Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 267, No. 10, Issue of April 5, pp. 6463–6466, 1992
© 1992 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

Cloning and Expression of a cDNA for Mouse Prostaglandin E Receptor EP_3 Subtype*

(Received for publication, December 12, 1991)

Yukihiko Sugimoto, Tsunehisa Namba‡, Akiko Honda, Yasunori Hayashi‡, Manabu Negishi, Atsushi Ichikawa, and Shuh Narumiya‡§

From the Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences and ‡Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

A functional cDNA clone for mouse EP_3 subtype of prostaglandin (PG) E receptor was isolated from a mouse cDNA library using polymerase chain reaction based on the sequence of the human thromboxane A_2 receptor and cross-hybridization screening. The mouse EP_3 receptor consists of 365 amino acid residues with putative seven-transmembrane domains. The sequence revealed significant homology to the human thromboxane A_2 receptor. Ligand binding studies using membranes of COS cells transfected with the cDNA revealed specific [^{3}H]PGE_2 binding. The binding was displaced with unlabeled PGs in the order of PGE_2 = PGE_1 > iloprost > $PGF_{2\alpha}$ > PGD_2. The EP_3-selective agonists, M&B 28,767 or GR 63799X, potently competed for the [^{3}H]PGE_2 binding, but no competition was found with EP_1- or EP_2-selective ligands. PGE_2 and M&B 28,767 decreased forskolin-induced cAMP formation in a concentration-dependent manner in Chinese hamster ovary cells permanently expressing the cDNA. Northern blot analysis demonstrated that the EP_3 mRNA is expressed abundantly in kidney, uterus, and mastocytoma P-815 cells and in a lesser amount in brain, thymus, lung, heart, stomach, and spleen.

Eicosanoids comprising various oxygenated metabolites of arachidonic acid such as prostaglandins (PGs)[1] and leukotrienes exert a variety of biological activities for maintenance of local homeostasis in the body (1, 2). These metabolites act on a cell surface receptor specific for each member to exert their actions. Among them, PGE_2 in particular, produces a broad range of biological actions in diverse tissues. PGE_2 receptors are pharmacologically subdivided into three subtypes, EP_1, EP_2, and EP_3 (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 (4–8). Pharmacological actions of these subtypes have been well characterized, and the EP_3 receptor has been suggested to be involved in inhibition of gastric acid secretion (7), modulation of neurotransmitter release in central and peripheral neurons (9), and inhibition of sodium and water reabsorption in kidney tubulus (8, 10, 11). In spite of this information, none of the receptors has been isolated, and their molecular characterization has been carried out only poorly. Recently we cloned a cDNA for the human TXA_2 receptor (12). Based on its sequence we carried out PCR to amplify a homologous sequence from mouse cDNA and, using this fragment as a hybridization probe, performed homology screening. Analysis of nucleotide sequence and expression of the isolated clone revealed that it encodes the mouse EP_3 receptor. We report here the complete nucleotide and deduced amino acid sequences of this receptor, and its ligand binding and biochemical properties analyzed in several mammalian expression systems. This study will be of help in understanding similarity and divergence of eicosanoid receptors.

EXPERIMENTAL PROCEDURES

Materials—M&B 28,767, GR 63799X, butaprost, and SC-19220 were generous gifts from Dr. M. P. L. Caton of Rhone-Poulenc Ltd., Dr. B. M. Bain of Glaxo Group Research Ltd., Dr. P. J. Gardiner of Bayer UK Ltd., and Dr. P. W. Collins of Searle, respectively. [α-^{32}P]dCTP (3,000 Ci/mmol) and [5,6,8,11,12,14,15-^{3}H]PGE_2 (185 Ci/mmol) were obtained from Du Pont-New England Nuclear. PGE_1, PGE_2, PGD_2, and $PGF_{2\alpha}$ were purchased from Funakoshi Pharmaceuticals (Tokyo, Japan). Iloprost and the ^{125}I-labeled cyclic AMP assay system were obtained from Amersham Corp. Forskolin and 3-isobutyl-1-methylxanthine were from Sigma. Sources of other materials are shown in the text.

