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Cloning of the rat and human prostaglandin $F_{2\alpha}$ receptors and the expression of the rat prostaglandin $F_{2\alpha}$ receptor

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Abstract We have cloned the FP receptor from rat corpus luteum and human uterus cDNA libraries, respectively. The coding DNA sequence in the rat cDNA is 1101 bp and is similar to the mouse cDNA coding for a receptor protein of 366 amino acids. The human sequence shows a 5 bp deficiency in the 3′ region, truncating the coding sequence to 359 amino acids. Northern blot analysis indicates highest expression in the ovary. Cell lines have been established giving stable expression of the FP receptor. Activation of the cloned FP receptor gave an increase in intracellular calcium, indicating signaling via phospholipase C-mediated phosphoinositide turnover. Using [^{3}H]$PGF_{2\alpha}$, binding of PGs showed the rank order of fluprostenol > PhXA70 > $PGF_{2\alpha} \geq$ PhXA85 > PGD_2 > PGE_2.

Key words: Prostanoid receptor; $PGF_{2\alpha}$; Cloning; Expression; Ligand binding; Northern analysis

1. Introduction

Although the presence of specific prostaglandin receptors has been recognized pharmacologically [1,2], only recently has the cloning of prostaglandin receptors been undertaken. Prostaglandin (PG) receptors are classically defined to be linked to the stimulation of second messengers such as cAMP, IP_3 or intracellular calcium release coupled via a G regulatory protein [10]. The activation of prostaglandin receptors may also result in various other responses, including inhibition of adenylyl cyclase activity, inhibition of phosphatidylinositol turnover and inhibition of Ca^{2+} mobilization [10,11]. The first prostaglandin receptor to be cloned was that of thromboxane A_2, (TP) [3]. Subsequently the receptors for PGE_2, the EP_1, EP_2 and EP_3, have been cloned [3–8]. All cloned prostaglandin receptors have been of the seven-transmembrane type, coupled to guanine nucleotide binding regulatory (G) proteins linked to either adenylate cyclase or the phospholipase C–inositol triphosphate pathway (GPCR), which are amongst the most studied receptor systems [9]. Recently, distinct PG receptors with identical or similar ligand binding specificity have been identified [12]. Such receptors are the isoforms of the EP_3 receptor [13] which have been shown to activate different second messenger systems [14], and the TP receptor have recently been shown to exist in at least two isoforms [15]. Thus, it seems that different isoforms of PG receptors functionally coupled to different second messenger systems exist. While the TP and EP receptors have been cloned and characterized there has not until recently been information available about the FP receptor [16–18]. However, the receptor ligand $PGF_{2\alpha}$ has well-known biological effects such as luteolysis, contraction of the uterus and bronchoconstriction [19,20], and this PG may play an important role in a variety of physiological events. Induction of luteolysis in corpus luteum has been shown in a variety of species from rat to man [21,22]. Astrocytes have been shown to express FP receptors [23], indicating functions of $PGF_{2\alpha}$ in the central nervous system as well. Agonists and antagonists of FP receptors may be important from a clinical therapeutic point of view in luteolysis, uterine constriction, and increasing the blood pressure etc. Prostaglandins, especially $PGF_{2\alpha}$ and esters of $PGF_{2\alpha}$, have also been shown to reduce intraocular pressure [24]. Recently, selective analogues of $PGF_{2\alpha}$, with a potential use as anti-glaucoma agents, have been identified [25] and may be used to treat glaucoma [26], whereas putative drugs which block FP receptors (antagonists) may be used therapeutically to treat pathological conditions, e.g. in the lungs and uterus. Despite their clinical utility, a problem with several currently available FP agonist drugs is their many side effects. These side effects are predominantly due to a lack of receptor specificity, since several of the FP receptor agonists described interact not only with FP receptors but with other receptors as well [10].

2. Material and methods

2.1. Molecular cloning and sequencing analyses of rat and human FP receptors

In order to clone an FP receptor, the polymerase chain reaction (PCR) method was used to amplify cDNA sequences from a rat corpus luteum cDNA library in the Lambda ZAPII vector (Stratagene, USA). Lambda DNA was prepared as described in [27] and submitted to 45 cycles of PCR amplification in a total reaction volume of 25 μl with 1 μM each of the following degenerated primers from transmembrane regions TM2, 5′ ATI I(CT)(CG) (TA)I(TC) (TC)TG GCI ITI ICC GAT 3′; and TM7, 5′ C(GT)(AG) AAI AGI AT(AG) TAI ACC CAI GGG TC 3′; and 200 μM dNTPs and 2 U *Taq* DNA polymerase (Perkin Elmer-Cetus). The timing used was 45 s (in the first cycle 3 min) at 95°C, 3 min at 50°C and 3 min at 72°C. The 72°C step was extended with 6 s for each cycle. Individual bands were excised from an agarose gel and were submitted separately to 20 cycles of PCR amplification in a total reaction volume of 20 μl with 100 μM of each of the primers in TM2, 5′ ATI I(CT)(CG) (TA)I(TC) (TC)TG GCI ITI ICC GAT 3′; and TM7, 5′ C(GT)(AG) AAI AGI AT(AG) TAI ACC CAI GGG TC 3′; 200 μM of dNTPs and 2.5 U *Taq* DNA polymerase, otherwise as in the first PCR reaction. PCR fragments were subsequently cloned into the PCR II vector using the TA cloning kit (Invitrogen) according to the instructions from the manufacturer, transformed into One Shot (Invitrogen) competent cells and the DNA sequenced. One insert was found to exhibit close sequence homology to a related receptor. This insert was used as a probe to screen a rat corpus luteum cDNA library to isolate a full-length clone from the rat corpus luteum cDNA library. Positively hybridizing purified phage clones were expanded in *E. coli*

