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Cloned human EP_1 prostanoid receptor pharmacology characterized using radioligand binding techniques

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Abstract

Prostaglandins such as prostaglandin E_2 (PGE_2) interact with EP-class prostanoid receptors including EP_1, EP_2, EP_3 and EP_4 subtypes. We have conducted a detailed pharmacological characterization of the binding of [^{3}H]-PGE_2 to recombinant human EP_1 prostanoid receptors expressed in human embryonic kidney (HEK-293) cells using a broad panel of natural and synthetic prostanoids. The receptor displayed high affinity (K_d = 16.0±0.69 nM; n = 3) for [^{3}H]-PGE_2, and was expressed at high levels (B_{max} = 3.69±0.30 pmol (mg protein)$^{-1}$) in cell membranes of HEK-293 cells. Specific binding constituted 97.5±1.4% (n = 12) of the total binding. In competition assays, the rank order of affinities of natural prostanoids for the receptor was $PGE_2 > PGE_1 > PGF_2 > PGI_2 > PGD_2$. PGE_2 was more effective than PGE_1 at displacing bound [^{3}H]-PGE_2 (K_i for PGE_2 = 14.9±2.2 nM; K_i for PGE_1 = 165±29 nM). The affinities of enprostil (K_i = 14.5±3.1 nM) and 17-phenyl-ω-trinor-PGE_2 (K_i = 7.3±2.7 nM) for the receptor were quite similar to that of PGE_2, while that of sulprostone (K_i = 137±13 nM) more closely resembled PGE_1. Some compounds historically classified as specific for DP prostanoid receptors bound with relatively high affinity to the recombinant human EP_1 receptor (e.g. ZK118182 (K_i = 73.4±8.6 nM) and ZK110841 (K_i = 166±20 nM)). All FP (e.g. travoprost acid, fluprostenol), IP (iloprost) and TP (SQ29548) receptor-specific ligands exhibited low affinity ($K_i \geq 1$ μM).

Introduction

Prostanoids, including prostaglandins such as prostaglandin D_2 (PGD_2) and PGE_2, have many functions in biological systems such as immunoregulation, platelet aggregation and smooth muscle contraction or relaxation (Coleman et al 1994). The different prostanoid classes and their corresponding receptors (DP, EP, FP, IP and TP) are linked through G-proteins to various intracellular signalling pathways (cAMP generation, phosphoinositide turnover, Ca^{2+} mobilization, etc.) (Coleman & Humphrey 1993; Coleman et al 1994). Recently, various prostanoid agonists have found utility in treating clinical conditions as diverse as male erectile dysfunction (Williams et al 1998) and increased intraocular pressure, a risk factor associated with glaucoma (Bito et al 1993; Hellberg et al 2002). EP, DP and FP receptors have been found in the ciliary muscle of the eye (Davis & Sharif 1999; Sharif et al 1999) and the trabecular meshwork (Anthony et al 1998), key structures for the maintenance of intraocular pressure. Different prostanoids apparently lower intraocular pressure by different mechanisms. Whereas $PGF_{2\alpha}$ appears to increase outflow via the uveoscleral route by promoting remodelling of the extracellular matrix in the ciliary muscle (Gabelt & Kaufman 1989; Lindsey et al

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1996), PGE_1 may lower intraocular pressure by stimulating conventional outflow through the trabecular meshwork by a cAMP-dependent mechanism (Dijkstra et al 1999). Within the E-series prostanoids, at least four subclasses of EP receptor (EP_1, EP_2, EP_3 and EP_4) are known (Coleman et al 1994). Subsequent to the cloning of these four receptor genes from cDNA in the mouse (Kiriyama et al 1997), rat (Boie et al 1997) and humans, the mechanisms of signal transduction for each subclass has been delineated. In the case of the EP_1 receptor, activation by PGE_2 leads to phosphoinositide turnover and mobilization of intracellular calcium (Coleman et al 1994), which ultimately causes the final biological response such as contraction of smooth muscle (Lawrence et al 1992; Ndukwu et al 1997). The EP_1 receptor has been detected in the human kidney, liver and heart where it may play a protective role in ionic balance (Guan et al 1998) and possibly recovery from ischaemic insult (Baek et al 1999). The cloning of the rat (Boie et al 1997), mouse (Kiriyama et al 1997) and human (Funk et al 1993) EP_1 receptors has been accomplished. The rodent and human EP_1 receptors appear to be quite homologous (Funk et al 1993; Boie et al 1997; Kiriyama et al 1997). While preliminary pharmacological studies have been conducted on rat and mouse EP_1 receptors (see above), little pharmacological characterization has been described for the human EP_1 receptor. Therefore, the aim of the present study was to define the binding profile and pharmacological characteristics of the cloned human EP_1 receptor expressed in HEK-293 cell membranes using a large number of natural and synthetic prostanoids.

Materials and Methods

Recombinant human EP_1 receptor membranes

Cell membranes from HEK-293 cells expressing the recombinant human EP_1 receptor were purchased from Receptor Biology, Inc. (Beltsville, MD). The membranes (Batch 1481; 1.4 pmol (mg protein)$^{-1}$) were stored in liquid nitrogen until use.