Amplification of a Mouse cDNA Fragment Homologous to the Human TXA_2 Receptor by PCR—First strand cDNA was synthesized from mouse lung total RNA by using random hexanucleotides as primers. PCR primers were designed based on the human TXA_2 receptor cDNA sequences corresponding to the putative third and sixth transmembrane domains of the receptor (12). Mouse lung cDNA served as template in 30 cycles of PCR with 1 min of denaturation at 95 °C, 0.5 min of annealing at 60 °C, and 1.5 min of extension at 72 °C on a Zymoreactor (Atto Corp., Tokyo, Japan). A single 418-base pair cDNA fragment was amplified and subcloned into pBluescript SK(+) (Stratagene). A clone isolated (LT3) showed a sequence 78% homologous to the corresponding region of the human cDNA.

Molecular Cloning by Cross-hybridization—Mouse lung cDNA prepared by an oligo(dT) priming method was size-selected (>1.8 kilobases) and inserted into the *Eco*RI site of λZAPII DNA (Stratagene) with *Eco*RI adaptors (New England Biolabs, Inc.). The 1.9×10^5 clones derived from the cDNA library were screened by hybridization with LT3. Hybridization was carried out at 58 °C in 6 × SSC (900 mM NaCl and 90 mM sodium citrate) containing 5 × Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin) and 0.5% sodium dodecyl sulfate, and filters were washed at 60 °C in 2 × SSC containing 1% sodium dodecyl sulfate. Among several clones hybridizing positively to LT3, we picked up one showing a signal apparently weaker than others and subjected it to further screening. Nucleotide sequencing of the isolated clone (ML64) revealed that it was a partial clone. Using this clone as a hybridization probe, we then screened the cDNA library of mouse mastocytoma P-815 cells for a full-length clone. From 7.2×10^5 clones

* This work was supported in part by Grants-in-aid for Scientific Research 03260102, 03454150, 03268218, 02404079, and 03671048 from the Ministry of Education, Science and Culture of Japan and by grants from the Naito Foundation and the Japanese Foundation on Metabolism and Diseases. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D10204.

§ To whom correspondence should be addressed.

[1] The abbreviations used are: PG, prostaglandin; G protein, heterotrimeric GTP-binding protein; IC_{50}, drug concentration to inhibit response by 50%; PCR, polymerase chain reaction; TX, thromboxane; CHO, Chinese hamster ovary.

Downloaded from www.jbc.org at Massachusetts Institute of Technology on February 28, 2009

of the P-815 λZAPII library, nine clones were isolated and subjected to sequence analysis. Nucleotide sequencing was carried out on double-stranded templates using the dideoxy chain termination method. One clone (MP660) was a full-length clone with a 1,095-base pair open reading frame.

cDNA Expression in COS-1 Cells and Binding Assay—The *Eco*RI insert of MP660 was subcloned into pcDNAI (Invitrogen), a modified eukaryotic vector, and the resultant plasmid DNA was transfected into COS-1 cells by the DEAE-dextran method (13). After culture for 72 h, the cells were harvested, and crude membranes were prepared as described (12). Using these membranes, [^{3}H]PGE_2 binding was determined, and protein was measured as described previously (14).

Stable Expression of the Cloned Receptor and cAMP Assay—cDNA transfection and cell line establishment were performed essentially as described by Nakajima *et al.* (15). The *Eco*RI fragment of MP660 was inserted into pdKCR-dhfr, a eukaryotic expression vector containing a mouse gene as a selection marker (16). The plasmids were transfected to CHO cells deficient in dihydrofolate reductase activity (CHO-dhfr$^-$) (17) by the calcium phosphate method (18). Cell populations expressing the cDNA together with dihydrofolate reductase were selected in α-modification of Eagle's medium lacking ribonucleosides and deoxyribonucleosides and containing penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% dialyzed fetal bovine serum (Cell Culture Laboratories) (16). From these cell populations, clonal cell lines were isolated by single-cell cloning. Expression of the cDNA was assessed by RNA blotting. As a control, CHO cells were mock-transfected (transfected only with the vector) and isolated. These cells gave no signal on RNA blotting. Cyclic AMP levels in these cells were determined as described previously (19). Inositol phosphates were measured as described (15).

