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European Journal of Pharmacology 377 (1999) 101–115

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A novel biological role for prostaglandin D_2 is suggested by distribution studies of the rat DP prostanoid receptor

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Received 4 May 1999; accepted 11 May 1999

Abstract

We report the cloning, functional expression and cell-specific localization of the rat homologue of the prostaglandin D_2 receptor (DP). In situ hybridization, utilizing multiple digoxigenin-labelled riboprobes and their complementary sense controls, was performed to determine the detailed distribution of DP receptor mRNA in the central nervous system and the gastrointestinal tract. Within the brain, the leptomeninges and choroid plexus expressed DP receptor mRNA. Transcripts detected in the spinal cord were localized to the sensory and motor neurons of the dorsal and ventral horns, respectively, suggesting a role for the DP receptor in the modulation of central nervous system processes, including pain transmission. Within the gastrointestinal tract (stomach, duodenum, ileum and colon) signals were highly localized to the mucous-secreting goblet cells and the columnar epithelium. These findings suggest a novel biological role for prostaglandin D_2-mediated activity at the DP receptor, namely mucous secretion. In addition, radioligand binding assays (saturation analyses and equilibrium competition assays) and functional assays (measuring cAMP accumulation) were performed to characterize the recombinant rat DP receptor expressed in human embryonic kidney (HEK) 293(EBNA) cells. A single site of binding ($K_D = 14$ nM, $B_{max} = 115$ fmol/mg protein) was measured for prostaglandin D_2-specific binding to the rat DP receptor. Prostaglandin D_2 proved to be a potent agonist at the rat DP receptor ($EC_{50} = 5$ nM). The rank order of efficacy for DP receptor specific agonists [prostaglandin D_2 = prostaglandin J_2 = BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropylhydantoin)) > L-644,698 ((4-(3-(3-(3-hydroxy-octyl)-4-oxo-2-thiazolidinyl) propyl) benzoic acid) (racemate)] reflected the affinity with which the ligands bound to the receptor.

Keywords: Prostanoid receptor; DP; Prostaglandin D_2; Central nervous system; Gastrointestinal tract; (Rat)

1. Introduction

The eicosanoids referred to as the primary bioactive prostanoids encompass prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostacyclin (prostaglandin I_2) and thromboxane A_2. They act through eight individual prostanoid receptors DP, EP (EP_1, EP_2, EP_3, EP_4), FP, IP and TP (for review, see Coleman et al., 1994) which form a distinct sub-family within the rhodopsin-type G-protein coupled receptor superfamily. To date, the rat prostanoid receptors EP_1, EP_2, $EP_{3\alpha}$, EP_4 (Boie et al., 1997), FP (Lake et al., 1994), IP (Sasaki et al., 1994), and TP (Kitanaka et al., 1995) have been cloned. In this report, we describe the cloning, functional expression and cell-specific localization of the rat DP prostanoid receptor.

Prostaglandin D_2 is the prostanoid interacting preferentially with the DP receptor. It is generated from arachidonic acid in a number of tissues by the sequential actions of prostaglandin H synthase and prostaglandin D synthase, and is associated with both central and peripheral physiological effects. Within the central nervous system, prostaglandin D_2 has been associated with sleep induction, modulation of body temperature, olfactory function, hormone release, nociception and neuromodulation. Peripherally, prostaglandin D_2 has been shown to mediate vasodilation, inhibition of platelet aggregation, glycogenolysis, bronchoconstriction and vasoconstriction (for review, see Hayaishi, 1988; Ito et al., 1989). As well, prostaglandin D_2 is the major prostanoid generated by mast cells upon

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00358-1

immunological challenge (Roberts et al., 1980). However, several of these effects may be due to interaction of prostaglandin D_2 with prostanoid receptors other than the DP receptor (e.g., bronchoconstriction potentially occurs via the FP receptor) (Liston and Roberts, 1985).

The cloning and characterization of the human (Boie et al., 1995) and mouse (Hirata et al., 1994) DP receptor homologues has previously been reported and limited distribution data described. In both cases, the detection of DP receptor mRNA transcripts in various tissues by Northern blot analysis was presented. For the human DP receptor, 19 tissues were investigated (brain, retina, heart, aorta, lung, liver, stomach, small intestine, pancreas, kidney, adrenal, spleen, thymus, mammary gland, placenta, uterus, ovary, testis and skeletal muscle) and only the retina and small intestine were positive for human DP receptor mRNA. In the case of the mouse DP receptor, 10 tissues were probed (brain, thymus, lung, heart, spleen, stomach, liver, ileum, kidney and uterus) and positive signals were identified in ileum, lung, stomach and uterus. Although species variation with respect to DP receptor-mediated activity is well-established (Giles and Leff, 1988, and references within), much of the observed biological activity of prostaglandin D_2 cannot be accounted for by the reported distribution of the DP receptor. In order to address the link between prostaglandin D_2-stimulated and DP receptor-mediated activities, the rat homologue of the DP receptor has been used to study the cell-specific localization of rat DP receptor-specific mRNA transcripts as identified by in situ hybridization in selected tissues.