Radioligand

[^{3}H]-PGE_2 (NEN Life Science Products, Inc., Boston, MA) was used in competitive binding studies. The radioligand (batch no. NET-428, lot 3281-134) had a specific activity of 200 Ci mol^{-1} and was supplied at 0.1 mCi mL^{-1}. This reagent was stored at -40°C until dilution in binding buffer.

Chemicals and prostanoid compounds

Ethylenediaminetetraacetic acid (EDTA), 2-[*N*-morpholino]ethanesulfonic acid (MES), polyethylenimine and manganese chloride were obtained from Sigma Chemical Co. (St Louis, MO). Potassium hydroxide (45%) was purchased from EM Sciences (Gibbstown, NJ). A range of prostanoid compounds were used to define the ligand-binding characteristics of the cloned human EP_1 receptor. The natural prostaglandins PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2 and PGD_2, as well as the following prostaglandin analogues, were obtained from Cayman Chemical Co. (Ann Arbor, MI): cloprostenol, iloprost, fluprostenol, sulprostone, misoprostol, 17-phenyl-ω-trinor-PGE_2, 11-deoxy-16,16-dimethyl- PGE_2, 11-deoxy-PGE_1 and 13,14-dihydro-PGE_1. The compounds AH22921X, AH23848B, BW245C and BWA868C were generous gifts from Glaxo-SmithKline (Stevenage, UK). An additional group of compounds was synthesized in the Medicinal Chemistry department at Alcon Research Ltd (Fort Worth, TX): latanoprost, travoprost acid, travoprost, PHXA85, enprostil, 16-*R*-butaprost, SQ27986, UF-021, AL-6556 (13,14-dihydro-ZK118182) and AL-6598 (isopropyl ester of AL-6556) (Hellberg et al 2001), as well as the putative EP_4 agonists (Burk 1997) AL-24615 (15-methoxy-17-(2-furanyl)-18,19,20-trinor-$PGF_{2\alpha}$) and AL-24620 (17-(5-methyl-2-furanyl)-18,19, 20-trinor-$PGF_{2\alpha}$) (Davis & Sharif 2000). The compounds AH6809 and SQ29548 were obtained from Tocris Cookson Inc. (Ballwin, MO) and Research Biochemicals Inc. (Natick, MA), respectively. ZK110841 and ZK118182 were generous gifts from Schering AG (Berlin, Germany). In addition, S-1033, SC19920 and RS93520 were generous gifts from Shionogi (Osaka, Japan), G.D. Searle (Skokie, IL) and Hoffman La-Roche (Basel, Switzerland), respectively.

Competitive binding assays

Assays were conducted in 10 mM 2-[*N*-morpholino] ethanesulfonic acid (MES) buffer (pH 6.0) containing 1 mM EDTA and 10 mM $MnCl_2$, pH 6.0 (binding buffer). Initial tissue linearity studies were carried out using 0.5–160 μg of HEK-293 cell membranes expressing the cloned human EP_1 receptor per 0.5-mL total volume in 96-well assay blocks (Matrix Technologies Corp., Hudson, NH), using 3 nM [^{3}H]-PGE_2. Membranes were thawed quickly, diluted to the desired concentration in binding buffer and mixed to a homogeneous suspension before dispensation. After addition of the radioligand, the blocks were incubated at 23°C for 90 min on a rotary shaker (60 rev min^{-1}). The assay mixture was then harvested on Whatman GF/B glass fibre filter mats

(previously soaked in 0.5% polyethyleneimine) using rapid vacuum filtration with cold 10 mM MES plus 1 mM EDTA (pH 6). The mats were dried in a microwave oven for 3 min before being sealed in a plastic bag with 30 mL Wallac Betaplate Scint scintillation fluid (Wallac Oy, Turku, Finland). Bound radioligand was then quantified by liquid scintillation spectrometry at 50% efficiency.

For routine assays, the HEK-293 cell membranes containing the EP_1 receptor were used at 8 μg per 0.5 mL total volume. A series of competitive binding assays was carried out with unlabelled PGE_2 to determine the dissociation constant (K_d) and maximal ligand binding (B_{max}) values for the membrane preparation using the specific activity dilution paradigm. In these assays, unlabelled PGE_2 was diluted in ten half-log steps and 50 μL of each dilution was added to the assay block in duplicate using a Biomek 2000 automated laboratory workstation (Beckman Instruments, Inc., Fullerton, CA). This was followed by the addition of 400 μL of cell membranes and 50 μL of the radioligand (final concentration 2 nM). Other assay parameters were as detailed above. For competitive binding determination of equilibrium inhibition constant (K_i) values, prostanoid compounds were diluted in five log steps and assayed in duplicate as described above. Non-specific binding in both assay formats was determined with 10 μM PGE_2, or in some instances, by the highest concentration of the test compound. Both methods yielded very similar data.

