization screening. Using this clone, we found that the EP_2 receptor mRNA is widely distributed throughout various mouse tissues and expressed most abundantly in the uterus. We also present here the primary structure of the mouse EP_2 receptor, together with those of the other seven mouse prostanoid receptors.

2. Materials and methods

2.1. Materials

Butaprost, SC-19220 and misoprostol, M&B 28767, and sulprostone were generous gifts from Dr. P.J. Gardiner of Bayer UK Ltd., Dr. P.W. Collins of Searle, Dr. M.P.L. Caton of Rhone-Poulenc Ltd., and Schering, respectively. [5,6,8,9,11,12,14,15-^{3}H]PGE_2 (181 Ci/mmol), iloprost, and the ^{125}I-labeled cyclic AMP assay system were obtained from Amersham Corp. [α-^{32}P]dCTP (3,000 Ci/mmol) and [α-^{32}P]UTP (3,000 Ci/mmol) were obtained from DuPont-New England Nuclear. PGE_2, 11-deoxy-PGE_1, 19(R)OH-PGE_2, 1-OH-PGE_1, and 16,16-dimethyl-PGE_2 were purchased from Cayman Chemical (Ann Arbor, MI). PGE_1, $PGF_{2\alpha}$ and PGD_2 were obtained from Funakoshi Pharmaceuticals (Tokyo, Japan). Sources of other materials are shown in the text.

2.2. Amplification of a cDNA fragment of the mouse EP_2 receptor by PCR

First-strand cDNA was synthesized from mouse lung total RNA using random hexanucleotides as primers. Degenerate PCR primers, 5′-CTGGGATCCATGCTCATGCT(CG)TT(CT)GC(ACGT)ATGG-C-3′ and 5′-GCAGAATTCAGACGGCGAA(CG)GT(AG)AT(GT)-GTCAT-3′, corresponding to the putative third and sixth transmembrane domains of the human EP_2 receptor [11] were synthesized. The thermal cycle program was 94°C for 0.8 min, 48°C for 1 min, 72°C for 3 min for the first 2 cycles, 94°C for 0.8 min, 50°C for 1 min, 72°C for

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The nucleotide sequence reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with the accession number D50589.

Abbreviations: PG, prostaglandin; PCR, polymerase chain reaction; TX, thromboxane.

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2 min for the next 3 cycles, followed by 94°C for 0.8 min, 55°C for 1 min, 72°C for 3 min for 27 cycles. Amplified cDNAs were subcloned into pBluescript II SK(+) (Stratagene). A 466-base pair clone showed a sequence highly homologous to the human EP_2 receptor cDNA.

2.3. Isolation of a functional cDNA clone

A mouse lung cDNA library was prepared as described previously [12]. Approximately 3.0×10^5 recombinant clones from this library were screened by hybridization with the cDNA fragment obtained by PCR. Hybridization was carried out under the conditions described previously [13]. Four positive clones were isolated, and a representative clone, ML202, was subjected to sequence and expression analyses. Nucleotide sequencing was carried out on both strands by the dideoxy chain termination method.

2.4. Expression in CHO cells and functional analyses

The *Eco*RI insert of ML202 was subcloned into pdKCR-dhfr [14], and the resultant plasmid DNA was transfected into Chinese hamster ovary (CHO) cells deficient in dihydrofolate reductase activity (CHO-dhfr$^-$) by the lipofection method [15]. Establishment of CHO cell clones stably expressing the receptor was performed essentially as described previously [9]. Using the crude membrane fraction prepared from a CHO cell clone expressing the receptor, [^{3}H]PGE_2 binding was determined as described previously [6]. The cAMP levels in the CHO cell clone were measured as described [6].