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XL1-Blue, and the resulting phage stocks were used to prepare cDNA-containing pBluescript plasmids by phagemid excision according to the Stratagene protocol. The 4 phagemids with the longest inserts were analyzed by DNA sequencing methods. We sequenced our cDNA with primers homologous to regions on the M13 multiple cloning site according to Applied Biosystems protocol for their Taq Dye Deoxy Terminator cycle sequencing kit. All sequence analysis were performed on a Applied Biosystem Model 373A DNA sequencing system. The generated primary data was processed on a VAX computer using the sequence analysis programs from Genetics Computer Group Inc., Madison, USA [28]. To obtain a human probe, amplification of 350 ng human genomic DNA was performed in a 50 μl reaction in 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.1% gelatin, 200 μM each of dATP, dCTP, dGTP, dTTP, 2.5 U *Taq* polymerase (Perkin-Elmer) and 3 μM of the oligonucleotides 5'-CAC GAC TTG CCA GAC GGA GAA C-3' and 5'-TTG TAG AAA CAC CAG GTC CT-3'. An initial denaturation step at 94°C followed by 30 cycles of annealing at 55°C 1 min, extension at 72°C 1 min, denaturation at 94°C 1 min, and finally one cycle of 55°C 1 min, 72°C 10 min. One DNA fragment was obtained and cloned and sequenced as above; a 80% homology to the rat sequence indicated that a fragment of the human *fp* gene had been found. To isolate a full-length cDNA of the human *fp* gene, a human uterus cDNA gt11 library (Clontech) was plated and screened with the human probe. Positive plaque pools were picked and subjected to a primary and a secondary PCR analysis with nested primers. PCR amplification was performed in a 50 μl reaction volume in 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin and the presence of 200 μM each of dATP, dCTP, dGTP, 2.5 U of *Taq* polymerase (Perkin-Elmer) and 30 μM of primer 5'-CAC GAC TTG CCA GAC GGA GAA C-3' and 5'-CCA GTC TTT GAT GTC TTC TGT G-3' each. Secondary PCR was carried out using 1 μl of the primary PCR reaction as template under the same conditions but with the primers 5'-CAG TAA TCT TCA TGA CAG TGT GG-3' and 5'-TTG TAG AAA CAC CAG GTC CT-3. Positive plaque pools were subjected to another round of hybridization and PCR amplification until single positive plaques could be discriminated. Extracted lambda DNA was digested with *Eco*RI and the DNA fragments were separated on a 0.8% agarose gel in 1× TAE. Three fragments were purified and ligated into pBluescriptSK$^+$. Screening of clones containing inserts was performed by restriction enzyme analysis. Positive clones were sequenced as above. PCR amplification of the full-length receptor gene for subsequent cloning into an expression vector was performed using the primers 5'-GTA CGT TCT AGA CTC GAG CCA CCA TGT CCA TGA ACA ATT CCA AAC AG-3' and 5'-ATG CAG GCT TCT AGA TGT CCT ATT AAG CTA GGT GCT TGC-3' under the same conditions as previously described. The resulting 1113 bp fragment was cloned into the PCR II vector according to the instructions from the manufacturer, transformed into One Shot (Invitrogen) competent cells and positive clones were screened by restriction enzyme analysis with *Eco*RI. Insert cDNA from rat and human was ligated into PCR/CMV (Invitrogen) and subsequently sequenced in both directions.

2.2. Transfections

A mutant cell line CHO:DG44 (kindly provided by Dr. L. Chasin, Columbia University) where the dihydrofolate reductase (DFHR) gene is deleted was used for transfection. The cells were routinely grown in a modified F12 medium (Gibco BRL, Paisly, Scotland) supplemented with 5% bovine serum (HyClone Laboratories Inc., USA). Cells wer transfected by the $CaPO_4$ precipitate method [30,31] with the above-mentioned rat and human vector constructs, and co-transfected with the *DHFR* gene in a ratio of 10:1, respectively. Transformants positive for DHFR were selected in a F12 medium lacking hypoxantine, glycine, thymidine (Gibco BRL), supplemented with 10% dialyzed fetal bovine serum (HyClone Lab Inc.). Methotrexate (Sigma Chemical Co, USA) was used to amplify the transfected genes.

2.3. Flow cytometry

To find high expressing clones, flow cytometry analysis was performed on rat FP-transfected CHO cells, amplified with 20 nM MTX and 100 nM, respectively. The antibodies used in the analysis were biotinylated rabbit anti-FP carboxy-terminal IgG (Pharmacia BioScience). Cells in PBS at a density of 10^7 cells/ml were incubated at 22°C in a concentration of 13 μg antibody/ml for 30 min. For detection, fluorescein-conjugated avidin (Molecular Probes A-2662) was used. Cells stained with avidin only were used as a negative control. Flow cytometryc analysis was performed using an EPICS 753(Coulter Electronics, Hialeah, FL).

2.4. Northern blot analysis

Nylon membranes blotted with 2 μg poly(A)$^+$ RNA from eight different rat tissues (heart, brain, spleen, lung, liver, skeletal muscle and testis) and eight human tissues (spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes (MTN-Blot, Clontech) were used for analysis. The rat blots were probed with a rat *fp* fragment and the human blot with a human *fp* fragment according to the protocol for QUIKHYB (Stratagene) and analyzed in a PhosphorImager (Molecular Dynamics).