Data analysis

Resulting disintegrations per min^{-1} (d min^{-1}) values from individual assays were analysed with spreadsheet-based non-linear, iterative curve-fitting computer programs using logistic functions (Bowen & Jerman 1995; Sharif et al 1998). In some cases, assay data were also analysed by the KELL/EBDA software package (McPherson 1983). For derivation of K_i values from the compound concentrations resulting in 50% inhibition of ligand binding (IC50 values), the method of Cheng & Prusoff (1973) was employed. The correlation plots were constructed in the Origin Scientific Graphics software package (Microcal Software, Northampton, MA).

Results

Tissue linearity

Specific binding of [^{3}H]-PGE_2 to HEK-293 cell membranes expressing the human cloned EP_1 receptors was linearly related to the amount of receptor protein (2–80 μg mL^{-1}; data not shown). Specific binding was at least 80% of the total binding across the entire tissue linearity range indicating a high level of specific binding to these receptors. To conserve membrane stocks and still preserve adequate signal strength, it was decided that 8 μg per 0.5 mL of the membrane preparation would be sufficient for subsequent assays. At this concentration, specific binding constituted 97.5±1.4% (n = 12) of the total binding.

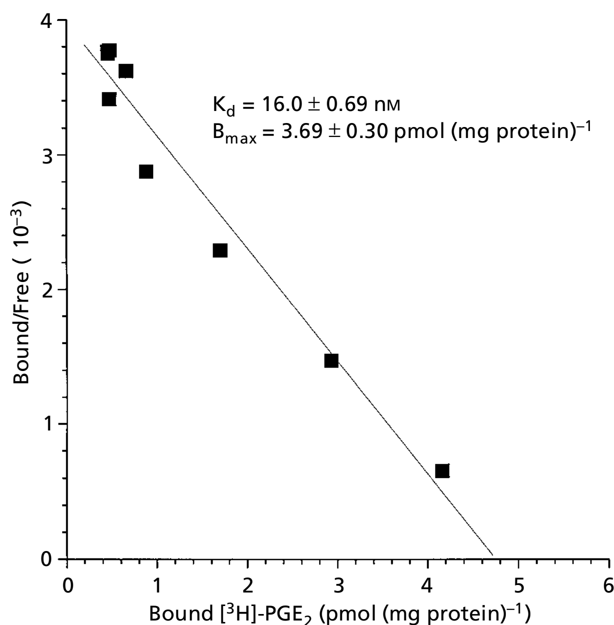

Figure 1 Scatchard analysis of competitive binding experiments with unlabelled PGE_2. Analysis was performed using data from competition curves obtained as described in the Methods. Data from a representative experiment are shown. ■ = mean of duplicate determinations.

Determination of K_d and B_{max} values

Unlabelled PGE_2 competed for [^{3}H]-PGE_2 in a concentration-dependent manner. Analysis of these specific activity dilution data produced a linear Scatchard plot (Figure 1). Computer analysis of these competition binding data yielded the following binding parameters: K_d = 16.0±0.69 nM, B_{max} = 3.69±0.30 pmol (mg protein)$^{-1}$ (n = 3).

Determination of K_i values for prostanoid compounds

A range of prostanoid compounds with differing prostanoid class and EP-subclass affinities were evaluated in competition assays against 2 nM [^{3}H]-PGE_2 to