2. Materials and methods

2.1. Cloning of the rat DP prostanoid receptor

A DNA probe (rDPTMD2-5) of size 416 bp was amplified from a rat genomic DNA library by polymerase chain reaction (PCR) using the primers xDP-fTMD2 (5′ ACG GTC ACC GAC TTG CTG GGC AAG N 3′) and xDP-rTMD5 (5′ CAT GAG GCT GGA GTA GAG CAC AGA N 3′). These DP receptor-specific primers, based on sequences conserved between the mouse (Hirata et al., 1994) and human (Boie et al., 1995) homologues, correspond to regions within the second and fifth transmembrane domains, respectively. The PCR conditions were: initial denaturation at 95°C, 2 min; followed by 35 cycles of denaturation at 95°C, 15 s; then annealing and extension at 60°C, 30 s; and then a final extension at 72°C, 10 min (GeneAmp 9600 system; Perkin-Elmer/Applied Biosystems, Foster City, CA).

A rat spinal cord cDNA library ($1.0–1.2 \times 10^6$ plaques) (Stratagene, La Jolla, CA) was screened with the rDPTMD2-5 cDNA probe which was random-labelled with [α-^{32}P]cytidine 5′-triphosphate (NEN, Mississauga, ON). Three positive clones were obtained and their cDNA inserts were subcloned into bluescript pKS (Stratagene, La Jolla, CA) at the *Eco*RI site and sequenced (ABI-373 Stretch automated sequencer; Perkin-Elmer/Applied Biosystems). Two independent clones were used to construct the open reading frame (ORF) of the rat DP receptor: 10-2-I which spanned from the 5′ untranslated region to a position 10 residues 3′ of the first in-frame stop codon, and 24-1-II which extended from the cDNA corresponding to the first transmembrane domain to the 3′ untranslated region. The complete cDNA for the receptor (pKS-rDP) was constructed by ligation of the 5′ end of 10-2-I and the 3′ end of 24-1-II at a unique *Ngo*MI site which occurs at a position 150 bp from the start codon.

2.2. Construction of the FLAG-epitope tagged rat DP and human DP receptors

The 5′-FLAG-epitope tagged rat DP receptor was generated by first amplifying by PCR the 10-2-I clone using the primers FLAGrDPf72 (5′ ACT AAG CTT ACC ATG GAC TAC AAG GAC GAC GAT GAC AAG TGG CAA GGA CTA GCT GCG AAT GAG TCC TAT CGC 3′) and xDP-rTMD5, to generate DNA corresponding to the FLAG-epitope tagged 5′ terminus of the rat DP receptor. The PCR protocol used was as described above. The amplified DNA was digested using *Hin*dIII and *Ngo*MI. The digested fragment was then ligated into the 24-1-II clone, which had previously been digested with *Hin*dIII and *Ngo*MI, to produce the full length 5′-FLAG-epitope tagged rat DP receptor in bluescript pKS (pKS-FLAG-rDP). The 5′-FLAG-epitope tagged human DP receptor was generated by first amplifying by PCR human DP receptor cDNA in pcDNA3 (Invitrogen, Carlsbad, CA, USA) (pcDNA3-hDP; described previously by Boie et al., 1995) using the primers FLAGhDPf72 (5′ AAG CTT ACC ATG GAC TAC AAG GAC GAC GAT GAC AAG CTG CTC CCG CAC GCC GCG AAG TCG CCG TTC TAC 3′) and hDP-m22 (5′ CTT GGC ACA ACG AGT TGT CCA A 3′), as described above. The amplified DNA was digested at a *Hin*dIII site which was engineered into the FLAGhDPf72 primer, as well as at the *Bst*EII site 207 bp from the start codon. The digested fragment was then ligated into pcDNA3-hDP, digested with *Hin*dIII and *Bst*EII, to produce the full length 5′-FLAG-epitope tagged human DP receptor in pcDNA3 (pcDNA3-FLAG-hDP).

2.3. Construction of pCEP4-rDP, pCEP4-FLAG-rDP and pCEP4-FLAG-hDP mammalian expression vectors

Digestion of both pKS-rDP and pKS-FLAG-rDP with *Hin*dIII and *Not*I released their respective fragments from pKS, which were subsequently subcloned into pCEP4 (Invitrogen) at the same restriction sites employed for construction of the pCEP4-rDP and pCEP4-FLAG-rDP expression vectors. Digestion of pcDNA3-FLAG-hDP with *Hin*dIII and *Xho*I released FLAG-human DP from pcDNA3, which was subsequently subcloned in pCEP4 at the same restriction sites for construction of the pCEP4-FLAG-hDP expression vector.