2.5. Northern blot analysis

Total RNAs from various mouse tissues were isolated by the acid guanidinium thiocyanate/phenol/chloroform method [16], and poly(A)$^+$ RNAs were purified using Oligotex dT30 < Super > (Takara Shuzo, Kyoto, Japan). For the detection of EP_2 transcripts, 5 μg of poly(A)$^+$ RNAs purified twice with the Oligotex from various tissues were separated by electrophoresis on a 1.2% agarose gel, and transferred onto a nylon membrane (Hybond-N, Amersham). An antisense RNA probe was synthesized with T7 RNA polymerase using linearized ML202 as the template. For the detection of the EP_4 mRNA, 5 μg of poly(A)$^+$-rich RNA (purified once with the Oligotex) was used. An antisense RNA probe was synthesized with T7 RNA polymerase using linearized MP412 with the *Xho*I fragment deleted as the template. Hybridization was carried out at 70°C for 4 h in 5 × SSC, 50% formamide, 5 × Denhardt's solution, 0.2% SDS, 0.05 M sodium phosphate (pH 6.5), 250 μg/ml heat-denatured salmon sperm DNA, 200 μg/ml yeast transfer RNA, and the ^{32}P-labeled probe. Filters were washed at 70°C in 2 × SSC and 1% SDS for 10 min, in 0.2 × SSC and 0.5% SDS for the next 10 min, and then in 0.1 × SSC and 0.1% SDS for 20 min. The filter for detecting the EP_4 mRNA was treated with 1 μg/ml RNase A at 20°C for 10 min, and washed again at 70°C in 0.1 × SSC and 0.1% SDS for 10 min.

3. Results and discussion

A mouse cDNA clone, ML202, was isolated by PCR and subsequent screening of a mouse lung cDNA library. Fig. 1 shows the nucleotide and deduced amino acid sequences of ML202. ML202 contains a 1.7-kilobase (kb) insert which has an open reading frame of 1,086 base pairs. The deduced amino acid sequence showed that it encodes a protein of 362 amino acid residues with an estimated molecular weight of 40,478. The ATG assigned as the initiation codon matched well to the Kozak consensus sequence for translation initiation [17]. Analysis of the predicted amino acid sequence indicated that it possesses seven hydrophobic segments typical of guanine nucleotide-binding protein-coupled rhodopsin-type receptors, and is 86% identical to that of the human EP_2 receptor [11]. Two potential *N*-glycosylation sites [18] were found in the N-terminal and the first extracellular loop regions. Five potential sites of phosphorylation by protein kinase C [19] were found in the second and third intracellular loops and the C-terminal regions.

Membranes of CHO cells expressing the cloned receptor showed specific binding of [^{3}H]PGE_2. Scatchard analysis of this binding yielded a dissociation constant of 26.7 nM and maximal binding of 822 fmol/mg protein. Fig. 2 shows the specificity of