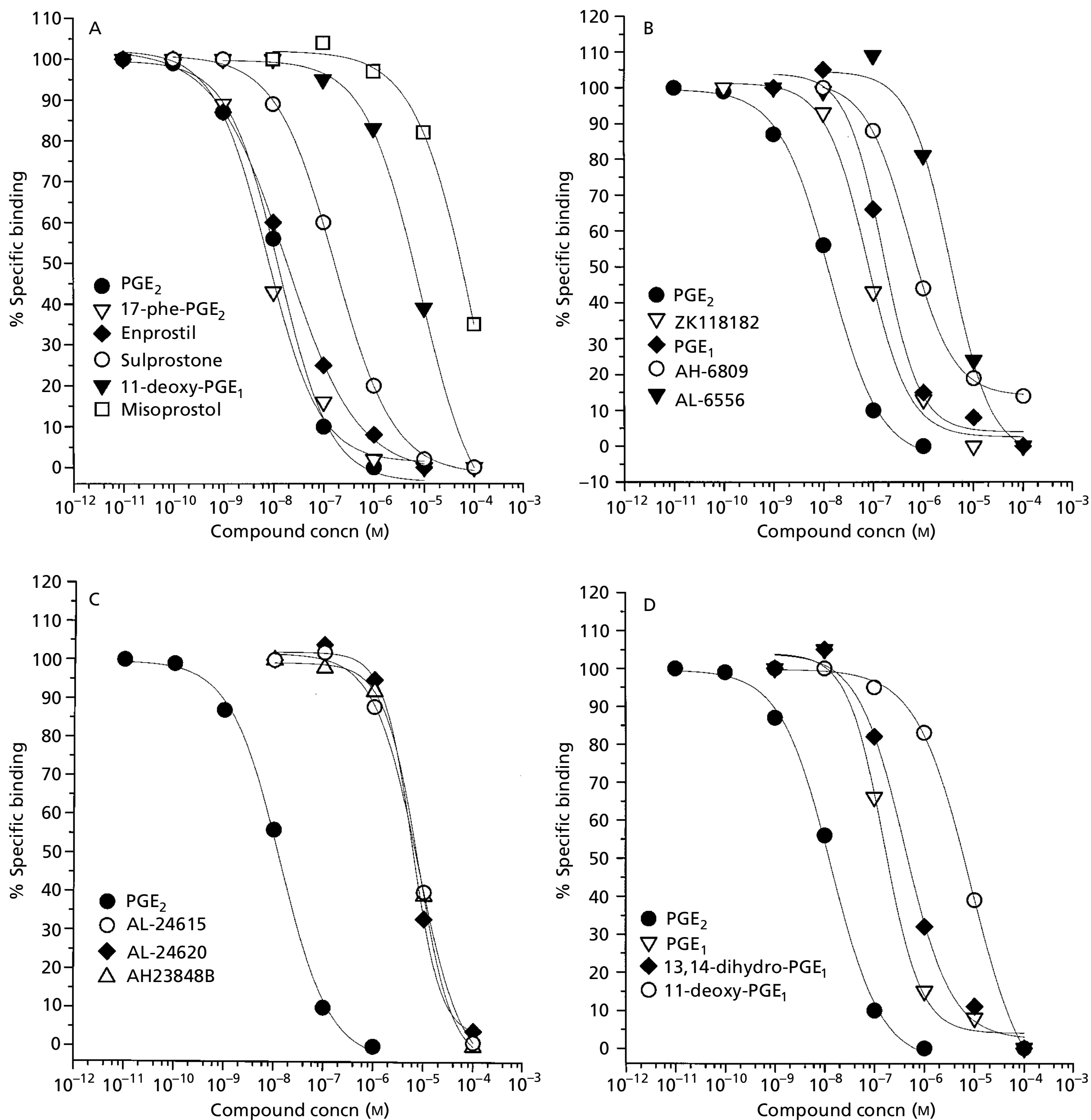

Figure 2 Competitive binding assays with natural prostaglandins and prostaglandin analogues. Data are derived from five-point titrations performed in duplicate, and the mean of ⩾ triplicate determinations is plotted. Error bars are omitted for clarity.

generate the competition curves. Analyses of the binding data from these experiments generated IC50 values that were converted to K_i values for each compound as detailed in the Methods section. Test compounds could be divided into groups depending on their source (natural prostaglandins as opposed to synthetic) or their reported affinity in the literature for specific prostanoid receptor classes. Plots depicting the titration curves for selected compounds are shown in Figures 2A–2D, while full summaries of derived K_i data can be found in Tables 1–3.

A comparison of $-\log K_i$ (pK_i) values resulting from this study with those previously obtained by Boie et al (1997) with recombinant rat EP_1 receptors expressed in

Table 1 Summary of competitive binding data for natural prostanoids.

Prostanoid	K_i (nM)	Hill coefficient (n_H)
PGE_2	14.9±2.2	1.15±0.10
PGE_1	165±29	1.06±0.09
$PGF_{2\alpha}$	594±12	1.40±0.39
PGI_2	15800±1940	0.88±0.08
PGD_2	19500±2930	1.14±0.13

Data are means±the s.e.m. from ⩾3 experiments performed in duplicate.

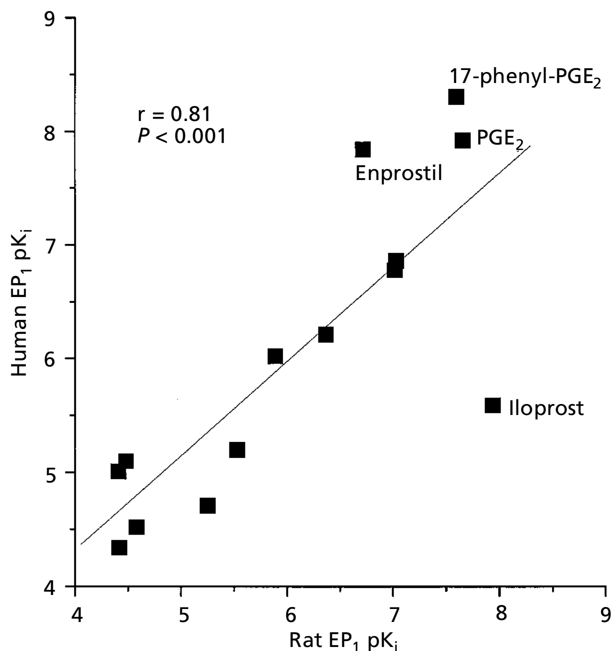

Figure 3 Correlation plot between the recombinant human EP_1 binding data generated in this study vs that previously reported by Boie et al (1997) for the rat EP_1 receptor. Data are expressed as mean pK_i ($-\log K_i$) values for individual compounds.

HEK-293 cells yielded a linear regression fit with a correlation coefficient of 0.81 (Figure 3). Comparison of our data with that of Kiriyama et al (1997) with the mouse EP_1 receptor was not as good, producing at best a correlation coefficient of 0.55 (data not shown).

