A functional cDNA clone for mouse prostaglandin (PG) F receptor was isolated from a mouse cDNA library using polymerase chain reaction based on the sequence of cloned prostanoid receptors, and cross-hybridization screening. The mouse PGF receptor consists of 366 amino acid residues with putative seven transmembrane domains. The sequence revealed the highest homology to the EP subtype of PGE receptor and thromboxane (TX) A₂ receptor. Ligand binding studies using membranes of COS-I cells transfected with the cDNA revealed specific [³²P]PGF₂α binding. The binding was displaced with unlabeled PGs in the order of PGF₂α, <i>p</i> luteum, and in a lesser amount in kidney, heart, and choroid plexus. Specific binding was demonstrated that the PGF receptor transcripts are abundantly expressed in luteal cells of corpus luteum and in a lesser amount in kidney, heart, stomach, and lung.

Eicosanoids comprising various oxygenated metabolites of arachidonic acid such as prostanoids (PGs) and leukotrienes exert a variety of biological activities for maintenance of local homeostasis in the body (1-3). These metabolites act on a cell surface receptor specific for each member to exert their actions (4). PGF₂α produces constriction of bronchia, trachea, stomach fundus, and lung strip and of other nonvascular and vascular smooth muscle preparations (5-7). It is widely accepted that PGF₂α is involved in luteolysis in various species of animals (8, 9). However, the precise mechanism of the action is still an enigma. PGF receptor in these tissues is suggested to couple to stimulation of phospholipase C and cytosolic Ca²⁺ mobilization (10-12). In spite of this information, PGF receptor has not been isolated, and their molecular characterization has been carried out only poorly. For a clear understanding of the physiological roles of PGF₂α, we performed cDNA cloning of the PGF receptor. We report here the primary structure, ligand binding and biochemical properties, and mRNA expression pattern of the mouse PGF receptor.

**EXPERIMENTAL PROCEDURES**

Materials—[15,16,17,18,19,20]-³²P]PGF₂α (168 Ci/mmol), [²⁵¹P]cGMP (3,000 Ci/mmol), and [²⁵¹P]cGMP binding was obtained from Du Pont NEN. PGF₂α, PGD₂, and PGF₂α were purchased from Funakoshi Pharmaceuticales (Tokyo, Japan). STA₃ was given by Ono Pharmaceuticals (Osaka, Japan). The cDNA and inositol and iproprost were obtained from Amersham Corp. Sources of other materials are shown in the text.

Amplification of a Bovine cDNA Fragment Homologous to Prostanoid Receptors by PCR—Because high levels of PGF receptor have been found in the corpus luteum (13), and because bovine corpus luteum was easily obtained with large quantity, we chose this bovine tissue as a source of PCR. PRF primers were designed based on highly conserved cDNA sequences of the human and mouse TXA₂ receptors and mouse three PGE receptor subtypes corresponding to the putative second extracellular loop region and seventh transmembrane domain (14-18). PCR primers used were 5'-GGATCCAG(CG)A(GT)ATA(AC)ACC-3' and 5'-GAATTCAC(A)TG(A)CG-3'. Amplification cycling program was 94 °C for 0.5 min, 50 °C for 1 min, 72 °C for 1 min, 94 °C for 0.5 min, 55 °C for 1 min, 72 °C for 3 min for 27 cycles. Amplified cDNAs were subcloned into plasmid pBluescript II SK(+) (Stratagene). Of 17 clones analyzed, 10 clones had an identical sequence homologous to human TXA₂ receptor, suggesting that it encodes bovine TXA₂ receptor. A 390-base pair clone (BCA2) showed a sequence 62.0 and 50.0% homologous to the corresponding region of the mouse EP₃ and human TXA₂ receptor cDNA, respectively.

**Isolation of a Functional Mouse cDNA Clone by Cross-hybridization**—Ovarian tissue from pregnant mice was prepared, and the poly(A)⁺ RNA was isolated. cDNA prepared by an oligo(dT) priming method was size-selected (<2.0 kilobases) and inserted into the EcoRI site of pZAPII DNA (Stratagene) with EcoRI adaptors. The resultant cDNA library (6.0×10⁶ clones) was screened by hybridization with the cDNA insert of BCA2, as described (19). A number of positive clones, 16 clones were isolated and subjected to sequence analyses. One representative clone, MC205 with a 1,098-base pair clone (BCA2) showed a sequence 62.0 and 50.0% homologous to the corresponding region of the mouse EP₃ and human TXA₂ receptor cDNA, respectively.