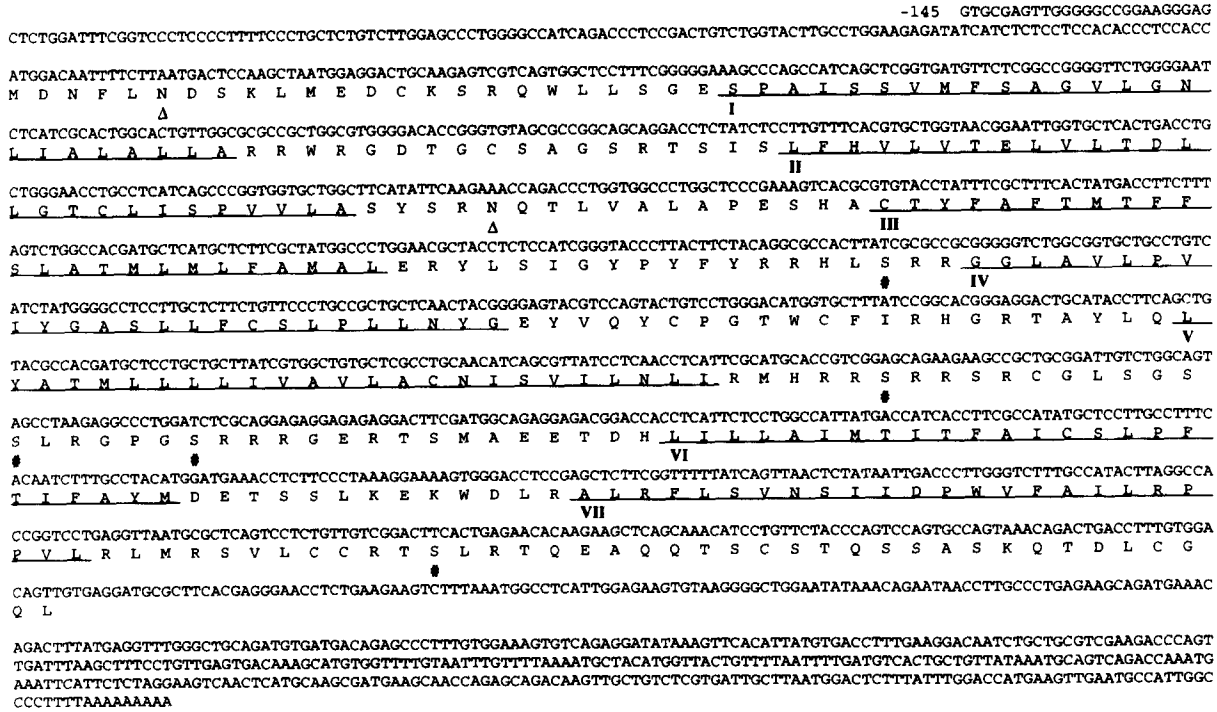

Fig. 1. Nucleotide and deduced amino acid sequences of ML202. The deduced amino acid sequence is shown beneath the nucleotide sequence using the single-letter code. The positions of the putative transmembrane segments, I–VII, are underlined. Triangles = potential *N*-glycosylation sites; parallel crosses = potential sites of phosphorylation by protein kinase C.