Competitive binding assays

The rank order of natural prostanoid affinity for the recombinant human EP_1 receptor was PGE_2 > PGE_1 > $PGF_{2\alpha}$ > PGI_2 > PGD_2 (Table 1). PGE_1 was approximately one log unit less effective at displacing bound [^{3}H]-PGE_2 than unlabelled PGE_2. When various prostaglandin analogues were evaluated, enprostil and 17-phenyl-ω-trinor-PGE_2 were closely aligned with PGE_2, while sulprostone mirrored the affinity of PGE_1 for the EP_1 receptor (Tables 1 and 2). The 11-deoxy derivative of PGE_1 was less active than native PGE_1 by a factor approaching two logs, while the affinity of misoprostol resembled the low affinities displayed by PGD_2 and PGI_2. When FP-receptor-specific prostaglandin analogues (e.g. travoprost acid) were tested, all displayed affinities at least two log units lower than PGE_2 (Table 3). However, the same could not be said for two prostaglandin analogues reported as being specific for the DP receptor. In fact, ZK110841 and ZK118182 displayed affinities for the cloned human EP_1 receptor that were equal to or greater than PGE_1 itself (Table 3, Figure 2B). Modification of ZK118182 to the 13,14-dihydro derivative (AL-6556) abolished this high affinity. All putative EP_4-receptor-specific ligands had affinities approximately 3 logs lower than that of PGE_2 (Figure 2C; Table 2). When synthetic derivatives of PGE_1 were evaluated, the 13,14-dihydro derivative retained much of the affinity of the native prostaglandin, while the 11-deoxy derivative was weaker by almost two logs (Figure 2D). A quantitative summary of all of the data from the competitive binding experiments for the human EP_1 receptors is presented in Tables 1–3.

A good correlation was found between the pharmacological profile of the cloned human and rat EP_1 receptors (r = 0.81, $P < 0.001$; Figure 3), but a lesser correlation (r = 0.55) existed between the cloned human and mouse EP_1 receptors (data not shown).

Discussion

At present, the existing literature dealing specifically with the EP_1 receptor is sparse. The cloning and initial pharmacological characterization of the human and rodent EP_1 receptor has been reported (Funk et al 1993; Boie et al 1997; Kiriyama et al 1997). The EP_1 receptor is a heptahelical G-protein coupled protein which couples to phospholipase C to generate inositol phosphates and diacylglycerol and mobilizes intracellular calcium (Funk et al 1993; Coleman et al 1994; Ichikawa et al 1996; Boie et al 1997; Kiriyama et al 1997). The rodent and human EP_1 receptors share a high degree of homology at the genetic and amino acid sequence levels but there are only very limited studies describing their respective pharmacological characteristics (see above).

This study evaluated the ligand binding and pharmacological properties of the recombinant human EP_1

Table 2 Summary of competitive binding data for EP receptor-specific prostanoids.

Prostanoid	Reported specificity	K_i (nM)	Hill coefficient (n_H)
17-phenyl-ω-trinor-PGE_2	EP_1/EP_3	7.3±2.7	0.95±0.32
Enprostil	EP_3/EP_1	14.5±3.1	1.60±0.97
Sulprostone	EP_1/EP_3	137±13	0.78±0.12
13,14-dihydro-PGE_1	EP	531±207	1.13±0.25
11-deoxy-16,16-dimethyl-PGE_2	EP	1300±976	0.96±0.11
AL-24615	EP_4	5730±679	1.15±0.14
AL-24620	EP_4	5390±1440	1.48±0.11
11-deoxy-PGE_1	$EP_2/EP_3/EP_4$	6340±1960	1.25±0.26
AH23848	EP_4	7920±1870	1.49±0.23
Butaprost acid	EP_2	9690±1400	2.21±0.57
SC19220	EP_1	30100±9570	1.37±0.32
Misoprostol	$EP_1/EP_3/EP_4$	45500±9420	1.00±0.11

Data are means±the s.e.m. from ⩾ 3 experiments performed in duplicate. Where evidence exists in the literature for an EP receptor subtype specificity it is listed.

Table 3 Summary of competitive binding data for DP-, FP- and IP-receptor-specific prostanoids.

Prostanoid	Reported specificity	K_i (nM)	Hill coefficient (n_H)
ZK118182	DP	73.4±8.6	1.08±0.19
ZK110841	DP	166±20.3	0.61±0.12
AH6809	DP	946±336	0.62±0.17
Cloprostenol	FP	1310±570	1.02±0.31
Latanoprost acid (PHXA85)	FP	2060±688	1.22±0.09
Iloprost	IP	2550±249	0.87±0.05
AL-6556	DP	3410±1050	1.22±0.30
AL-6598	DP	9590±958	1.93±0.61
Travoprost acid	FP	9540±1240	1.96±0.50
SQ27986	DP	9570±1630	0.91±0.16
Fluprostenol	FP	12300±1240	4.20±1.48
Unoprostone (UF-021)	FP	11700±2710	2.16±0.98
S-1033	FP	13500±1670	2.56±0.43
Travoprost	FP	13800±2820	3.14±0.73
RS93520	DP	15600±7580	1.05±0.26
Latanoprost	FP	16700±7000	1.55±0.28
BWA245C	DP	24000±5800	0.65±0.14
BWA868C	DP	27000±9420	2.35±0.33
SQ29548	TP	85600±31100	1.10±0.21