2.5. Ligand binding

All steps were carried out at +4°C. Cells were washed once with PBS and then scraped from roller bottles into cold PBS and spun down. The cells were homogenized using a glas homogenisator (Dounce) and centrifuged. The supernatant was then centrifuged at 100,000 × *g* for 60 min using a Beckman ultracentrifuge, and the final pellet was suspended in 50 mM Tris-HCl, pH 7.0, with 2.5 mM $MnCl_2$. Protein concentration was determined by the method of Lowry [32]. Cell membranes (100 μg) were incubated with [^{3}H]$PGF_{2\alpha}$ (specific activity 154 Ci/mmol; New England Nuclear) in 50 mM Tris-HCl, pH 5.8, containing 2.5 mM $MnCl_2$ in a total volume of 0.2 ml for 60 min at room temperature. After incubation the samples were filtered through GF filters (particle retention size of 1.0 μm) using a Skatron cell harvester. Specific binding was calculated by subtracting the values for non-specific binding in the presence of 10^{-5} M unlabelled $PGF_{2\alpha}$. All samples were assayed in triplicate. In saturation analysis ('hot' Scatchard plot) concentrations of 0.5–15 nM of [^{3}H]$PGF_{2\alpha}$ were used. For competitive binding, cell membranes were incubated with 3 nM [^{3}H]$PGF_{2\alpha}$ together with varying concentrations (5×10^{-13} to 10^{-5} M) of different unlabelled ligands (prostaglandins $F_{2\alpha}$, E_2, D_2, fluprostenol, PhXA70 (17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$) and PhXA85 (13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$) [25]). Ligand binding experiments with CHO cells expressing the human FP receptor were carried out with intact cells cultured in 96-well plates at a density of 10,000 cells/well, incubated with [^{3}H]$PGF_{2\alpha}$ (0.5–10 nM) in 50 mM Tris-HCl, pH 5.8, containing 2.5 mM $MnCl_2$ in a total volume of 0.2 ml for 45 min at room temperature. The cells were lysed by adding NaOH to each well and the lysed cells were transferred to scintillation vials, mixed with scintillation cocktail (Ultima Gold; Packard) and counted. Non-specific binding was determined in the presence of 10^{-5} M unlabelled $PGF_{2\alpha}$. Data were analyzed using the EBDA/LIGAND program [33]. Data were fitted to single and multiple site models and compared for statistically significant differences. The high and low affinity binding sites were designated as R_H and R_L and their corresponding affinity constants as K_H and K_L, respectively. The concentration (IC_{50}) of each ligand to cause 50% inhibition of the binding of [^{3}H]$PGF_{2\alpha}$ was derived from binding curves. EBDA was used to perform Scatchard analysis for determination of maximum number of binding sites (B_{max}) and dissociation constant (K_d) for [^{3}H]$PGF_{2\alpha}$.

2.6. Functional analysis

An analysis of the functional activation of the cellular signal transduction was performed as an activation of intracellular calcium responses in a spectrofluorometer (PTI, USA; data not shown). cAMP analysis gave, as expected, no altered levels in $PGF_{2\alpha}$-challenged cells. The functionality was tested in a microphysiometer as well (Cytosensor; Molecular Devices, USA). Activation of second messenger systems have been shown to change the extracellular pH around cells due to an increased metabolism [34].

3. Results and discussion

3.1. Isolation and characterization of full-length cDNA clones for a $F_{2\alpha}$ receptor: cloning and sequencing analyses of $F_{2\alpha}$ receptor cDNA

In order to clone the FP receptor, a PCR method was used to selectively amplify cDNA sequences from a rat corpus luteum cDNA library. Ovine and bovine corpus luteum has

previously been shown to express this receptor subtype [12,36]. PCR amplification was performed with pairs of highly degenerated primers derived from the second and seventh, and third and sixth, transmembrane regions, respectively, of previously cloned seven-transmembrane receptor super family members and in particular the TP receptor. This process resulted in the amplification of several cDNA fragments.

The amplified cDNA fragments were preliminarily characterized by DNA sequence analysis. One of the cDNA fragments was found to exhibit considerable sequence homology to the previously cloned G protein-coupled TP receptor and was subsequently used to screen the rat corpus luteum cDNA library in order to isolate a full-length clone. Twenty-four cDNA clones with insert sizes ranging from about 1.7 to 3.3 kb were isolated, all of which strongly hybridized with the ^{32}P-labeled PCR probe on southern analysis (data not shown). One of these clones, with an insert of about 3 kb, was sequenced and found to exhibit more than 55% amino acid sequence homology to