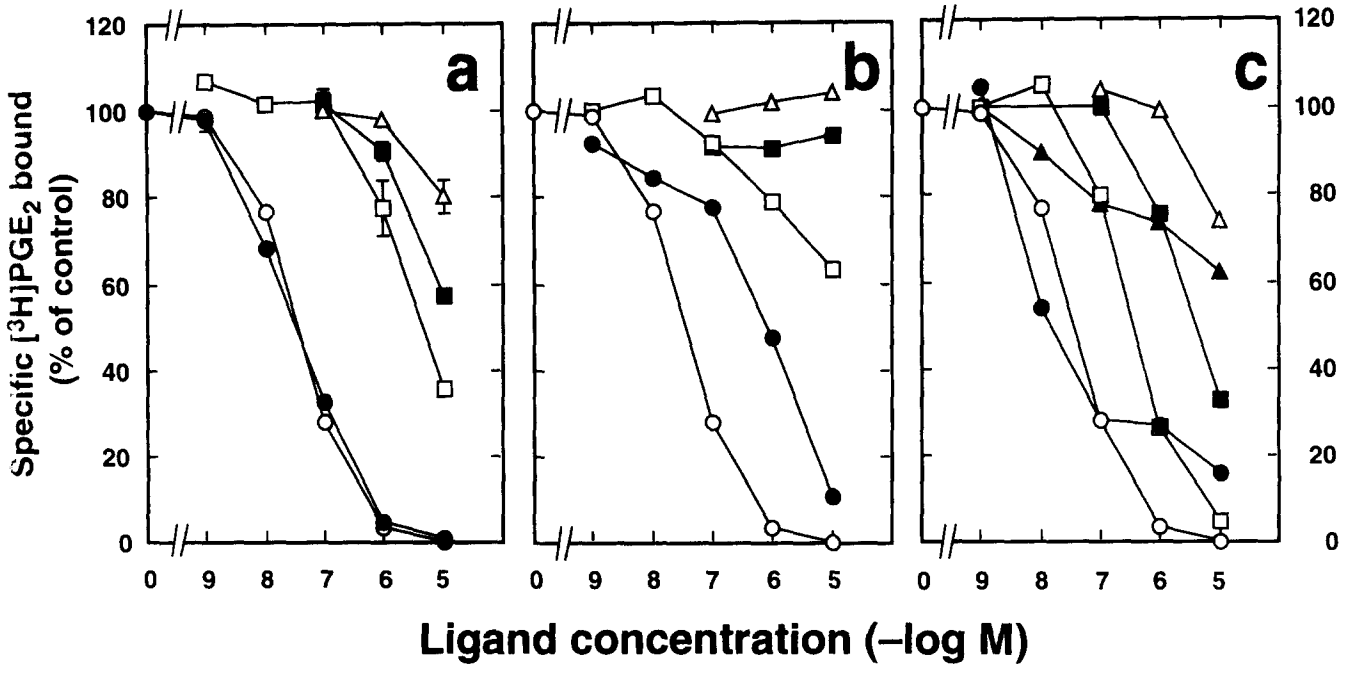

Fig. 2. Binding of $[^3H]PGE_2$ to ML202-expressing CHO cell membranes. (a) Displacement of $[^3H]PGE_2$ binding by various prostanoids. Membranes of CHO cells expressing ML202 were incubated with 4 nM $[^3H]PGE_2$ and various unlabeled prostanoids at the concentrations indicated. ○ = PGE_2; ● = PGE_1; □ = iloprost; ■ = $PGF_{2\alpha}$; △ = PGD_2. (b) Displacement of $[^3H]PGE_2$ binding by ligands for PGE receptor subtypes. ● = butaprost; ▯ = M&B 28767; ■ = sulprostone; △ = SC-19220. (c) Displacement of $[^3H]PGE_2$ binding by other EP agonists. ● = 16,16-dimethyl-PGE_2; □ = 11-deoxy-PGE_1; ■ = misoprostol; △ = 19(R)OH-PGE_2; ▲ = 1-OH-PGE_1. The same data for PGE_2 as shown in (a) are displayed in (b) and (c).

this binding. As shown in Fig. 2a, specific $[^3H]PGE_2$ binding was displaced by unlabeled prostanoids in the order of PGE_2 = $PGE_1 \gg$ iloprost, a stable PGI_2 agonist > $PGF_{2\alpha}$ > PGD_2. This result indicates that ML202 encodes a specific PGE receptor. As shown in Fig. 2b, further analyses using several ligands specific to PGE receptor subtypes demonstrated that the binding was also inhibited by butaprost (an EP_2 agonist) and to a lesser extent by M&B 28767 (an EP_3 agonist), but not by sulprostone (an EP_1 and EP_3 agonist) and SC-19220 (an EP_1 antagonist). These results indicate that ML202 encodes the mouse EP_2 receptor. We further characterized the binding specificity of the mouse EP_2 receptor for various ligands including EP_2 agonists. As shown in Fig. 2c, specific $[^3H]PGE_2$ binding was also inhibited by several ligands in the order of 16,16-dimethyl-PGE_2 (a nonselective EP agonist) [6,11] > 11-deoxy PGE_1 (an EP_2, EP_3, and EP_4 agonist) [6,11] > misoprostol (an EP_2, EP_3, and EP_4 agonist) [10,20,21] > 1-OH-PGE_1 (an EP_4 partial agonist) [6] > 19(R)OH-PGE_2 (an EP_2 agonist) [11,22]. This binding characteristic was in good agreement with that of the human EP_2 receptor [11], except for the poor reactivity of 19(R)OH-PGE_2 to the mouse receptor. This may have been at least in part due to species difference between the two receptors.

Fig. 3 shows the comparison of the amino acid sequences among the cloned mouse prostanoid receptors [8–10, 12,13,23,24]. The EP_2 receptor showed a higher degree of homology to the DP, IP, and EP_4 receptors than to the other prostanoid receptors. It is intriguing phylogenetically that the EP_2, DP, IP, and EP_4 receptors are all coupled to stimulation of adenylate cyclase. Among these receptors, the EP_2 receptor possesses higher sequence homology with the DP and IP receptors rather than with the EP_4 receptor; the EP_2 receptor shares 44%, 39%, and 31% overall amino acid identity with the DP, IP, and EP_4 receptors, respectively. This indicates that the divergence between the EP_2 and EP_4 receptors occurred before the derivation of the DP and IP receptors from the EP_2 receptor [25].