Northern Blots—Total RNAs from various mouse tissues were isolated by the acid guanidinium thiocyanate-phenol-chloroform method (20), and poly(A)$^+$ RNAs were purified using Oligotex dT_{30} (Takara Shuzo, Kyoto, Japan). Poly(A)$^+$ RNAs (10 μg) from each tissue were separated by electrophoresis on a 1.2% agarose gel, transferred onto nylon membranes (Hybond-N, Amersham Corp.), and hybridized with a ^{32}P-labeled *Eco*RI/*Bam*HI fragment of MP660 clone. Hybridization was carried out at 68 °C in 6 × SSC, and filters were washed at 68 °C in 1 × SSC.

RESULTS AND DISCUSSION

Fig. 1 shows nucleotide and deduced amino acid sequences of MP660. The amino acid sequence was assigned from the longest open reading frame of the cDNA. The nucleotide sequence surrounding the initiation codon agrees reasonably well with the consensus sequence (21). The polypeptide consists of 365 amino acid residues with an estimated molecular weight of 40,077. The hydropathicity profile determined by the Kyte and Doolittle method (22) and the sequence homology analysis indicated that it possesses seven hydrophobic segments and shares a significant sequence similarity with other members of G protein-coupled receptors (23), especially with the human TXA_2 receptor (12) (38% in total). Similar to other G protein-coupled receptors (24, 25), potential *N*-