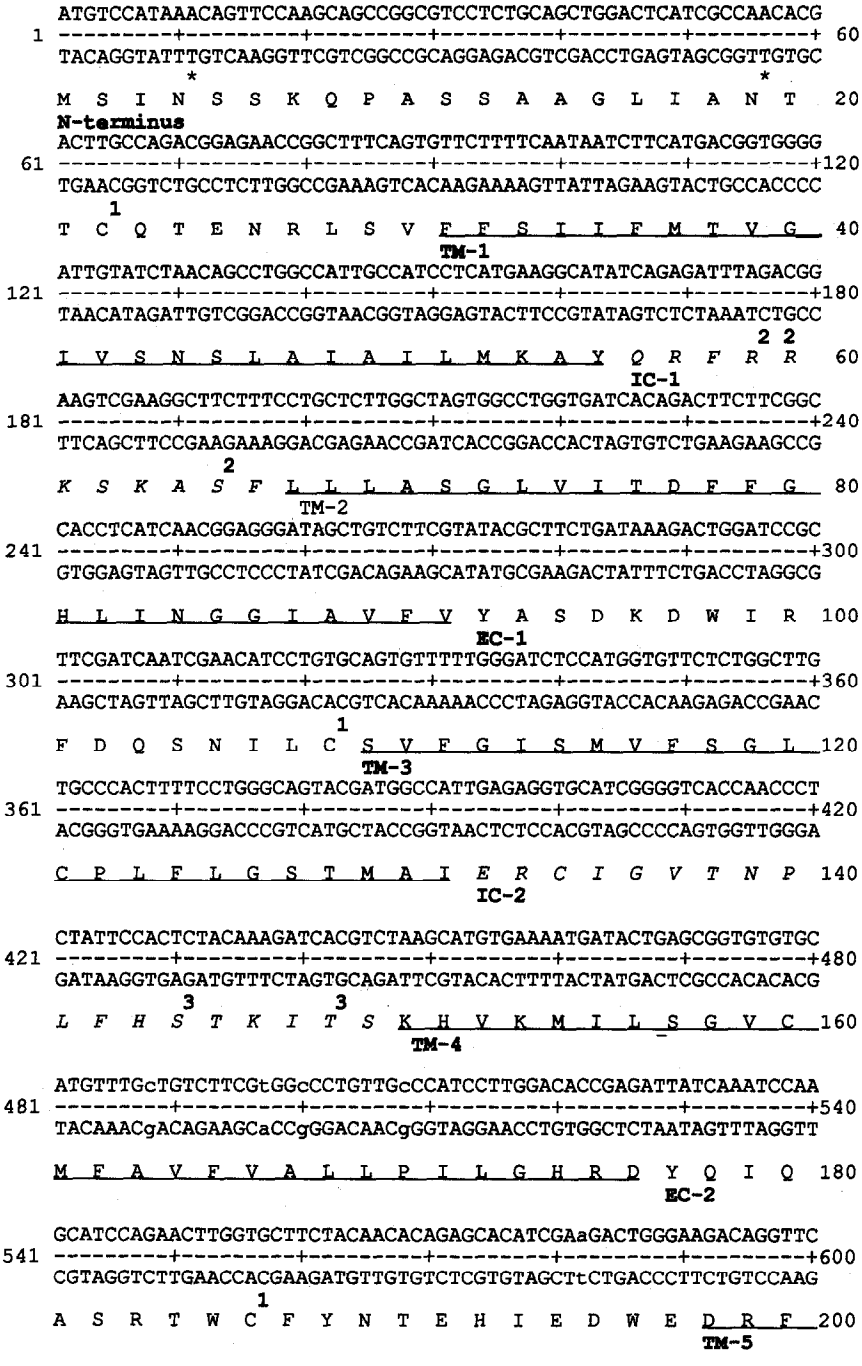

Fig. 1. The nucleotide sequence of a FP receptor along with the deduced amino acid sequence of the longest open reading frame. The nucleotide sequence is numbered from the putative initiator methionine and indicated at the left of each line, while the amino acid numbers are indicated at the right of each line. Transmenbrane regions are indicated by heavy lines, intracellular regions are in italics and extracellular regions are in normal font. Consensus sites are indicated as follows: *, N-glycosylation; 1, disulphide bond; 2, PKA phosphorylation; 3, PKC phosphorylation; and 4, cysteine myristylation.

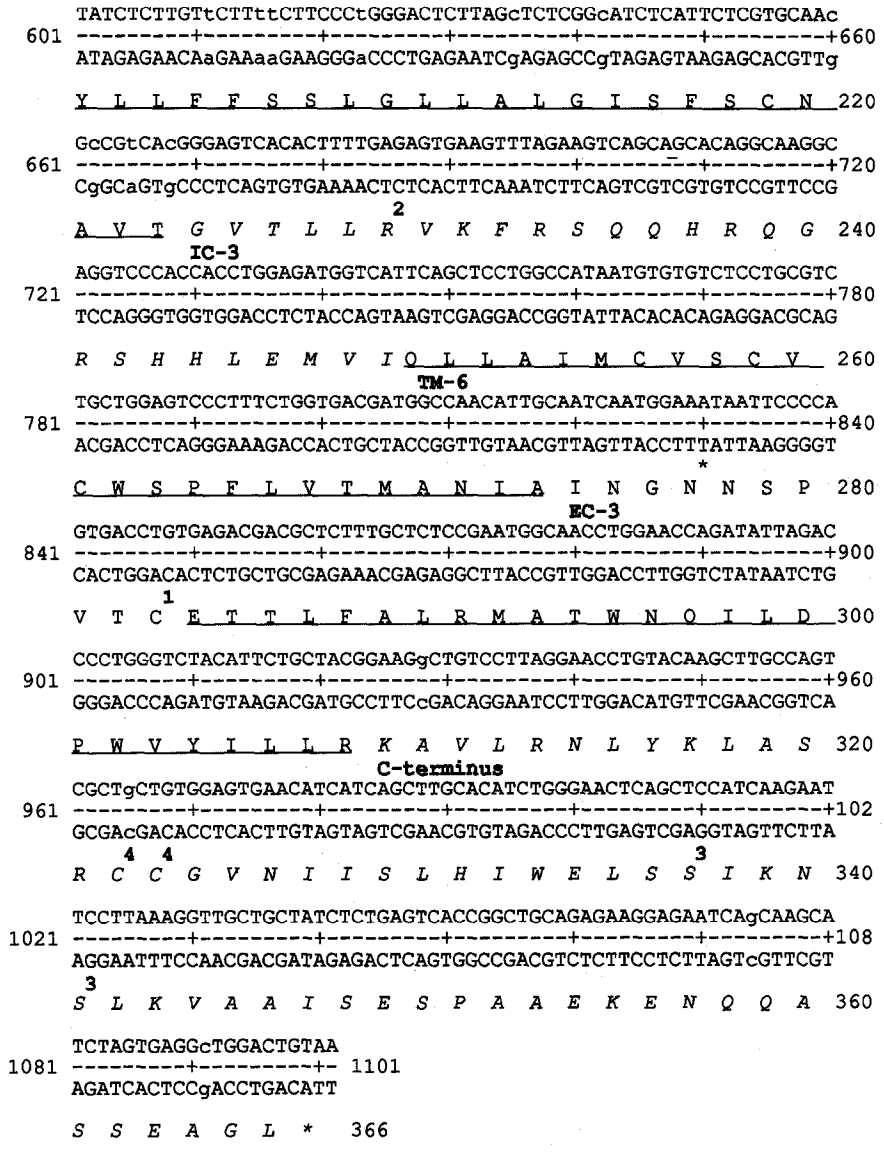

Fig. 1. (continued).

related PG receptors in the coding regions of the sequence. The nucleotide and deduced amino acid sequence for the cloned FP receptor is shown in Fig. 1. The longest open reading frame in this cDNA codes for a 366 residue protein with a theoretical molecular weight of 41 kDa. Putative glycosylation as well as other post-translational modifications may increase the molecular weight. Although there are neighboring sequences with ATG in the reading frame similar to Kozak's consensus initiation sequence [37], the Met codon at position 1 provides the most probable initiation site (Fig. 1). A Kyte and Dolittle hydrophobicity analysis of the translated protein reveals seven clusters of about 25 hydrophobic residues, predicted to represent transmembrane-spanning domains, connected by three extracellular and three intracellular loops. This pattern is similar to that observed for other cloned G protein-coupled receptors where the amino-terminus is proposed to be extracellular and the carboxy-terminus projects into the cytoplasm [38].