We next examined the signal transduction pathway of the mouse EP_2 receptor. As shown in Fig. 4, PGE_2 generated cAMP in a concentration-dependent manner in CHO cells expressing the EP_2 receptor. In addition, butaprost and 11-deoxy-PGE_1 were also effective in stimulating cAMP formation in the cells, although their half-maximal concentrations for this effect were about 10-fold higher than that of PGE_2. 19(R)OH-PGE_2 and 1-OH-PGE_1 were less effective than the ligands described above. We also examined whether PGE_2 induces accumulation of inositol phosphates, but PGE_2 did not evoke phosphatidylinositol hydrolysis in the cells (data not shown), as in the case of the EP_4 receptor [10].

PGE_2 has been shown to increase the cAMP level in many tissues and cells [1] through its interaction with the EP_2 and/or EP_4 receptors. To elucidate which EP receptor subtype is involved in cAMP-mediating actions of PGE_2 in each tissue, we compared the expression patterns of the EP_2 and EP_4 receptors by Northern hybridization. As shown in Fig. 5a, the level of EP_2 expression was much lower than that of the EP_4 in most of the tissues examined. In the previous report, using standard hybridization analysis, Regan et al. detected an EP_2 message only in the mRNA from human placenta and lung, but not in the other tissues [11]. We, therefore, utilized highly purified poly(A)$^+$ RNAs for more sensitive detection (Fig. 5b). As a result, a positive band at 2.2 kilobase was detected in a number of tissues

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                                                       I                              II
mEP2                          MDNFLNDSKLMEDCKSRQWLLSGESP-AISSVMFSA-GVLGNLIALALLARRWRGDTGCSAGSRTSISLFHVLVT  73
mDP                                    MNESYRCQTSTWVERGSSA-TMGAVLFGA-GLLGNLLALVLLARSGLGSCRPGPLHPPP-SVFYVLVC  65
mIP     MKMMASDGHPGPPSVTPGSPLSAGGREWQGMAGGCWNITYVQDSVGP-ATSTLMFVA-GVVGNGLALGIL------GARRRSH-PS--A-FAVLVT  84
mEP4     MAEVGGTIPRSNRELQRCVLLTTTIMSIPGVNASFSSTPERLN-SPVTIPAVMFIF-GVVGNLVAIVVL------CKSRKEQKET--T-FYTLVC  84
mEP3α                       MASMWAPEHSAEAHSNLS-STTDDCGSVSV-AFPITMMVT-GFVGNALAM-LLVSRSY--RRRESKRKK----FLLCIG  70
mEP1                   MSPCGLNLSLADEAATCATPRLPNTSVVLPTGDNGTSP-ALPI-FSMTLGAVSNVLALALLAQVAGRMRRRRSAA----T-FLLFVA  80
mFP                         MSMNSSKQPVSPAAGLIANTTCQTENRLSV-FFSI-IFMTVGILSNSLAIAILMKAYQRF-RQKSKA----S-FLLLAS  71
mTP                         MWPNGTSLGACFRPVNIT--LQERRAIASP-WFAA-SFCALGLGSNLLALSVLAGA-----RPGAGPRS--S-FLALLC  67

                                      III                                        IV
mEP2    ELVLTDLLGTCLISPV-VLASYSRNQTLVALAPES--HACTYFAFTMTFFSLATMLMLFAMALERYLSIGYPYFYRRHLSR-RGGLAVLPVIYGAS 165
mDP     GLTVTDLLGKCLISPM-VLAAYAQNQSLKELLPASGNQLCETFAFLMSFFGLASTLQLLAMAVECWLSLGHPFFYQRHVTL-RRGVLVAPVVAAFC 159
mIP     GLAVTDLLGTCFLSPA-VFVAY--G-LAHGGT-M----LCDTFDFAMTFFDLASTLILFAMAVERCLALSHPYLYAQLDGP-PCARFALPSIYAFC 177
mEP4    GLAVTDLLGTLLVSPV-TIATYMKG-QWPGDQ-A----LCDYSTFILLFFGLSGLSIICAMSIERYLAINHAYFYSHYVDK-RLAGLTLFAIYASN 172
mEP3α   WLALTDLVGQLLTSPV-VILVYLSQRRWEQLDP-SGR-LCTFFGLTMTVFGLSSLLVASAMAVERALAIRAPHWYASHMKT-R-ATPVLLGVWLSV 161
mEP1    SLLAIDLAGHVIPGAL-VLRLYTAGRA-PAGG-A-----CHFLGGCMVFFGLCPLLLGCGMAVERCVGVTQPLIHAARVSVAR-ARLALAVLAAMA 167
mFP     GLVITDFFGHLINGGIAVFV-YASDKDWIRFD-QSNI-LCSIFGISMVFSGLCPLFLGSAMAIERCIGVTNPIFHSTKITSKH-VKMILSGVCMFA 163
mTP     GLVLTDFLGLLVTGAI-VASQHAALLDWRATDP-SCR-LCYFMGVAMVFFGLCPLLLGAAMASERFVGITRPFSRPTATSR-R-AWATVGLVWVAA 158