**Cloning and Expression of a cDNA for Mouse Prostaglandin F Receptor**

Yukihiro Sugimoto, Ken-yuh Hasumoto, Tsuchisaka Namba, Atsushi Irie, Masato Katsuyama, Manabu Negishi, Akira Kakizuka, Shuh Narumiya, and Atsushi Ichikawa

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

*This work was supported in part by Grants-in-aid for Scientific Research 05404020, 04255103, 05771975, 05671816, and 05454568 from the Ministry of Education, Science and Culture of Japan and by grants from the Mitsubishi Foundation and the Takeda Science Foundation. 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 nomenclature sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D17433. To whom correspondence should be addressed: Dept. of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan, Tel.: 81-75-753-4827, Fax.: 81-75-753-4557.

1. The abbreviations used are: PG, prostaglandin; G protein, heterotrimeric GTP-binding protein; PCR, polymerase chain reaction; TX, thromboxane; IP₃, inositol trisphosphate; CTPαS, cytidine 5′-e-thio-triphosphate.
Structure and Expression of Cloned Prostaglandin F Receptor

-223

FIG. 1. Nucleotide and deduced amino acid sequences of MC205. The deduced amino acid sequence is shown beneath the nucleotide sequence. Positions of the putative transmembrane segments I–VI are indicated by underlines below 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. Triangles, potential N-glycosylation sites in the extracellular regions.

RESULTS AND DISCUSSION

A mouse cDNA clone, MC205, was isolated through PCR-mediated DNA amplification and subsequent screening of a mouse pregnant ovary cDNA library. Fig. 1 shows nucleotide and deduced amino acid sequences of MC205. The amino acid sequence was assigned from the longest open reading frame of the cDNA. The polypeptide consists of 366 amino acid residues with an estimated molecular weight of 40,077. The hydrophobic profile (24) indicated that it possesses seven hydrophobic segments, and the sequence homology analysis showed it shares a significant sequence similarity with other members of G protein-coupled receptors (25), especially with the mouse TXA2 receptor (15) and EP1 subtype of PGE receptor (26). After electrophoresis on a 1.2% agarose gel, transferred onto nylon membranes (Hybond-N, Amersham Corp.), and hybridized with a 32P-labeled NotI/HindIII fragment (1,706 base pairs) of MC205 clone. The resultant riboprobes were degraded to -150 bases by alkaline hydrolysis. Early pregnant (day 4 or 7) or non-pregnant mouse ovarian tissue was frozen and cut into sections of 8-μm thickness on a cryostat. Sections were fixed with 4% formaldehyde, treated with 0.25% acetic anhydride, and dehydrated. Slides were hybridized with 35S-labeled antisense riboprobe at 57 °C for 1-2 weeks at 37 °C. The slides were air-dried and dipped in NTB2 emulsion (Kodak) diluted 1:1 with distilled water. After exposure for 1-2 weeks at 4 °C, the dipped slides were developed in Kodak D-19 developer, fixed, and counterstained with hematoxylin and eosin.

Ligand Concentration (~log M)
receptor at least three prostanoid receptors. The putative transmembrane regions are indicated by boxed regions.

1358

yielded a dissociation constant of 1.32 nM, which agrees well with that previously reported on binding of ['H]PGF2α to bovine corneal membranes (28). The average density of binding sites in three experiments was 629 fmoles/mg protein of membranes of mock-transfected cells. Specificity of this binding is shown in Fig. 2. The binding of ['H]PGF2α was inhibited by unlabeled PGs in the order of PGF2α > 9α,11β-PGF2α > PGD2 > STA2 (a stable TXA2 agonist) > PGE2 > iloprost (a stable PGI2 agonist). This binding specificity order was in good agreement with that of the PGF receptor previously characterized (28, 29). These results established that MC205 encodes the mouse PGF receptor.

To identify a ligand for this receptor, cDNA insert of MC205 was expressed in COS-1 cells, and membranes of the transfected cells were subjected to binding assays using various radiolabeled PGs. Among the PGs tested, ['H]PGF2α specifically bound to the membranes. Scatchard analysis of this binding yielded a dissociation constant of 1.32 nM, which agrees well with that previously reported on binding of ['H]PGF2α to bovine corneal membranes (28). The average density of binding sites in three experiments was 629 fmoles/mg protein of the transfected COS-1 cell membranes. ['H]PGF2α did not bind to membranes of mock-transfected cells. Specificity of this binding is shown in Fig. 2. The binding of ['H]PGF2α was inhibited by unlabeled PGs in the order of PGF2α > 9α,11β-PGF2α > PGD2 > STA2 (a stable TXA2 agonist) > PGE2 > iloprost (a stable PGI2 agonist). This binding specificity order was in good agreement with that of the PGF receptor previously characterized (28, 29). These results established that MC205 encodes the mouse PGF receptor.