The human FP receptor cDNA was identified and cloned from a human uterus cDNA library using a human probe obtained with rat-specific PCR primers. Homology analysis showed homology with the published sequence for the human FP receptor [17] except in positions 63 and 213 where cytosine to thymidine substitutions were shown. However, these base substitutions do not give rise to any amino acid substitutions. Comparison between mouse and rat FP cDNA sequences show 83.5% and 80.5% homology, respectively (Table 1). It is interesting to note a 5 bp deletion in the 3′ end of the human sequence compared to the mouse and rat sequences, giving a

Table 1
Percent identity and similarity between the FP receptor in man, rat and mouse.

	Rat		Human
Mouse	Id	95.6%	86.7%
	Sim	97.5%	93.6%
Rat			85.6%
			93.1%

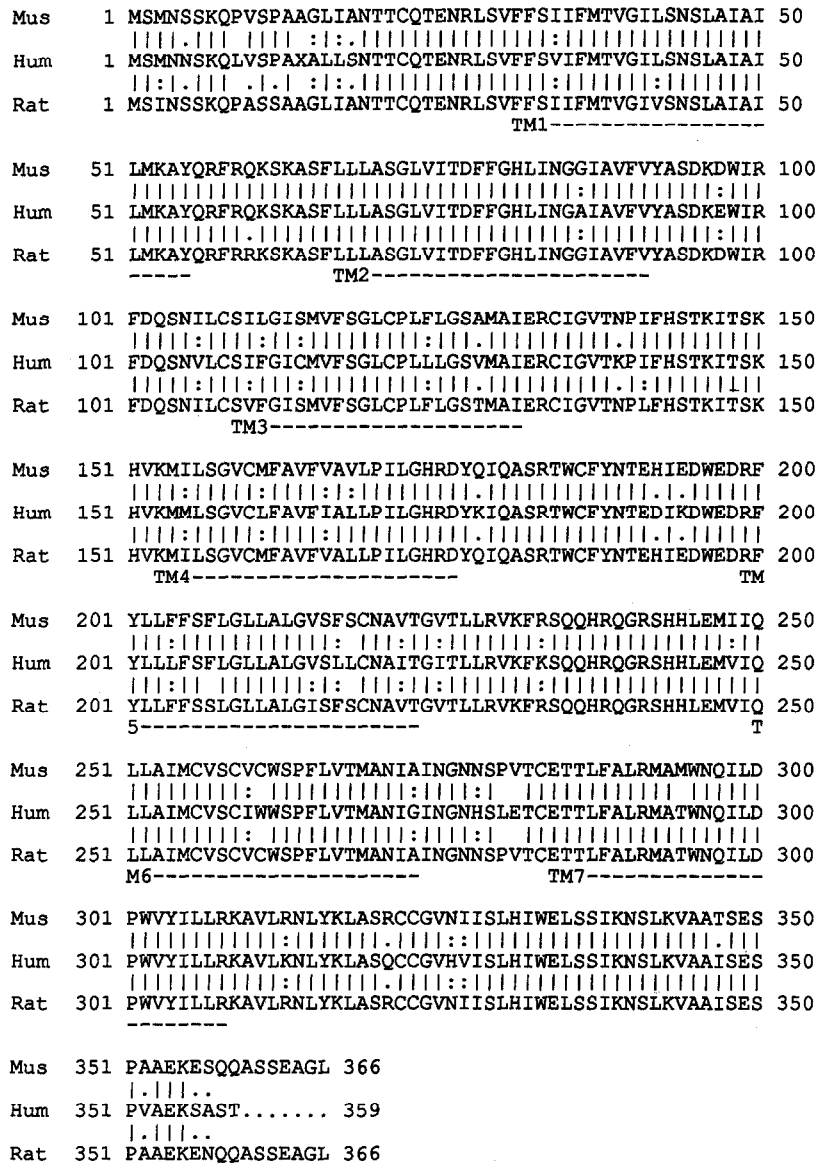

Fig. 2. Comparison between the FP receptor amino acid sequences in mouse, man and rat.

translational frame shift and truncating the carboxy-terminus in the human amino acid sequence (Fig. 2).

3.2. *Characterization of the amino acid sequences for the rat FP receptor clone*

Comparison of the deduced amino acid sequence for the cDNA clones with the sequences of various rodent prostaglandin receptors is shown in Fig. 3. As can be seen, the regions of highest identity appear to occur within the predicted transmembrane-spanning domains. Within these regions, the FP protein exhibits the highest sequence homologies with the mouse EP_1 and TP receptor. The amino- and carboxy-termini and the extracellular and intracellular loops are significantly more divergent among these receptors.

The long carboxy-terminus contains several serine residues, possibly representing additional sites for regulatory phosphorylation. The amino acid sequence reveals, by a theoretical analysis, the following: two possible N-glycosylation sites are located in the amino-terminus and one in the third extracellular (EC) loop. Four cysteines are located in the putative extracellular parts, giving the possibility for two disulfide bonds. Except for the Cys–Cys bond found in most, if not all, GPCR, in EC-1 and EC-2, FP also has another possible pair in the amino-terminus and the EC-3, a feature shared by the EP_3 but not other PG receptors. Other GPCR's having similar located cysteines seem to belong to the small peptide-ligand receptor family, e.g. the IL-8 receptor.