2.4. pCEP4-rDP, pCEP4-FLAG-rDP and pCEP4-FLAG-hDP bulk stable expression in HEK 293(EBNA) cells, cell culture and membrane preparation

Transfection of pCEP4-rDP, pCEP4-FLAG-rDP and pCEP4-FLAG-hDP plasmids into HEK 293(EBNA) for bulk stable expression of the rat DP, FLAG-rat DP and FLAG-human DP receptor constructs was achieved as previously described (Wright et al., 1998). Cells were maintained in culture for 48 h post transfection and then grown in the presence of 200 μg/ml hygromycin B (Calbiochem, La Jolla, CA, USA) for 2 weeks, to select for resistant colonies expressing the rat DP, FLAG-rat DP or the FLAG-human DP receptors. Maintenance of HEK 293(EBNA) cells in culture was as previously described (Wright et al., 1998) for subsequent analyses by radioligand binding assays, immunoblot and cAMP accumulation assays.

In order to prepare membranes from HEK293(EBNA) cells (all procedures at 4°C), they were first resuspended in the presence of protease inhibitors (2 mM phenylmethylsulfonylfluoride, 10 μM E-64, 100 μM leupeptin and 0.05 mg/ml pepstatin) by Dounce homogenization (pestle B, 10 strokes). Cells were next disrupted by nitrogen-cavitation at 800 psi for 30 min on ice. The resulting cell suspension was subjected to two centrifugation steps: 1000 $\times g_{max}$ for 10 min followed by 100 000 $\times g_{max}$ for 30 min. The resulting pellet was resuspended to 1/10th the original volume in 10 mM HEPES/KOH (pH 7.4) containing 1 mM EDTA (tetrasodium salt) by Dounce homogenization (pestle A, 10 strokes), and aliquots were quickly frozen in liquid nitrogen and then stored at −80°C at a protein concentration of 8–10 mg/ml.

2.5. [^{3}H]prostaglandin D_2 binding to FLAG-rat DP and FLAG-human DP receptors expressed in HEK 293(EBNA) cell membranes

Radioligand binding assays were carried out as previously described (Wright et al., 1998). Briefly, assays were performed in 0.2 ml of 10 mM HEPES–KOH (pH 7.4), 1 mM EDTA, 8 nM [^{3}H]prostaglandin D_2 (115 Ci/mmol) and 10 mM $MnCl_2$. Compounds were added in dimethylsulphoxide (Me_2SO) at 1% (v/v) of the final incubation volume (vehicle concentration was constant throughout). The reaction was initiated by the addition of 200 μg (FLAG-rat DP receptor) or 50 μg (FLAG-human DP receptor) cell membrane protein to all tubes and the samples were incubated at room temperature for 1 h. The reaction was terminated by rapid filtration at 4°C in 4 ml of 10 mM HEPES–KOH (pH 7.4) through GF/C filters (Brandel) which had been presoaked in the same buffer. The residual [^{3}H]prostaglandin D_2 bound to the filter was determined in 5 ml per well of Ultima Gold (Packard, Meriden, CT, USA) scintillation cocktail (59% efficiency). Non-specific binding was determined in the presence 1 μM prostaglandin D_2. Analysis of [^{3}H]prostaglandin D_2 binding was performed as previously described (Wright et al., 1998). In particular, sigmoidal curves from equilibrium competition assays were analyzed by proprietary software custom designed, based on the work described in Kenakin (1997), which employs a non-linear least-squares fitting routine based on the four parameter logistic equation: $y = (m_1 - m_2)(1 + (m_0/m_3)e^{m_4})^{-1} + m_2$; where m_1 and m_2 represent the maximum and minimum of the curve, m_3 represents the inflection point (IP), m_4 represents the slope of the curve at the IP, m_0 represents the concentration of the competing ligand and y represents the percentage of [^{3}H]prostaglandin D_2-specific binding. K_i values were calculated from the equation $K_i = IP/1 + [radioligand]/K_D$ (Cheng and Prusoff, 1973). The integrity of the radioligand was maintained throughout the incubation in the presence of HEK293(EBNA) membranes.

2.6. cAMP assays with FLAG-rat DP and FLAG-human DP receptor-expressing HEK 293(EBNA) cell membranes

Assays of cAMP accumulation were carried out as previously described (Wright et al., 1998). Briefly, FLAG-rat DP or FLAG-human DP receptor-expressing HEK 293(EBNA) cells were harvested at 80% confluence by resuspension in 10 ml of enzyme-free cell dissociation buffer and washed in phosphate-buffered saline (PBS) by centrifugation (300 $\times g_{max}$, 6 min, room temperature). Cells were then washed in 10 ml of Hank's balanced salt solution (HBSS) by centrifugation under the same conditions as above and resuspended in HBSS at 4×10^6 cells/ml. Cell viability was determined to be >95% by Trypan blue exclusion. The generation of cAMP was performed in a final incubation volume of 0.2 ml of HBSS containing 100 μM Ro 20-1724 to abrogate cAMP hydrolysis. Compounds were added in Me_2SO, kept constant at 1% (v/v) of the final incubation volume. The reaction was initiated by the addition of 2×10^4 cells per incubation and the samples were incubated for 10 min at 37°C with shaking. Reactions were halted by the incubation of samples in boiling water for 3 min and cAMP was subsequently measured by use of a [^{125}I]cAMP scintillation proximity assay (Albano et al., 1974). Sigmoidal concentration–response curves were analyzed by custom designed software to determine EC_{50} values. Maximal stimulation was defined as the quantity of cAMP produced by incubation of a given receptor construct (either the FLAG-rat DP or FLAG-human DP receptor) with 1 μM prostaglandin D_2.