Fig. 3 shows the comparison of the amino acid sequences of the mouse PGF receptor, three subtypes of mouse PGE receptor, and mouse TXA2 receptor. The sequence identity in the putative transmembrane segments between PGF receptor and other prostanoid receptors are as follows: 47.2% between EP1 receptor and PGF receptor, 47.8% between EP2 receptor and PGF receptor, 43.4% between EP3 receptor and PGF receptor, and 30.2% between EP2 receptor and PGF receptor. PGF receptor shows higher homology to the TXA2 receptor, especially in the segment VI (81% each) and segment VII, such as Arg-291 in PGF receptor, is conserved in all prostanoid receptors. Considering that retinal is attached to Lys-296 of bovine rhodopsin in segment VI (30), the structure and expression of cloned prostaglandin F receptor.
tural features of highly conserved segment VII including Arg residue may reflect the acidic nature of the ligand for the prostaglandin receptors as first suggested by the structure of the human TXA₂ receptor (14).

Possible association of the PGF receptor with IP₃ formation and intracellular Ca²⁺ mobilization has been indicated (10, 11). We tested this possibility by expressing the cDNA in COS cells and examining response of the cells to PGF₂α. As shown in Fig. 4, the transfected COS cells displayed a concentration-dependent increase in cellular IP₃ accumulation when exposed to PGF₂α. In untransfected cells, PGF₂α at 10 μM showed no significant increase in IP₃ formation (data not shown). We further investigated the electrophysiological response of Xenopus laevis oocytes expressing this receptor. An inward current was elicited by PGF₂α, 9α,11β-PGF₂α, and PGF₁α (1 μM each), but neither by PGD₂ nor PGE₂ (data not shown). These results demonstrate that this receptor is coupled to stimulation of phospholipase C and resultant intracellular Ca²⁺ mobilization.

RNA blot analysis showed two major positive bands at estimated mRNA sizes of 2.3 and 6.0 kilonucleotides, and minor hybridizing bands at 2.0, 7.0, and 9.1 kilonucleotides (Fig. 5). The most abundant expression of PGF receptor mRNA was seen in the pregnant ovary, and this reflects high content of corpus luteum. A prominent expression of this receptor was also observed in kidney. PGF₂α is one of major products of cyclooxygenase in glomerulus (31), and that PGF₂α stimulates intracellular Ca²⁺ mobilization in cultured rat mesangial cells (32). These results suggest that PGF receptor is involved in renal function at glomerulus. The PGF receptor was also expressed in lung, consistent with a report that PGF₂α causes bronchoconstriction (6, 33). Although significant bands were also detected in heart and stomach, the functions of this receptor in these tissues are not known at present.

In situ hybridization analysis was performed to examine the expression of PGF receptor mRNA in the ovarian tissue. PGF receptor mRNA expression was observed exclusively in the corpus luteum in the early pregnant (day 4) or non-pregnant ovarian tissue (Fig. 6, a–c). No significant hybridization was observed in parallel experiments using the same probe in the presence of an excess of unlabeled probe (Fig. 6d). High expression of this mRNA was seen in large luteal cells (Fig. 6e). This result suggested that the PGF receptor is involved in luteal cell function by stimulating inositol lipid metabolism.

In summary, we present in this paper the primary structure of the mouse PGF receptor and provide direct proof that this receptor is functionally coupled to stimulation of phospholipase C and is abundantly expressed in corpus luteum. This work will
contribute to understanding of the luteolytic function of and facilitate cloning of other members of eicosanoid receptors.

Acknowledgments—We thank Drs. Y. Hayashi and H. Oida of the Department of Pharmacology, Drs. Y. Gotoh and H. Fujiwara of the Department of Obstetrics and Gynecology, and Dr. R. Shigemoto of the Department of Morphological Brain Science, Kyoto University Faculty of Medicine, for helpful advice and encouragement. We also thank A. Yamazaki and K. Morimoto of the Department of Physiological Chemistry, Kyoto University Faculty of Pharmaceutical Sciences, for technical assistance in the molecular cloning.

REFERENCES