Data are means±the s.e.m. from ⩾ 3 experiments performed in duplicate.

receptor expressed in HEK-293 cells using several classes of natural and synthetic prostanoids. The initial binding studies yielded a high degree of specific binding expected of a uniquely expressed recombinant protein like other cloned receptors (e.g. EP_4 receptors; Davis & Sharif 2000). The dissociation constant (K_d = 16 nM) for [^{3}H]-PGE_2 binding to the cloned human EP_1 receptor protein in our studies correlated reasonably well with that reported by Funk et al (1993) of 1 nM, and this was also in good agreement with that of the recombinant rat receptor (K_d = 24 nM; Boie et al 1997) and mouse receptor (K_d = 21 nM; Kiriyama et al 1997). The overall receptor density (B_{max}) value we obtained (3.69 pmol (mg protein)$^{-1}$) compared well with that for the rat receptor (3.3 pmol (mg protein)$^{-1}$; Boie et al 1997), but not for the mouse receptor (200 fmol (mg protein)$^{-1}$;

Kiriyama et al 1997) or the cloned human EP_1 receptor expressed in COS-M6 cells (Funk et al 1993). These similar and disparate expression levels may explain the general agreement of our drug inhibition data with those generated using the rat receptor but not with those of Kiriyama et al (1997) with the mouse receptor. Unfortunately, the previous pharmacological data for the cloned human EP_1 receptor (Funk et al 1993) are rather limited and direct comparisons are not possible with our studies.

In terms of the pharmacological characteristics of the EP_1 receptor, our data compared favourably with the limited data of Boie et al (1997) for the rat EP_1 receptor, and for some compounds tested, our values also agreed well with those generated by Kiriyama et al (1997) for the mouse EP_1 receptor. For the natural prostaglandins, this was most apparent with PGE_2, where we obtained a K_i value of 14.9 nM versus 22 nM reported by Boie et al (1997) and 20 nM by Kiriyama et al (1997). However, our K_i values for PGE_1 were significantly higher (165 nM) than those of Boie et al (1997) (95 nM) or Kiriyama et al (1997) (36 nM). There are also other instances in which our results vary significantly from those studies using cloned rat or mouse EP_1 receptors. For instance, iloprost yielded a K_i value of 2.6 μM in our hands, whereas the rat and mouse receptors yielded $K_i$values of 11.5 nM and 21 nM, respectively (Boie et al 1997; Kiriyama et al 1997). Other cases illustrate the general agreement seen with results generated using the rat receptor as depicted in Figure 3. Sulprostone yields a K_i value of 137 nM in our system, and 94 nM with the rat receptor. However, Kiriyama et al (1997) reported a K_i value of only 21 nM. The relatively high affinity of enprostil at the EP_1 receptor is not surprising since it has EP_3/EP_1 pharmacology (Coleman et al 1994). The relatively low affinity of SC-19220 at the EP_1 receptor is also consistent with the reported low potency of this compound in other systems (Coleman et al 1994).

The most surprising finding was that the DP-receptor-specific compounds ZK118182 and ZK110841 had high affinity for the recombinant human EP_1 receptor. Other compounds known to be partially specific for the EP_1 receptor along with other receptor classes such as enprostil, sulprostone, and 17-phenyl-ω-trinor-PGE_2, produced expected binding profiles. There is a recent report of a biphenylene dibenzazocine class of compounds that appears to be strongly selective for the EP_1 receptor subclass in comparison with all other prostanoid receptors (Ruel et al 1999). Unfortunately, we did not have access to these latter compounds for our studies.

There are certainly many reports dealing with effects of prostanoid compounds in which elevated intracellular calcium plays a pivotal role, but there is controversy regarding the receptors mediating these processes. For example, even though PGE_2 and 17-phenyl-ω-trinor-PGE_2 increase intracellular calcium levels in rat microglia (Caggiano & Kraig 1999) and in human ciliary muscle cells (Chen et al 1997), the involvement of the EP_1 receptor here is still undefined since neither of these agents is specific for the EP_1 receptor. Thus, these reports imply either a direct effect on the EP_1 receptor in increasing intracellular calcium or an indirect effect resulting from stimulation of another prostanoid receptor. Other investigations have probed the ability of EP_1 receptor agonists to inhibit cation inflow through different ionic channels, either the L-type Ca^{2+} channel or the Ca^{2+}-activated potassium channel (Clapp et al 1998; Yamamoto et al 1999). There are also reports of EP_1 receptors in the nuclear membrane of cultured cells, implying a role in activation/regulation of transcription (Bhattacharya et al 1998). In the cat eye, agonists acting at the EP_1 receptor appear to lower intraocular pressure, and selective EP_1 agonists are being explored for their ability to control increased intraocular pressure in humans (Stjernschantz et al 1999). The multitude of functional responses generated in different cell models by prostanoid compounds presents a complicated picture of inter-related receptors and second messenger molecules. As the natural prostaglandins and many prostaglandin analogues interact with multiple prostaglandin receptor classes, assignment of downstream events to a particular class can be problematic. Therefore, the use of pharmacological agents described herein may be useful to help delineate whether EP_1 receptors are involved in these various processes.