                                      V
mEP2    LLFCSLPLLNYGEY-VQYCPGTWCFIRHGR-------------TAYLQLYATMLLLLIVAVLACNISVIL-NLIRMHRRSRRSRCGLSGSSLR--- 243
mDP     LAFCALPFAGFGKF-VQYCPGTWCFIQMIHKERS------FSVIGFSVLYSSLMALLVLATVVCNLGAMY-NLYDMHRRHRHYPHRCSRDRAQ--- 244
mIP     CLFCSLPLLGLGEHQ-QYCPGSWCFIRM-R--SAP------GGCAFSLAYASLMALLVTSIFFCNGSVTL-SLYHMYRQQRRHHGSFVP------- 255
mEP4    VLFCALPNMGLGRSERQY-PGTWCFIDWTT--NVT------AYAAFSYMYAGFSSFLILATVLCN-VLVCGALLRMHRQFMRRTSLGTEQHHAAAA 258
mEP3α   LAFALLPVLGVGRYSVQW-PGTWCFISTGPAGNETDPAREPGSVAFASAFACLGLLALVVTFACN-LATIKALVSR-------------------- 235
mEP1    LAVALLPLVHVGRYELQY-PGTWCFISLGPRG------GWR-QALLAGLFAGLGLAALLAALVCNTLSGL-ALLRA-RWRRRRSRRFRKTAGPDDR 253
mFP     VFVAVLPILGHRDYQIQA-SRTWCFYNTEHIE------DWE-DRFYLLFFSFLGLLALGVSFSCNAVTGV-TLLRV-KF--RSQ----------- 235
mTP     GALGLLPLLGLGRYSVQY-PGSWCFLTLGT---------QRGDVVFGLIFALLGSASVGLSLLLNTVSVA-TL---------------------- 220

                                                       VI                                   VII
mEP2    ---------------------------GPGSRRRGERTSMAEETDHLI-LL-AIMTITFAICSLPFTIFAYMDETSSL------------KEKW-DL 298
mDP     ----------------------------SGSDYRHGSLHPLEELDHFV-LL-ALMTVLFTMCSLPLIYRAYYGAFKLENKAEG--D----SE---DL 302
mIP     ------------------------------------TSRAREDEVYHLI-LL-ALMTVIMAVCSLPLMIRGFTQAIAPDS-----------REMG-DL 303
mEP4    AAVASVACRGHAGASPALQRLSDF-----RRRRSFRRIAGAEIQMVI-LLIATS-LVVLICSIPLVVRVFINQLYQPNVV---KDIS----RNPDL 340
mEP3α   -------CRAKAAVSQS----------------SAQWGRI-TTETAIQLM-GIM-CVLSVCWSPLLIMMLKMIFNQMSVEQCKTQMGKEKECNSFL 305
mEP1    RRWGSRGPRLASASSASSITSATATLRSSRGGGSARRVHAHDVEMVGQLV-GIM-VVSCICWSPLLVLVV-LAIGGWNSNSL--Q----RPL--FL 338
mFP     ---------------------------------QHRQGRSHHLEMIIQLL-AIM-CVSCVCWSPFLVTMANIAINGNNSPVTC-------E--TTL 287
mTP     -------CRVYHT----------------R--EATQRPRDCEVEMMVQLV-GIM-VVATVCWMPLLVFIMQTLLQTPPVMSFSGQLLRATEHQ-LL 288