2.7. Protein assays

Protein concentrations were measured by the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) using bovine serum albumin as the standard.

2.8. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis

Samples (66 μg of protein) from the $100\,000 \times g_{max}$ membrane fraction were solubilized in 10 μl of 2% (w/v) SDS with shaking for 1 h at room temperature. Samples were subsequently diluted 10-fold in 10 mM $NaPO_4$ (pH 7.4) containing 10 mM EDTA, 1% (v/v) β-mercaptoethanol, 1% (w/v) *n*-octylglucoside and 2 mM phenylmethylsulfonylfluoride, and treated with *N*-glycosidase F (0.01 U/μg protein) (Boehringer Mannheim) with shaking for 1 h at room temperature. Samples (33 μg of protein equivalent) were diluted 3:1 (v/v) in SDS-PAGE sample buffer (Laemmli, 1970) and subjected to SDS-PAGE on a 10% gel (Novel Experimental Technology, San Diego, CA, USA), and transferred onto nitrocellulose by electroblotting. Samples were not boiled prior to SDS-PAGE because previous experiments in this laboratory and others (Herberg et al., 1984) have shown that heating samples results in subsequent aggregation and retention in the stacking gel during electrophoresis. Immunoreactive proteins were detected using a primary anti-FLAG m2 monoclonal antibody (IBI) and a secondary sheep anti-mouse immunoglobulin linked to horseradish peroxidase (Kodak). FLAG-bacterial alkaline phosphatase (IBI) was used as a positive control. Immunoreactivity was detected with the Renaissance chemiluminescence kit (NEN).

2.9. In situ hybridization

2.9.1. Tissue preparation and storage

All procedures were approved by the Animal Care Committee at the Merck Frosst Centre for Therapeutic Research in accordance with the guidelines established by the Canadian Council on Animal Care. Male Sprague–Dawley rats weighing 200–250 g were administered a lethal injection of sodium pentobarbital (85–100 mg/kg, intraperitoneally). Transcardial infusion of 0.9%-sodium chloride was followed by perfusion with 4.0% paraformaldehyde in cold 0.1 M PBS (pH 7.3). Tissues were removed immediately and processed using standard procedures for paraffin-embedding. Tissue blocks were cut into 4 μM thick sections and mounted on aminoalkylsilane-coated microscope slides. All solutions and glassware were pretreated to destroy nuclease activity.

2.9.2. Preparation of cRNA probes

PCR was used to amplify three non-overlapping sequences of 300–400 bp found within the rat DP receptor using the following primer pairs: Probe 1: rDP-ATG (5′ ATG AAT GAG TCC TAT CGC TGT CAG GCA 3′) and rDP-ri2 (5′ CAG AGA CAG CCA GCA CTC TAG TG 3′); Probe 2: rDP-fi2 (5′ CAC TAG AGT GCT GGC TGT CTC TG 3′) and rDP-re3 (5′ CAT GCC TGT AGT CTG AGC CTG ACT G 3′); and Probe 3: rDP-fe3 (5′ AGT CAG GCT CAG ACT ACA GGC ATG 3′) and rDP-TGA (5′ AGC CCT CAC AAA GTG GAT TCC ATG T 3′). Probes 1–3 were cloned into pCR2.0 (Invitrogen) which contains a SP6 polymerase binding site and a T7 polymerase binding site flanking the multiple cloning site at the 5′ and 3′ termini, respectively. pCR2.0-rDP1, pCR2.0-rDP2, and pCR2.0-rDP3 were generated using the TA cloning kit according to the manufacturers instructions. Probes 1–3 in pCR2.0 were digested at the multiple cloning site at either the *Xho*I site (5′ of the cloned insert) or the *Spe*I site (3′ of the cloned insert). The linearized probes were then treated with proteinase K (0.2 μg/μg DNA) at 37°C for 45 min, followed by phenol/chloroform extraction and ethanol precipitation. The DNA was subsequently resuspended in diethyl pyrocarbonate (DEPC)-treated H_20 and the concentration determined by UV spectrophotometry. Digoxigenin-labelled cRNA riboprobes were prepared by transcription of 1 μg of linearized probe template DNA with SP6 or T7 polymerases in the presence of digoxigenin-UTP using an RNA labelling kit (Boehringer Mannheim). Following the labelling reaction the samples were treated with 20 units of Dnase I to remove the DNA template prior to storage of the digoxigenin-labelled riboprobes in hybridization buffer [75% (v/v) formamide, 15% (v/v) 20 × NaCl, sodium citrate (SSC) buffer, 2% (v/v) 50 × Denhardt's, 4% yeast tRNA (5 mg/ml), 5% 1 M $NaPO_4$ (pH 7.4), 10% (w/v) dextran sulfate] at −20°C.