Consensus recognition sites for phosphorylation of protein kinases of both the PKC and PKA type are found in the intracellular (IC) loops and the carboxy-terminus; all together there are seven Ser and Thr residues as possible sites. These phosphorylations are proposed for the regulation of transmembrane signaling and desensitization of the receptor [39]. Probable Cys myristylation site(s) are located in the carboxy-terminus. EP_3 and EP_2 also have Cys in similar positions. These myristylatitions give a fourth intracellular loop which may in-

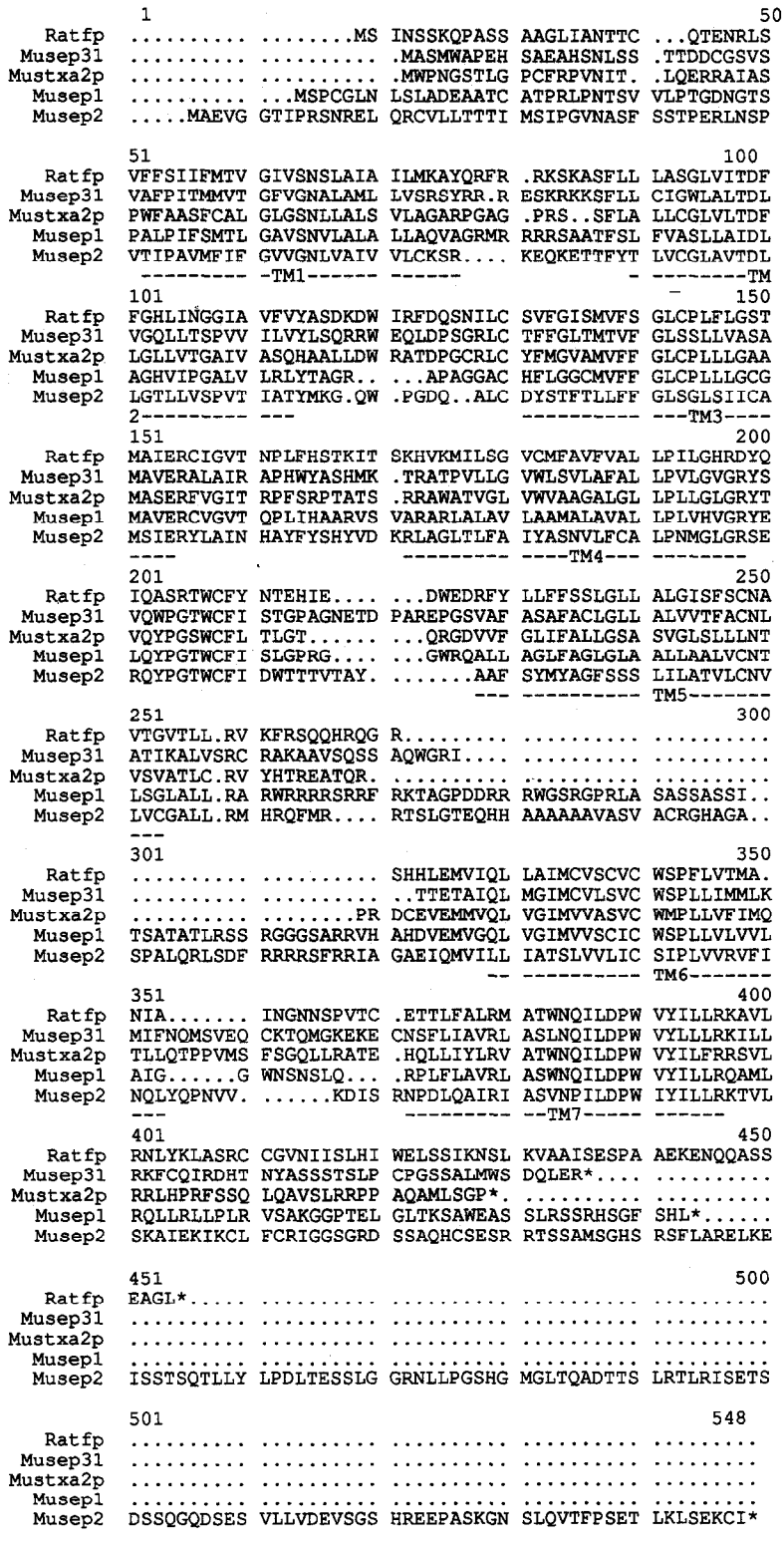

Fig. 3. Comparison of the rat FP receptor amino acid sequence with that of other known prostaglandin receptors. Amino acid sequences of the mouse TP receptor, the mouse EP_1, EP_2 and EP_3 receptor were aligned to optimize the homology with a rat FP receptor sequence. The putative transmembrane (TM) regions are indicated by the intermittent lines.

teract with G proteins [40] and affect the phosphorylation of the carboxy-terminus [41]. A major difference in size of the third IC loop is observed between FP (together with the EP_3 and TP) and EP_1 and EP_2. The C-terminus of the EP_2 is considerably longer than that of the FP and of the rest of the PG receptors.

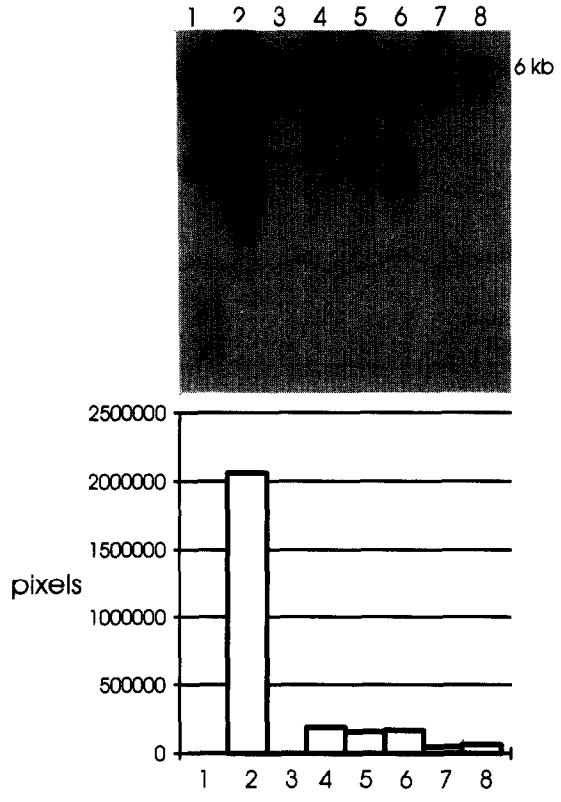

Fig. 4. Northern blot, rat. Lane 1, heart; 2, brain; 3, spleen; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, testis. The lower diagram shows a densiometric analysis of the 6 kb band and the relative expression of it in the presented tissues.