mEP2    RALRFLSVNSIIDPWVFAILRPPVLRL-MRSVLCCRTSLRTQEAQQTSCSTQSSASKQTDLCGQL                           362
mDP     QALRFLSVISIVDPWIFIIFRTSVFRM-LFHKVFTRPLIYRNWSSHSQQSNVESTL                                    357
mIP     LAFRFNAFNPILDPWVFILFRKAVFQR-LKFWLCCLCARSVHGDLQAPLSRPASGRRDPPAPTSLQAKEGSWVPLSSWGTGQVA(30)    416
mEP4    QAIRIASVNPILDPWIYILLRKTVLSKAIEKIKCLFCRIGGSGRDSSAQHCSESRRTSSAMSGHSRSFLARELKEISSTSQTL(90)     513
mEP3α   IAVRLASLNQILDPWVYLLLRKILLRK-FCQIRDHTNYASSSTSLPCPGSSALMWSDQLER                               365
mEP1    -AVRLASWNQILDPWVYILLRQAMLRQ-LLRLLPLRVSAKGGPTELGLTKSAWEASSLRSSRHSGFSHL                       405
mFP     FALRMATWNQILDPWVYILLRKAVLRN-LYKLASRCCGVNIISLHIWELSSIKNSLKVAAISESPAAEKESQQASSEAGL            366
mTP     IYLRVATWNQILDPWVYILFRRSVLRR-LHPRFSSQLQAVSLRRPPAQAMLSGP                                      341
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Fig. 3. Comparison of the amino acid sequences of mouse prostanoid receptors. The deduced amino acid sequences of the mouse EP_2 receptor (*mEP2*), mouse PGD receptor (*mDP*), mouse PGI receptor (*mIP*), mouse EP_4 (*mEP4*), mouse $EP_{3\alpha}$ receptor (*mEP3α*), mouse EP_1 receptor (*mEP1*), mouse PGF receptor (*mFP*), and mouse TXA_2 receptor (*mTP*) are aligned to optimize homology. Identical amino acid residues in four or more sequences are indicated by *bold characters*. The approximate positions of the putative transmembrane regions are indicated *above* the amino acid sequences.

in which the EP_2 receptor has been identified in the pharmacological studies [3]; the tissues include the uterus, lung, ileum, liver, stomach, thymus and spleen. Another hybridizing band was also seen at 2.8 kilobase in the uterus, lung, thymus and spleen. These data demonstrate that the EP_2 receptor was widely distributed throughout the mouse tissues.

The highest level of EP_2 mRNA expression was seen in the uterus, in which EP_4 was also expressed. As reported previously, EP_3 is also expressed in the uterus [9]. PGE_2 can cause both relaxation and contraction of myometrium [26]. Therefore, in the uterus the relaxant activity of PGE_2 may be exerted via EP_2 and/or EP_4, and the contractile response may be mediated via EP_3. Both EP_2 and EP_4 are expressed in the ileum and thymus. PGE_2 and butaprost were reported to cause relaxation of ileal smooth muscle [27], while PGE_2 also stimulates intestinal secretion via elevation of mucosal cAMP level [28–30]. It is likely that EP_2 and EP_4 are involved in relaxation of intestinal smooth muscle and stimulation of intestinal secretion, respectively. The EP_2 transcript was detected in the liver, in which no signals for the EP_4 receptor were detected. PGE_2 was reported to be involved in liver regeneration [31], and PGE_2 and other cAMP-elevating agents can induce the expression of hepatocyte growth factor [32]. EP_2 might be involved in these actions of PGE_2. EP_2 was also expressed in the spleen, lung, and stomach. In the lung, the other PGE receptors are also expressed [8,9], but EP_2 may be partly involved in PGE_2-induced relaxation of the trachea [7].