2.9.3. In situ hybridization reaction

All procedures were performed at room temperature unless otherwise noted. Glass slides with tissue slices were treated as follows: xylene [3 × 15 min], graded ethanol (100% ethanol: 2 × 10 min; 95%, 80%, 70%, 50% ethanol in DEPC–H_20, each 5 min) and 10 mM PBS, pH 7.4 (2 × 10 min). The tissues were then treated with 0.1 μg/ml proteinase K in 100 mM Tris–HCl pH 8 (10 min, 37°C), washed sequentially in DEPC–H_2O (5 min), 0.1 M triethanolamine pH 8 (5 min), 0.25% (v/v) fresh acetic anhydride in triethanolamine (10 min), 2 × SSC (5 min), then briefly dipped in graded ethanol (50%, 70%, 80%, 90%, 100%, 100% ethanol) and air dried. The tissues were then incubated with the digoxigenin-labelled probes in hybridization buffer (30 μl per slide) covered with Parafilm in a chamber humidified with 75% (v/v) formamide solution in H_2O at 56–57°C for 16 h. The concentrations of the digoxigenin-labelled antisense and sense probes added to tissues were standardized by comparison on a dot blot prior to each experiment. Parafilm coverslips were removed by dipping slides in 2 × SSC. The tissues were then treated with 40 μg/ml RNase A (Boehringer Mannheim) (45 min), and washed as follows: 2 × SSC (10 min), 1 × SSC (10 min), 0.5 × SSC (10 min), 0.1 × SSC (60°C, 45 min). Digoxigenin-labelling was then detected by immunochemistry at room temperature in a H_2O-humified chamber. Non-specific antibody sites were

blocked by incubation with Buffer 1 (100 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing 3% bovine serum albumin for 30 min. The tissues were incubated with Immunopure peroxidase suppressor (Pierce) for 30 min and washed in Buffer 1 (3×10 min). The slides were incubated with horseradish peroxidase-linked anti-digoxigenin antibody (Boehringer Mannheim) (1:75 in Buffer 1 containing 3% BSA) for 2 h and then washed in Buffer 1