In summary, the recombinant human EP_1 receptor expressed in HEK-293 cells provides us with an appropriate model to evaluate the binding of prostanoid compounds to probe and define the pharmacological properties of this receptor. Future evaluation of synthetic prostanoids may provide truly specific ligands which would be invaluable tools in the characterization of EP_1-receptor-dependent functional responses in human cells and tissues.

Conclusion

It is concluded that the cloned human EP_1 receptor transfected into HEK-293 cells can be expressed at relatively high levels. The expressed EP_1 receptor protein binds [^{3}H]-PGE_2 with a high affinity yielding very high specific binding. The overall pharmacological characteristics of the cloned human EP_1 receptor have been

defined by use of numerous natural and synthetic prostanoids. Collectively, these agents and data may be useful in ascribing EP_1 receptor function to the physiological and pharmacological actions of PGE_2 in biological systems in-vitro and in-vivo.

References

Anthony, T. L., Pierce, K. L., Stamer, W. D., Regan, J. W. (1998) Prostaglandin $F_{2\alpha}$ receptors in the human trabecular meshwork. *Invest. Ophthalmol. Vis. Sci.* **39**: 315–321

Baek, Y., Nakano, H., Kumada, K., Nagasaki, H., Kigawa, G., Sasaki, J., Kaneda, M., Htakeyama, T., Kitamura, N., Sanada, Y., Midorikawa, T., Yamaguchi, M. (1999) Administration of prostaglandin E1 reduces post-operative hepatocellular damage and restores hepatic integrity in patients undergoing hepatectomy. *Hepatogastroenterology* **46**: 1836–1841

Bhattacharya, M., Peri, K. G., Almazan, G., Ribeiro-Da-Silva, A., Shichi, H., Durocher, Y., Abramovitz, M., Hou, X., Varma, D. R., Chemtob, S. (1998) Nuclear localization of prostaglandin E_2 receptors. *Proc. Natl Acad. Sci. USA* **95**: 15792–15797

Bito, L. Z., Stjernschantz, J., Resul, B., Miranda, O. C., Basu, S. (1993) The ocular effects of prostaglandins and the therapeutic potential of a new $PGF_{2\alpha}$ analog, PhXA41 (latanoprost) for glaucoma management. *J. Lipid Mediat.* **6**: 535–543

Boie, Y., Stocco, R., Sawyer, N., Slipetz, D. M., Ungrin, M., Neuschafer-Rube, F., Puschel, G., Metters, K. M., Abramovitz, M. (1997) Molecular cloning and characterization of the four rat prostaglandin E_2 prostanoid receptor subtypes. *Eur. J. Pharmacol.* **340**: 227–241

Bowen, W. P., Jerman, J. (1995) Nonlinear regression using spreadsheets. *Trends Pharmacol. Sci.* **16**: 413–417

Burk, R. M. (1997) Cyclopentane heptan(ene)oic acid, 2-heteroarylalkenyl derivatives as therapeutic agents. *International Patent Application* WO 97/31895

Caggiano, A. O., Kraig, R. P. (1999) Prostaglandin E receptor subtypes in cultured rat microglia and their role in reducing lipopolysaccharide-induced interleukin-1beta production. *J. Neurochem.* **72**: 565–575

Chen, W., Andom, T., Bhattacherjee, P., Paterson, C. (1997) Intracellular calcium mobilization following prostaglandin receptor activation in human ciliary muscle cells. *Curr. Eye Res.* **16**: 847–853

Cheng, Y. C., Prusoff, W. H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**: 3099–3108

Clapp, L. H., Turcato, S., Hall, S., Baloch, M. (1998) Evidence that Ca^{2+}-activated K^+ channels play a major role in mediating the vascular effects of iloprost and cicaprost. *Eur. J. Pharmacol.* **356**: 215–224

Coleman, R. A., Humphrey, P. P. A. (1993) Prostanoid receptors: their function and classification. In: Vane, J. R., O'Grady, J. (eds) *Therapeutic applications of prostglandins.* Edward Arnold, London, pp 15–36

Coleman, R. A., Smith, W. L., Narumiya, S. (1994) VIII. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.* **46**: 205–229

Davis, T. L., Sharif, N. A. (1999) Quantitative autoradiographic visualization and pharmacology of FP-prostaglandin receptors in human eyes using the novel phosphor-imaging technology. *J. Ocular Pharmacol.* **15**: 323–336

Davis, T. L., Sharif, N. A. (2000) Pharmacological characterization of [^{3}H]-prostaglandin E_2 binding to the cloned human EP_4 prostanoid receptor. *Br. J. Pharmacol.* **130**: 1919–1926