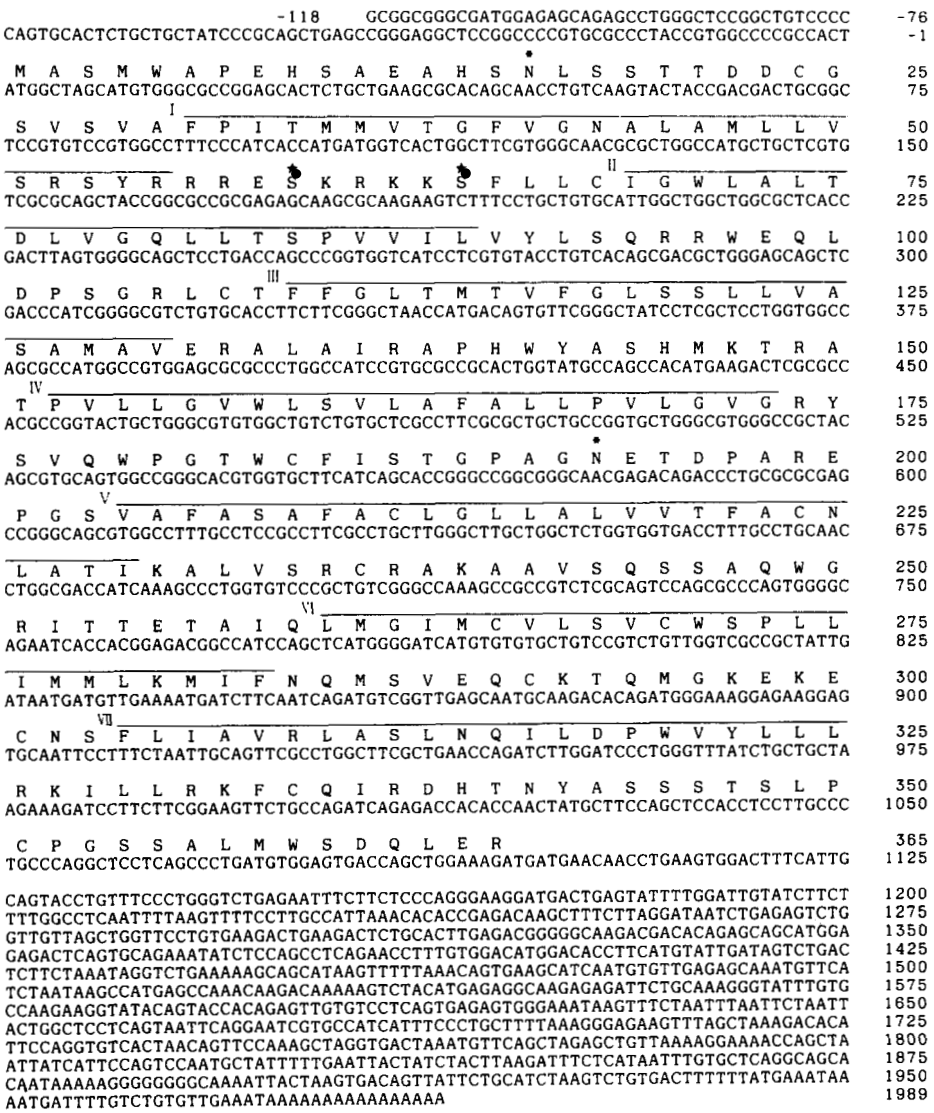

FIG. 1. **Nucleotide and deduced amino acid sequences of MP660.** The deduced amino acid sequence is shown above the nucleotide sequence using single-letter code. Positions of the putative transmembrane segments I-VII are indicated by *overlines* above the amino acid sequence. The termini of each segment are tentatively assigned on the basis of a hydropathicity 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.

Downloaded from www.jbc.org at Massachusetts Institute of Technology on February 28, 2009

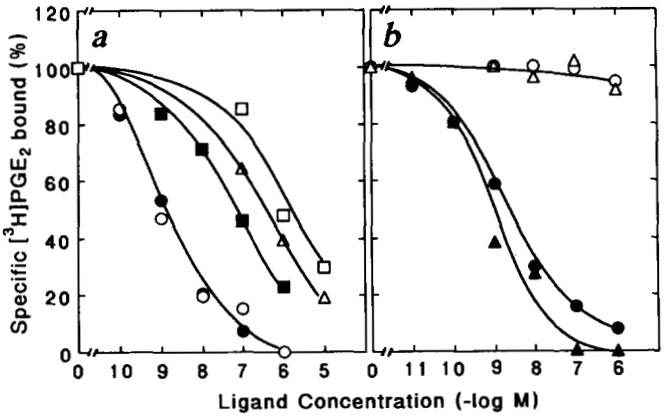

FIG. 2. **Binding of [^{3}H]PGE_2 to MP660-transfected COS-1 cell membranes.** *a*, displacement of [^{3}H]PGE_2 binding by various prostaglandins. Unlabeled prostaglandins were added to the binding assay mixture at indicated concentrations, and [^{3}H]PGE_2 binding was determined as described under "Experimental Procedures." ○, PGE_2; ●, PGE_1; ■, iloprost; △, $PGF_{2\alpha}$; and □, PGD_2. *b*, displacement of [^{3}H]PGE_2 binding by subtype-selective PGE analogues. Ligands used are M&B 28,767 (▲), GR 63799X (●), butaprost (△), and SC-19220 (○).

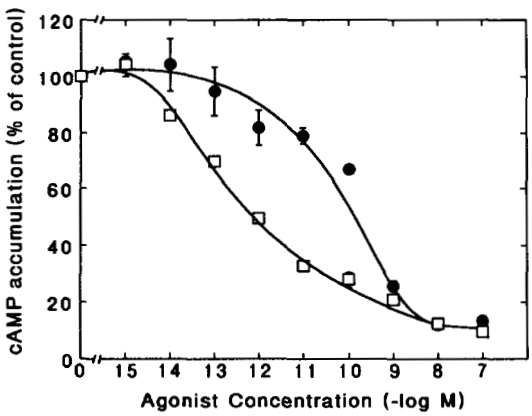

FIG. 3. **Inhibition by PGE_2 and EP_3-selective agonist of forskolin-induced cAMP accumulation in CHO cells permanently expressing the EP_3 receptor.** CHO cells permanently expressing the EP_3 receptor were incubated with 1 μM forskolin in the presence of the indicated concentrations of PGE_2 (●) or M&B 28,767 (□), and cAMP accumulation was determined as described (19). The incubation buffer contained 1 mM 3-isobutyl-1-methylxanthine. Each *point* represents the mean ± S.E. of triplicate determinations.

glycosylation sites are seen at the amino-terminal and the second extracellular loop regions, and nine serine and threonine residues at the carboxyl-terminal region as possible phosphorylation sites.