In summary, we presented in this paper the primary structure, ligand binding profile, signaling pathway, and tissue distribution of the mouse PGE receptor EP_2 subtype. Distribution analyses of EP_2 and EP_4 in the mouse tissues suggested that PGE_2 exerts multiple functions via these receptor subtypes differentially expressed in the body. This work will be useful for understanding the diverse physiological roles of PGE_2 mediated through multiple receptor subtypes.

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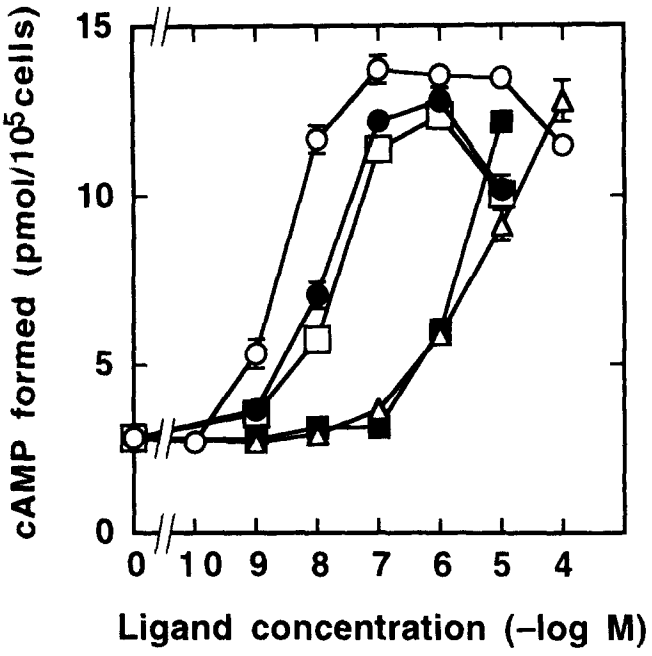

Fig. 4. Effects of PGE_2 and several ligands on the cAMP level in ML202-expressing CHO cells. CHO cells were incubated with the indicated concentrations of each ligand, and then the cAMP level was determined as described elsewhere [6]. ○ = PGE_2; ● = butaprost; □ = 11-deoxy-PGE_1; ■ = 19(R)OH-PGE_2; △ = 1-OH-PGE_1.

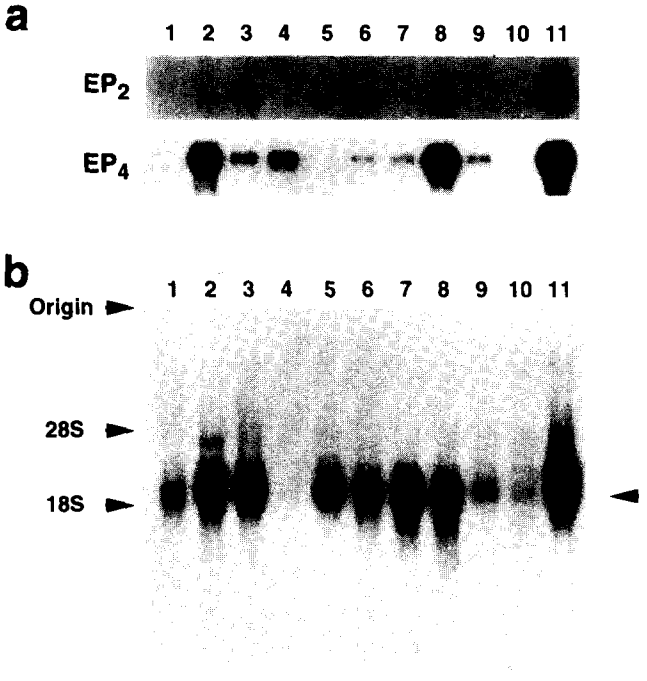

Fig. 5. Northern blot analysis of RNAs isolated from various mouse tissues. (a) Comparison of the levels of expression of the mouse EP_2 receptor (upper) and EP_4 receptor (lower). Poly(A)$^+$-rich RNA (5 μg) was applied to each lane. (b) Tissue distribution of the mouse EP_2 receptor. Highly purified poly(A)$^+$ RNA (5 μg) was applied to each lane. Hybridization analysis was carried out as described in section 2. 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.

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