Dijkstra, B. G., Schneemann, A., Hoyng, P. F. (1999) Flow after prostaglandin E_1 is mediated by receptor-coupled adenylyl cyclase in human anterior segments. *Invest. Ophthalmol. Vis. Sci.* **40**: 2622–2626

Funk, C. D., Furci, L., FitzGerald, G. A., Grygorczyk, R., Rochette, C., Bayne, M. A., Abramovitz, M., Adam, M., Metters, K. M. (1993) Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. *J. Biol. Chem.* **268**: 26767–26772

Gabelt, B. T., Kaufman, P. L. (1989) Prostaglandin $F_{2\alpha}$ increases uveoscleral outflow in the cynomolgus monkey. *Exp. Eye Res.* **49**: 389–402

Guan, Y., Zhang, Y., Breyer, R. M., Fowler, B., Davis, L., Hebert, R. L., Breyer, M. D. (1998) Prostaglandin E_2 inhibits renal collecting duct Na^+ absorption by activating the EP_1 receptor. *J. Clin. Invest.* **102**: 194–201

Hellberg, M. R., Sallee, V., Mclaughlin, M., Sharif, N. A., Desantis, L., Dean, T. R., Zinke, P. W. (2002) Identification and characterization of the ocular hypertensive efficacy of travoprost, a potent and selective FP prostaglandin agonist and AL-6598 a DP prostaglandin agonist. *Surv. Ophthalmol.* In press

Ichikawa, A., Sugimoto, Y., Negishi, M. (1996) Molecular aspects of the structures and functions of the prostaglandin E receptors. *J. Lipid Mediat. Cell Signal.* **14**: 83–87

Kiriyama, M., Ushikubi, F., Kobayashi, T., Hirata, M., Sugimoto, Y., Narumiya, S. (1997) Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.* **122**: 217–224

Lawrence, R. A., Jones, R. L., Wilson, N. H. (1992) Characterization of receptors involved in the direct and indirect actions of prostaglandins E and I on the guinea-pig ileum. *Br. J. Pharmacol.* **105**: 271–278

Lindsey, J. D., Kashiwagi, K., Boyle, D., Kashiwagi, F., Firestein, G. S., Weinreb, R. N. (1996) Prostaglandins increase proMMP-1 and proMMP-3 secretion by human ciliary smooth muscle cells. *Curr. Eye Res.* **15**: 869–875

McPherson, G. A. (1983) A practical computer based approach to the analysis of radioligand binding experiments. *Computer Prog. Biomed.* **17**: 107–114

Ndukwu, I. M., White, S. R., Leff, A. R., Mitchell, R. W. (1997) EP_1 receptor blockade attenuates both spontaneous tone and PGE_2-elicited contraction in guinea pig trachealis. *Am. J. Physiol.* **273**: L626–L633

Ruel, R., Lacombe, P., Abramovitz, M., Godbout, C., Lamontagne, S., Rochette, C., Sawyer, N., Stocco, R., Tremblay, N. M., Metters, K. M., Labelle, M. (1999) New class of biphenylene dibenzazocines as potent ligands for the human EP_1 prostanoid receptor. *Bioorg. Med. Chem. Lett.* **9**: 2699–2704

Sharif, N. A., Xu, S. X., Williams, G. W., Griffin, B. W., Crider, J. Y., Davis, T. L. (1998) Pharmacology of [^{3}H]Prostaglandin E_1/[^{3}H]Prostaglandin E_2 and [^{3}H]Prostaglandin $F_{2\alpha}$ binding to EP_3 and FP receptor binding sites in bovine corpus luteum: characterization and correlation with functional data. *J. Pharmacol. Exp. Ther.* **286**: 1094–1102

Sharif, N. A., Davis, T. L., Williams, G. W. (1999) [^{3}H]AL-5848 ([^{3}H]9-β-[+]fluprostenol): carboxylic acid of travoprost (AL-6221),

a novel FP prostaglandin to study the pharmacology and autoradiographic localization of the FP receptor. *J. Pharm. Pharmacol.* **51**: 685–694

Stjernschantz, J., Resul, B., Lake, S. (1999) Prostaglandin derivatives devoid of side effects for the treatment of glaucoma. *International Patent Application* WO 99/02165

Williams, G., Abbou, C. C., Amar, E. T., Desvaux, P., Flam, T. A., Lycklama, A., Nijeholt, G. A., Lynch, S. F., Morgan, R. J., Muller, S. C., Porst, H., Pryor, J. P., Ryan, P., Witzsch, U. K., Hall, M. M., Place, V. A., Spivack, A. P., Gesundheit, N. (1998) Efficacy and safety of transurethral alprostadil therapy in men with erectile dysfunction. MUSE Study Group. *Br. J. Urol.* **81**: 889–894

Yamamoto, T., Habuchi, Y., Tanaka, H., Suto, F., Morikawa, J., Kashima, K., Yoshimura, M. (1999) EP receptor-mediated inhibition by prostaglandin E_1 of cardiac L-type Ca^{2+} current of rabbits. *Am. J. Physiol.* **277**: H1369–H1374