To identify a ligand for this receptor, MP660 was expressed in COS-1 cells, and membranes of the transfected cells were subjected to binding assays using various radioactive PGs. Among the PGs tested, [^{3}H]PGE_2 specifically bound to the membranes. Scatchard analysis of this binding yielded a dissociation constant (K_d) of 2.9 nM, which agrees well with that previously reported on binding of [^{3}H]PGE_2 to canine renal medullary membranes (26). The average density of binding sites in three experiments was 770 fmol/mg of protein of the transfected COS cell membranes. Specificity of this binding is shown in Fig. 2*a*. The binding of [^{3}H]PGE_2 was inhibited by unlabeled PGs in the order of $PGE_2 = PGE_1 >$ iloprost, a PGI_2 analogue $> PGF_{2\alpha} > PGD_2$. This characteristic of binding specificity was in good agreement with the PGE receptor previously characterized in various tissues (26, 27). Because PGE receptor is pharmacologically subdivided into three receptor subtypes, EP_1, EP_2, and EP_3, with different agonist and antagonist profiles (4), we further characterized the specificity of this [^{3}H]PGE_2 binding using ligands specific for PGE receptor subtypes. As shown in Fig. 2*b*, among various PGE analogues, only EP_3-specific agonists, GR 63799X and M&B 28,767, specifically competed for the [^{3}H]PGE_2 binding with equal potency, and they were more potent than PGE_2 itself. On the other hand, no competition was found at all with either an EP_1-specific antagonist, SC-19220, or an EP_2-specific agonist, butaprost. [^{3}H]PGE_2 did not bind to membranes of untransfected cells. These results established that MP660 encodes the EP_3 subtype of PGE receptor.

Possible association of the EP_3 receptor with inhibition of adenylate cyclase has been indicated (4). We tested this possibility by permanently expressing the cDNA in CHO cells and examining response of the cells to PGE analogues. As shown in Fig. 3, the transfected CHO cells showed a dose-dependent decrease to PGE_2 in forskolin-induced cellular cAMP accumulation. M&B 28,767, an EP_3-specific agonist, also inhibited forskolin-induced cAMP synthesis and was more potent than PGE_2 (IC_{50} of M&B 28, 767 $= 1 \times 10^{-12}$ M; IC_{50} of $PGE_2 = 1 \times 10^{-10}$ M). This potency of PGE_2 correlates well with that found in canine kidney and rat uterine membranes (11, 28). Either agonist alone did not increase cAMP accumulation. We also examined phosphatidylinositol turnover in the transfected CHO cells. Addition of up to 1 μM M&B 28,767 revealed no significant increase in inositol phosphate content over the basal levels (data not shown). These results demonstrated that the EP_3 receptor is coupled exclusively to inhibition of adenylate cyclase.

The mouse EP_3 and human TXA_2 receptors (12) are significantly similar in size and show highly homologous amino acid sequences, especially in the putative seven-transmembrane segments except segments I and V (Fig. 4). The most highly conserved regions are segment VII and that spanning the latter half of segment IV to the first 12 amino acids in the second extracellular loop (from Leu-165 to Phe-184), 63.6 and 80.0% homology, respectively. As in the human TXA_2 receptor, there is no Asp in the third transmembrane segment of the EP_3, a residue which is presumed to bind the amino group of ligands in the adrenergic receptors (29). Furthermore, Arg-309 in the EP_3 is equivalent to Arg-295 in the TXA_2 receptor, which are located at the position analogous to Lys-296 of bovine rhodopsin in the seventh transmembrane segment. The latter amino acid residue was assigned for retinal attachment in the rhodopsin molecule (30). These structural features may reflect the acidic nature of the ligand for the prostanoid receptors. EP_3 receptor has the two potential phosphorylation sites by cAMP-dependent protein kinase (31) in the first cytoplasmic loop, which may be relevant to the finding that [^{3}H]PGE_2 binding is affected by cAMP-dependent phosphorylation in brain membranes (32).