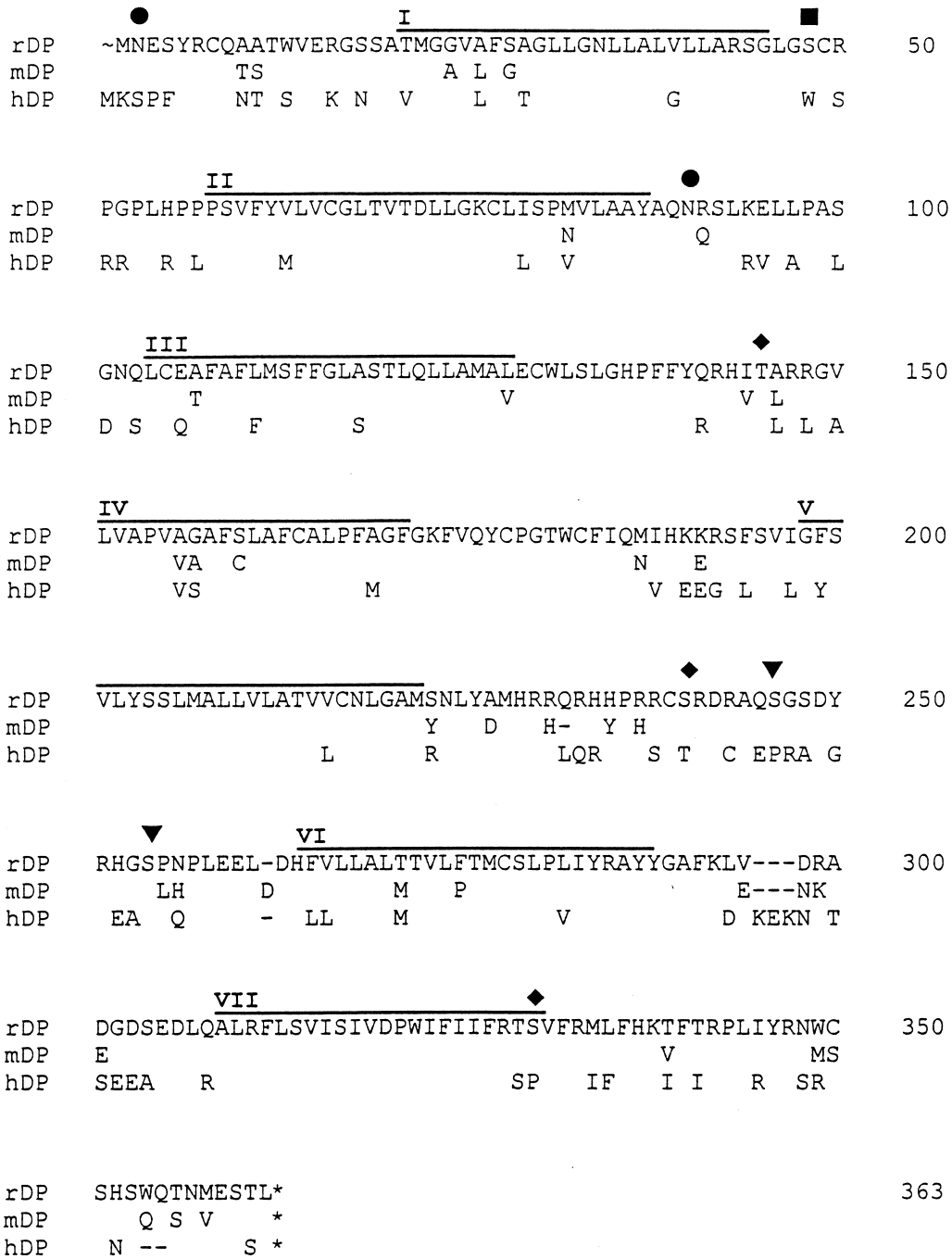


Fig. 1. Amino acid sequence alignments of the rat, mouse and human DP receptors. Alignments were performed using GCG Wisconsin DNA software. Shown are the amino acids which are not conserved in the human DP and mouse DP receptors compared to the rat DP receptor. DNA sequences corresponding to transmembrane domains I–VII are indicated by overlines. Gaps in the sequences to facilitate alignment are indicated by dashes, and the termination codon by an asterisk. Consensus sites within the rat DP receptor are indicated above the alignment for *N*-glycosylation (●), protein kinase C (■), protein kinase A (▼), and both protein kinase C and protein kinase A (◆). Two residues varied between our predicted amino acid sequence and a previously published sequence submitted to GenBank™ under accession number U92289. The current study identified aspartate instead of histidine at position 71, and threonine instead of alanine at position 338. The nucleotide and peptide sequences identified for the rat DP receptor have been submitted to the GenBank™/EMBL Data Bank with accession number AF120101.

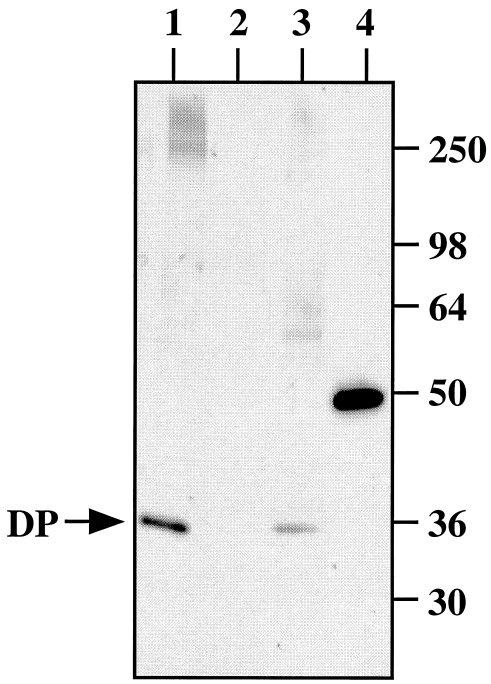


Fig. 2. Immunoblot of FLAG epitope-tagged rat and human homologues of the DP receptor expressed in HEK 293(EBNA) cell membranes using the anti-FLAG m2 antibody. Lane 1: FLAG-rat DP receptor; Lane 2: rat DP receptor; Lane 3: FLAG-human DP receptor; Lane 4: FLAG-bacterial alkaline phosphatase (FLAG-BAP) (Mr = 55 000). Cell membranes were solubilized, treated with *N*-glycosidase F, analyzed by SDS-PAGE, transferred to nitrocellulose and immunoblotted as described under Section 2. Lanes were loaded with 33 μg of original membrane protein except for lane 4 where 25 μg of FLAG-BAP was used.

(3 × 5 min), and positive labelling was visualized by incubation with diaminobenzidine (DAB) in substrate buffer (Pierce) for 15–45 min. The substrate reaction was stopped by overnight incubation in 10 mM Tris–HCl, 1 mM Na_2EDTA buffer, pH 8. Tissues were counterstained with Gill's hematoxylin (Fisher Scientific) and mounted using aqueous mounting media (Immunon).

2.10. Reagents

Prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, U46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$), prostaglandin J_2 and Ro-20-1724 (4-(3 butoxy-4-methoxybenzyl)-2-imidaxlidinone) were from Biomol Research Laboratories (Plymouth Meeting, PA, USA). BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropylhydantoin)) was a generous gift from The Wellcome Foundation (Beckenham, Kent, UK). L-644,698 ((4-(3-(3-(3-hydroxyoctyl)-4-oxo-2-thiazolidinyl) propyl) benzoic acid) (racemate) was synthesized at Merck Research Laboratories by Dr. J.B. Bicking. Iloprost (5-(hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1-octen-6-ynyl)-2(1*H*)-pentalenylidene) pentanoic acid) and [^{125}I]cAMP scintillation proximity assay kits were from Amersham (Oakville, ON, Canada). [^{3}H]prostaglandin D_2 was from Dupont NEN (Boston, MA, USA).