The similarity between FP and any PG receptor in the TM regions vary from about 45% in TM4 and TM5 to 76% in TM6 and TM7, the identity, i.e. the same aa in the same position vary from 4% in TM5 to 44% in TM7. The 16 aa most adjacent to the cytoplasm in TM7 are almost all identical, 10/16, an outstanding hallmark of the PG receptors. As pointed out by Hirata earlier [3], the Arg in TM7, conserved in all prostanoid receptors, may reflect the binding site of the acidic group in substances such as the prostaglandins [3,42,43]. It is interesting to note that within the third putative transmembrane domain of FP, as well as in the other PG receptors, there is not a conserved aspartate residue which is common to all biogenic amine receptors that have been sequenced thus far. Moreover, the fifth transmembrane-spanning domain of FP also contains two serine residues conserved among catecholamine receptors and critical for the recognition of agonist ligands possessing a catechol group [44]. Other PG receptors except EP_2 lacks these serines.

3.3. Northern analysis

A common transcript at about 6 kb was shown in all rat tissue (Fig. 4). Heart, brain and skeletal muscle showed additional transcripts of lower size, ranging from 2 to 6 kb. Highest expression was found in brain followed by lung and skeletal muscle. Heart, spleen, liver and testis all expressed the transcript while the kidney showed no detectable expression. The pattern in brain and skeletal muscle were similar. The variation of expression pattern, as shown in our rat tissue blot, differs from previously published thromboxane and prostaglandin E receptors both in the size of transcript and the multiplicity of transcripts in the tissues of heart, brain and muscle. The possibility that subtypes of the FP receptor exist can not be ruled out since a cross-hybridization with other PG receptors seem unlikely according to the published sizes of major PG receptor transcripts in mice. A study of the various sized bands in rat tissues has currently started. The human tissues examined did not show the size variability in transcripts found in rat (Fig. 5). A major transcript of about 6 kb was found in all tissues expressing the FP receptor, with the highest expression in the ovary followed by the small intestine, prostate, spleen and testis. Weak expression was shown in the colon and thymus but no expression was detectable in leukocytes. In tissues with a high expression, a minor transcript of a larger size of 6.5 kb could be detected. These different mRNA expression data may indicate a species difference in putative subtypes of the FP receptor.

Table 2
$[^3H]PGF_{2\alpha}$ and [^{3}H]PhXA85 saturation binding characteristics in membrane preparations of CHO cells expressing the transfected rat FP receptor.

Ligand	K_d (nM)	B_{max} (fmol/mg protein)
$PGF_{2\alpha}$	8.3 ± 2.5	78 ± 23
PhXA85	18.5 ± 0.1	158 ± 18

Data are mean ± SE of 3 independent experiments

3.4. Ligand binding

Ligand binding with radiolabeled $[^3H]PGF_{2\alpha}$ and competition with other prostaglandins on membranes prepared from the transfected cells is one way of confirming the identity of the cloned cDNA. Scatchard analysis showed a specific $[^3H]PGF_{2\alpha}$ binding to these membranes with a K_d value of 8.3 ± 2.5 nM

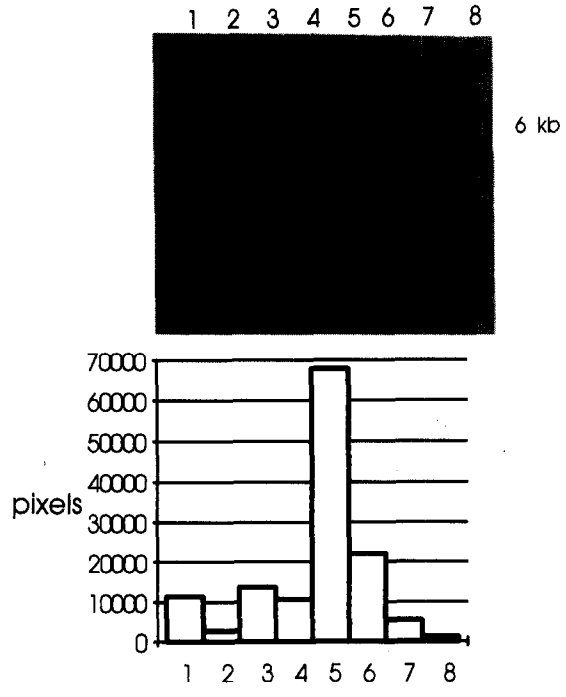

Fig. 5. Northern blot, human. Lane 1, spleen; 2, thymus; 3, prostate; 4, testis; 5, ovary; 6, small intestine; 7, colon; 8, leukocyte. Otherwise as Fig 4.