Poly $(A)^+$ RNAs were prepared from various mouse tissues and hybridized with the *Eco*RI/*Bam*HI fragment of MP660 (Fig. 5). A positive band was seen at 2.3 kilobases in a number of tissues in which PGE_2 has pharmacological effects and/or specific binding sites (4). Another hybridizing band was detected at an estimated mRNA size of 7.0 kilonucleotides in kidney, uterus, brain, and mastocytoma P-815 cells. Identity of this latter band is not known at present. The tissue most highly expressing EP_3 mRNA was kidney in which PGE_2 exerts an inhibitory effect on sodium and water reabsorption by inhibiting adenylate cyclase via G_i (8, 33). A significant band was also observed in stomach, suggesting that the receptor we cloned is indeed involved in inhibition of histamine-induced gastric acid secretion in this tissue (7). This analysis

Downloaded from www.jbc.org at Massachusetts Institute of Technology on February 28, 2009

```
                                                    I
PGER  MASMWAPEHSAEAHSNLSSTTDDCGSVSVAFPITMMVTGFVGNALAMLLVSRSYRRRESKR  61
                                                  *  *     *  *   * **       *   *
TXAR   MWPNGSSLGPCFRPTNITLEERRLIASPWFAASFCVVGLASNLLALSVLA-GARQGGSHT  59
                 II                                                 III
PGER  KKSFLLCIGWLALTDLVGQLLTSPVVILVYLSQRRWEQLDPSGRLCTFFGLTMTVFGLSSL  122
       ***     * ***  * * *   *          *   **  *** * *  *  **** *
TXAR  RSSFLTFLCGLVLTDFLGLLVTGTIVVSQHAALFEWHAVDPGCRLCRFMGVVMIFFGLSPL  120
                                                  IV
PGER  LVASAMAVERALAIRAPHWYASHMKTRATPVLLG-VWLSVLAFALLPVLGVGRYSVQWPGT  182
      *   *** ** * *  *          *      * **   **  *** ******* ** **
TXAR  LLGAAMASERYLGITRPFSRPAVASQRRAWATVGLVWAAALALGLLPLLGVGRYTVQYPGS  181
                                    V
PGER  WCFISTGPAGNETDPAREPGSVAFASAFACLGLLALVVTFACNLATIKALVSRCRAKAAVS  243
      ***   *       * * ***   *  ** *     *  *       *        *
TXAR  WCFLTLG---------AESGDVAFGLLFSMLGGLSVGLSFLLNTVSVATLCHVYHGQEAAQ  233
                          VI                                    VII
PGER  QSSAQWGRITTETAIQLMGIMCVLSVCWSPLLIMMLKMIFNQMSVEQCKTQMGKEKECNSF  304
      *         *   ** *** * **** ***                 *       *
TXAR  QRPRD---SEVEMMAQLLGIMVVASVCWLPLLVFIAQTVLRNPPAMSPAGQLSRTTE-KEL  291

PGER  LIAVRLASLNQILDPWVYLLLRKILLRKFCQIRDHTNYASSSTSLPCPGSSALMWSDQLER  365
      **  * *  ********* * *   **   *  *   *  * *       * *
TXAR  LIYLRVATWNQILDPWVYILFRRAVLRRL-QPRLSTRPRSLSLQPQLTQRSGLQ         344
```

FIG. 4. **Comparison of the amino acid sequences of the mouse EP_3 receptor and the human TXA_2 receptor.** The amino acid sequences of the mouse EP_3 (*upper*) and the human TXA_2 receptor (*lower*) are aligned to achieve the maximal homology. *Asterisks* indicate exact matches between the two sequences. *Hyphens* show deletions of the amino acid residues when compared between the two sequences.