3. Results

3.1. Cloning of the rat DP receptor

The rat DP prostanoid receptor open reading frame (ORF) was constructed as described in Section 2. The ORF consists of 1074 nucleotides which encode a polypeptide of 357 amino acids with a predicted molecular mass of 39 800. There are two potential *N*-glycosylation sites at positions 2 and 89, four potential protein kinase C phosphorylation sites at residues 47, 144, 239 and 325, and five protein kinase A consensus phosphorylation sites at positions 144, 239, 245, 253 and 325. The rat DP receptor is 92% and 78% identical at the nucleic acid level, falling to 90% and 73% at the amino acid level, to the mouse and human homologues, respectively (Fig. 1).

3.2. Immunodetection of FLAG-rat DP and FLAG-human DP receptors expressed in HEK 293(EBNA) cell membranes

Membranes from HEK 293(EBNA) cells expressing rat DP, FLAG-rat DP, and FLAG-human DP receptors were solubilized, deglycosylated, and then analysed by SDS-PAGE followed by immunoblotting as described in Section 2 (Fig. 2). For both the FLAG-rat DP and FLAG-human DP receptors (lanes 1 and 3, respectively), a major immunoreactive species was detected at a molecular mass of approximately 36 000, close to that predicted for the DP receptor. This probably represents the DP receptor because this band was not detected in lane 2 containing the recombinant rat DP receptor without the FLAG epitope. In addition, several less prominent higher molecular weight forms of immunoreactive material were detected, including species migrating at approximately 72 kDa, double the

Table 1
Inhibitor constants for competing ligands at HEK 293(EBNA) cell membranes expressing either FLAG-rat DP or FLAG-human DP receptors
Inhibitor constant (K_i) values are shown for prostanoids and synthetic prostanoid analogues. Values were derived from a single experiment.

Ligand	K_i (nM)	
	FLAG-rat DP receptor	FLAG-human DP receptor
Prostaglandin D_2	25	2.8
L-644,698	173	0.5
BW 245C	15	3.8
Prostaglandin E_2	> 1000	> 1000
Prostaglandin $F_{2\alpha}$	> 1000	> 1000
Iloprost	> 1000	> 1000
U46619	> 1000	> 1000

molecular weight of the DP receptor. These type of SDS-resistant receptor dimers have been observed for a number of G-protein coupled receptors including D2 dopamine receptors (Ng et al., 1996), β_2-adrenoceptors (Hebert et al., 1996) and δ-opioid receptors (Cvejic and Devi, 1997). Although there are reports that these receptor dimers are physiologically relevant, this remains to be definitely established (Hebert et al., 1996; Cvejic and Devi, 1997). FLAG-bacterial alkaline phosphatase (molecular weight of 55 kDa) (lane 4, positive control) was also detected by the anti-FLAG m2 antibody. Membranes prepared from pCEP4-transfected HEK293(EBNA) cells have also been analyzed by Western blot and did not demonstrate any immunoreactive species (Deborah Sliptez, personal communication).

3.3. Expression of the FLA-rat DP receptor in HEK 293(EBNA)

3.3.1. Receptor binding studies

The affinity (K_D) of prostaglandin D_2 for the expressed DP receptors and the maximum number of detectable prostaglandin D_2 specific binding sites (B_{max}) were identified by saturation analysis (data not shown). Specific binding of [^{3}H]prostaglandin D_2 to the FLAG-rat DP receptor was best described by a single site model with a K_D value of 14 nM and a B_{max} value of 115 fmol/mg protein. Similarly, [^{3}H]prostaglandin D_2 specific binding to the FLAG-human DP receptor was also fitted most appropriately by a one-site model with a K_D value of 3.8 nM and a B_{max} value of 84 fmol/mg protein.