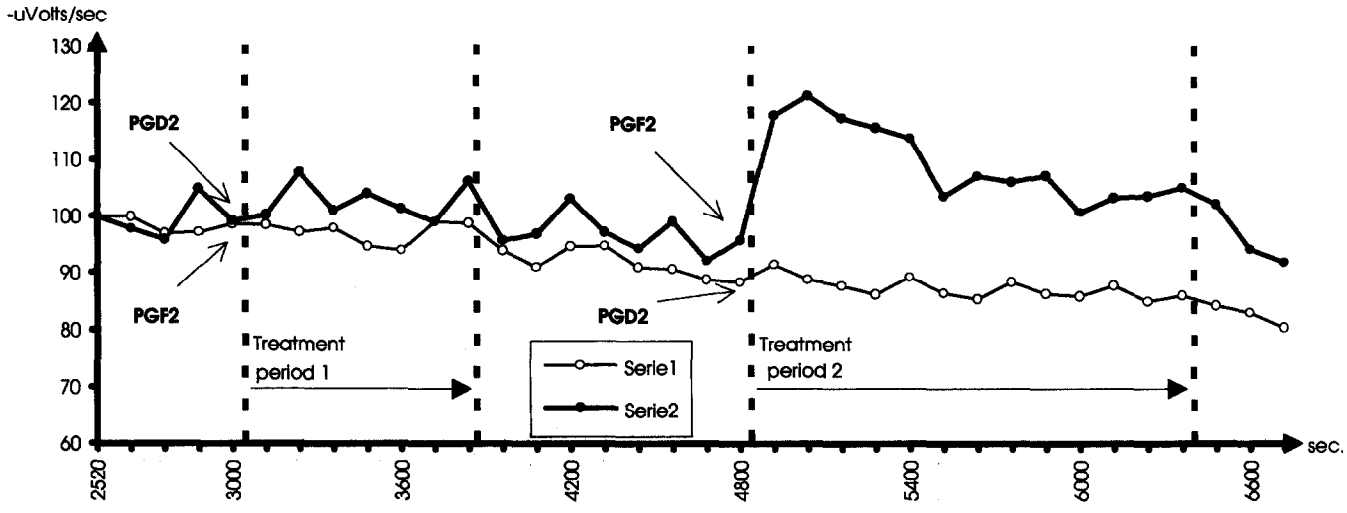

Fig. 6. Microphysiometer analysis of CHO cells expressing the transfected rat FP receptor (series 2) and untransfected CHO cells (series 1). Prostaglandins D_2 and $F_{2\alpha}$ are tested in this experiment by challenging the cells in the two treatment periods, respectively. % change in $-\mu V/s$ on the x-axis and time in seconds after the start of the microphysiometer on the y-axis.

and a B_{max} of 78 ± 23 fmol/mg protein (Table 2). The rank order of potency of prostaglandins competing for [^{3}H]$PGF_{2\alpha}$-specific binding at the FP receptor was fluprostenol > PhXA70 > $PGF_{2\alpha}$ ≥ PhXA85 > PGD_2 > PGE_2 (Table 3). A two-site model gave the best fit with the two ligands $PGF_{2\alpha}$ and fluprostenol; PhXA85 and the other gave best fit with a one-site model (for K_i, IC_{50} and B_{max} values see Table 4). These values are in agreement with published values of tissue preparations expressing the FP receptor. Comparing these ligand binding data with the ones presented for the cloned mouse and human FP receptors [16,17], there is a difference in the binding of PGD_2 to the rat and mouse FP receptor compared to the human receptor. The binding of PGD_2 and $PGF_{2\alpha}$ to the human FP receptor does not differ significantly, while in the rat and mouse there is a difference in binding of 10–100 times between PGD_2 and $PGF_{2\alpha}$.

Untransfected CHO cells as well as FP receptor transfected cells were stimulated with prostaglandins. The $PGF_{2\alpha}$ stimulations gave alterations in pH around the transfected CHO cells but not in the untransfected host CHO cells. Stimulations with PGD_2 gave no response in any of the two cell lines (Fig. 6). The functional analysis of the FP receptor-expressing CHO cells showed a coupling to PLC and an activation of intracellular calcium as expected (data not shown). It was also possible to analyze physiological responses when challenging the stable FP-expressing CHO cell line with various PGs (Fig. 6). These observations suggest that our rat cDNA clone encodes an endogenous prostaglandin $F_{2\alpha}$ receptor and that we have a functional response in our transfected rat FP-expressing CHO cells.

A major goal of clinical pharmacology and the pharmaceutical industry is the development of more selective drugs with greater efficacy than those currently in use. Impediments for such a process regarding targeting of the FP receptor are the low abundance of the receptor protein available to study in tissue, and the lack of suitable homogeneous model systems of the receptors with which to screen drugs. Commonly cells used for $PGF_{2\alpha}$ receptor analysis are obtained from bovine corpus luteum preparations, which also contain EP receptors [35].

A novel approach to the solution of this problem is to clone cDNAs encoding FP receptors, construct expression vectors containing these cDNAs, and create a series of stable transfected mammalian cell lines or prokaryotic cells which express functional FP receptors in abundance. These cell lines, which would express a homogeneous population of FP receptors, can be used by the pharmaceutical industry or others to screen drugs and study the FP receptors using a variety of biochemical, physiological and pharmacological techniques. To accomplish this goal, we have isolated the cDNA encoding a rat FP receptor linked to the activation of second messengers as measured by intracellular calcium. This cDNA was inserted

Table 3
Binding parameters of prostanoid agonists to membranes prepared from CHO cells expressing the transfected rat FP receptor.

Ligand	K_i (nM)	IC_{50} (nM)	B_{max} (fmol/mg)	Two site model*			
				K_H (nM)	K_L (nM)	R_H (%)	R_L (%)
PGF_{2a}	11 ± 2	18 ± 3	146 ± 4	3.9 ± 1.6	34 ± 10	55 ± 8	45 ± 8
PGE_2	400 ± 100	660 ± 170	153 ± 8				
PGD_2	470 ± 170	800 ± 254	184 ± 18				
PhXA 85	11 ± 2	18 ± 4	136 ± 11				
PhXA 70	1.1 ± 0.2	1.7 ± 0.3	48 ± 2				
Fluprostenol	0.4 ± 0.1	7.5 ± 1.5	114 ± 3	0.2 ± 0.9	22 ± 10	65 ± 3	35 ± 3

Data are means ± SE from 3-4 independent experiments
K_H and K_L = affinity constants for high and low binding sites
R_H and R_L = proportion of high and low affinity binding sites
*The two-site fit is significant better ($P > 0.05$) than the single site analysis

Table 4
K_d values for [^{3}H]PGF$_{2\alpha}$ specific FP receptor binding to intact CHO cells expressing the rat and human FP receptor, respectively.

Receptor	Kd (nM)	n
Rat	2.25 ± 0.34	4
Human	1.63 ± 0.39	3

mean values ± SE

into different eukaryotic expression vectors and used in the construction of cell lines expressing a functional protein. The resulting FP receptor-expressing cell lines were used to investigate the affinity and efficacies of agonist and putative antagonist drugs of a FP receptor using various techniques, such as radioligand binding and in mammalian cell line second messenger assays.

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