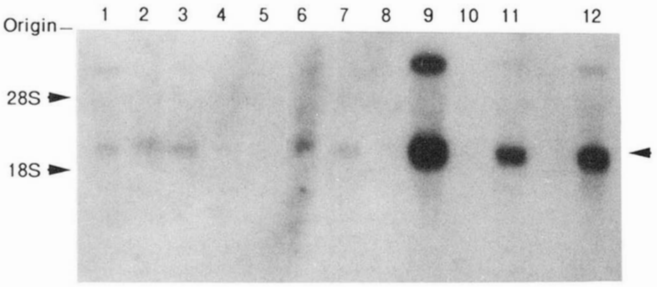

FIG. 5. **Northern blot analysis of RNAs isolated from various mouse tissues and P-815 cells.** Poly(A)$^+$ RNAs were isolated from the tissues listed below and a cell line, and 10 μg of RNA was applied in each *lane* except that 5 μg was used for P-815 cells. Hybridization analysis was carried out using the 1,072-base pair *Eco*RI-*Bam*HI fragment excised from clone MP660 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.

also showed that the uterus expressed the mRNA much higher than most tissues. It is known that PGE_2 exerts contractile response in uterine smooth muscle. The EP_3 receptor we found may mediate this contractile action. Uterine contraction has been observed as a side effect of several EP_3 agonists used as gastric anti-secretory prostanoid drugs (34). The EP_3 receptor was also expressed in heart, lung, thymus, and spleen. Although PGE_2 causes inhibition of sympathetic neurotransmitter release in some of these tissues (35), major functions of this receptor remain to be investigated. Our results also showed that EP_3 mRNA is significantly expressed in brain. The exact function of this receptor in this tissue is again not known at present. On the other hand, EP_3 mRNA was not detectable in testis, and little was found in liver and ileum. In summary, we present here the complete amino acid sequence of the mouse EP_3 subtype PGE receptor and provide direct proof that this receptor functionally couples to adenylate cyclase in an inhibitory manner. This work will contribute to our understanding on individual functions for three subtypes of PGE receptors and facilitate cloning of other members of eicosanoid receptors.

Acknowledgments—We thank Dr. M. Hirata of the Department of Pharmacology, and Drs. T. Abe, R. Kageyama, and Prof. S. Nakanishi of Institute for Immunology, Kyoto University Faculty of Medicine, for useful advice. We also thank Drs. K. Yatsunami and J. Yamamoto of the Department of Physiological Chemistry, Kyoto University Faculty of Pharmaceutical Sciences, for helpful discussions.

REFERENCES

1. Moncada, S., Flower, R. J., and Vane, J. R. (1985) in *The Pharmacological Basis of Therapeutics* (Gilman, A. G., Goodman, L. S., Rall, T. W., and Murad, F., eds) 7th Ed., pp. 660–673, Macmillan Publishing Co., New York
2. Samuelson, B., Goldyne, M., Granström, E., Hamberg, M., Hammarström, S., and Malmsten, C. (1978) *Annu. Rev. Biochem.* **47,** 997–1029
3. Coleman, R. A., Kennedy, I., Sheldrick, R. L. G., and Tolowinska, I. Y. (1987) *Br. J. Pharmacol.* **91,** 407P
4. Coleman, R. A., Kennedy, I., Humphrey, P. P. A., Bunce, K., and Lumley, P. (1989) in *Comprehensive Medicinal Chemistry* (Hansch, C., Sammes, P. G., Taylor, J. B., and Emmett, J. C., eds) Vol. 3, pp. 643–714, Pergamon Press, Oxford
5. Yokohama, H., Tanaka, T., Ito, S., Negishi, M., Hayashi, H., and Hayaishi, O. (1988) *J. Biol. Chem.* **263,** 1119–1122
6. Brunton, L. L., Wiklund, R. A., Van Arsdale, P. M., and Gilman, A. G. (1976) *J. Biol. Chem.* **251,** 3037–3044
7. Chen, M. C. Y., Amirian, D. A., Toomey, M., Sanders, M. J., and Soll, A. H. (1988) *Gastroenterology* **94,** 1121–1129
8. Sonnenburg, W. K., and Smith, W. L. (1988) *J. Biol. Chem.* **263,** 6155–6160
9. Hedqvist, P., and von Euler, U. S. (1972) *Neuropharmacology* **11,** 177–187
10. Nakao, A., Allen, M. L., Sonnenburg, W. K., and Smith, W. L. (1989) *Am. J. Physiol.* **256,** C652–C657
11. Garcia-Perez, A., and Smith, W. L. (1984) *J. Clin. Invest.* **74,** 63–74
12. Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y., Kageyama, R., Nakanishi, S., and Narumiya, S. (1991) *Nature* **349,** 617–620
13. Sussmann, D. J., and Milman, G. (1984) *Mol. Cell. Biol.* **4,** 1641–1643
14. Hashimoto, H., Negishi, M., and Ichikawa, A. (1990) *Prostaglandins* **40,** 491–505
15. Nakajima, Y., Tsuchida, K., Negishi, M., Ito, S., and Nakanishi, S. (1992) *J. Biol. Chem.* **267,** 2437–2442
16. Oikawa, S., Inuzuka, C., Kuroki, M., Matsuoka, Y., Kosaki, G., and Nakazato, H. (1989) *Biochem. Biophys. Res. Commun.* **164,** 39–45
17. Urlaub, G., and Chasin, L. A. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 4216–4220
18. Graham, F. L., and van der Eb, A. J. (1973) *Virology* **52,** 456–467
19. Negishi, M., Hashimoto, H., Yatsunami, K., Kurozumi, S., and Ichikawa, A. (1991) *Prostaglandins* **42,** 225–237
20. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159
21. Kozak, M. (1989) *J. Cell Biol.* **108,** 229–241
22. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132
23. O'Dowd, B. F., Lefkowitz, R. J., and Caron, M. G. (1989) *Annu. Rev. Neurosci.* **12,** 67–83
24. Hubbard, S. C., and Ivatt, R. J. (1981) *Annu. Rev. Biochem.* **50,** 555–583
25. Lefkowitz, R. J., and Caron, M. G. (1988) *J. Biol. Chem.* **263,** 4993–4996
26. Watanabe, T., Umegaki, K., and Smith, W. L. (1986) *J. Biol. Chem.* **261,** 13430–13439
27. Negishi, M., Ito, S., Tanaka, T., Yokohama, H., Hayashi, H., Katada, T., Ui, M., and Hayaishi, O. (1987) *J. Biol. Chem.* **262,** 12077–12084
28. Krall, J. F., Barrett, J. D., Jamgotchian, N., and Korenman, S. G. (1984) *J. Endocrinol.* **102,** 329–336
29. Strader, C. D., Sigal, I. S., Candelore, M. R., Rands, E., Hill, W. S., and Dixon, R. A. F. (1988) *J. Biol. Chem.* **263,** 10267–10271
30. Findlay, J. B. C., and Pappin, D. J. C. (1986) *Biochem. J.* **238,** 625–642
31. Kennelly, P. J., and Krebs, E. G. (1991) *J. Biol. Chem.* **266,** 15555–15558
32. Morii, H., Tanemura, M., and Watanabe, Y. (1991) *J. Neurochem.* **57,** 1281–1287
33. Watanabe, T., Shimizu, T., Nakao, A., Taniguchi, S., Arata, Y., Teramoto, T., Seyama, Y., Ui, M., and Kurokawa, K. (1991) *Biochim. Biophys. Acta* **1074,** 398–405
34. Collins, P. W. (1986) *J. Med. Chem.* **29,** 437–443
35. Hedqvist, P. (1972) in *Prostaglandins* (Ramwell, P., and Shaw, J., eds) pp. 410–415, Plenum Press, New York

Downloaded from www.jbc.org at Massachusetts Institute of Technology on February 28, 2009