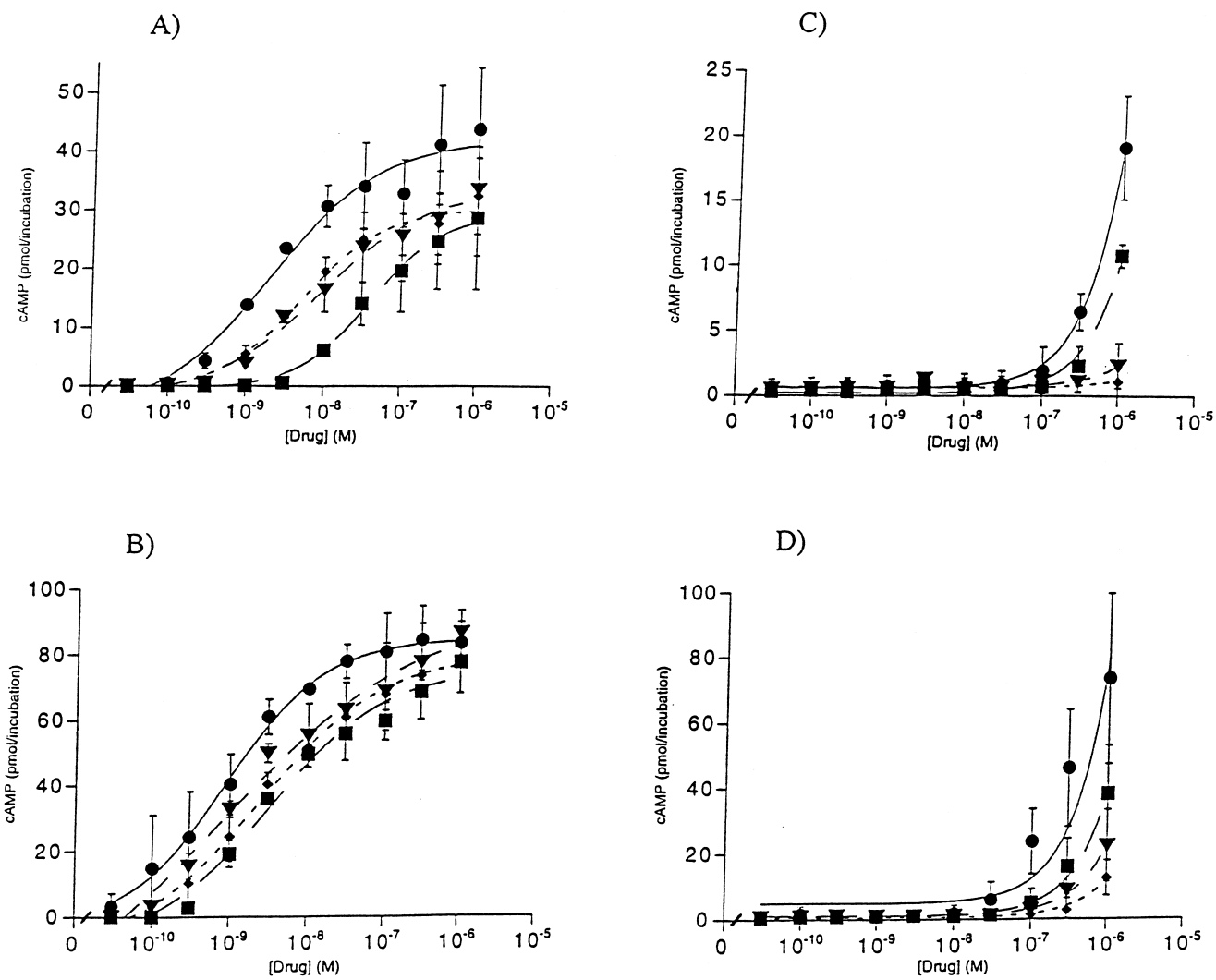


Fig. 3. cAMP Production by HEK 293(EBNA) cells expressing FLAG epitope-tagged rat or human homologues of the DP receptor. FLAG-rat DP (Panels A,C) or FLAG-human DP (Panels B,D) receptor-expressing cells were challenged with either DP receptor-specific prostanoids and prostanoid analogues, or prostanoids and prostanoid analogues of other specificities. The incubation medium contained 0.03–1000 nM of (in Panels A,B): prostaglandin D_2 (♦), prostaglandin J_2 (▼), L-644,698 (■), or BW 245C (●); or (in Panels C,D): prostaglandin E_2 (●), prostaglandin $F_{2\alpha}$ (■), iloprost (▼), or U46619 (♦). Data points are the mean of two experiments each performed in duplicate.

Table 2
Efficacies for ligands at HEK 293(EBNA) cells expressing either FLAG-rat DP or FLAG-human DP receptors.
EC_{50} values ± S.D. and maximal responses (percentage of maximally stimulated control values ± S.D.) are shown for prostanoids and synthetic prostanoid analogues. Values were derived from two separate experiments.

Ligand	FLAG-rat DP receptor		FLAG-human DP receptor	
	EC_{50} (nM)	Percentage of maximal stimulation of cAMP production[a]	EC_{50} (nM)	Percentage of maximal stimulation of cAMP production[a]
Prostaglandin D_2	4.8 ± 2.6	100	1.4 ± 0.1	100 ± 6
Prostaglandin J_2	6.9 ± 3.2	103 ± 12	0.4 ± 0.1	118 ± 27
L-644,698	32 ± 16	90 ± 21	4.1 ± 2.7	105 ± 11
BW 245C	1.9 ± 1.2	130 ± 8	0.8 ± 1.0	114 ± 2
Prostaglandin E_2[b]	2120 ± 360	–	169 ± 22	–
Prostaglandin $F_{2\alpha}$[b]	296	–	580 ± 20	–
Iloprost[b]	–	–	665 ± 313	–
U46619[b]	–	–	> 1000	–

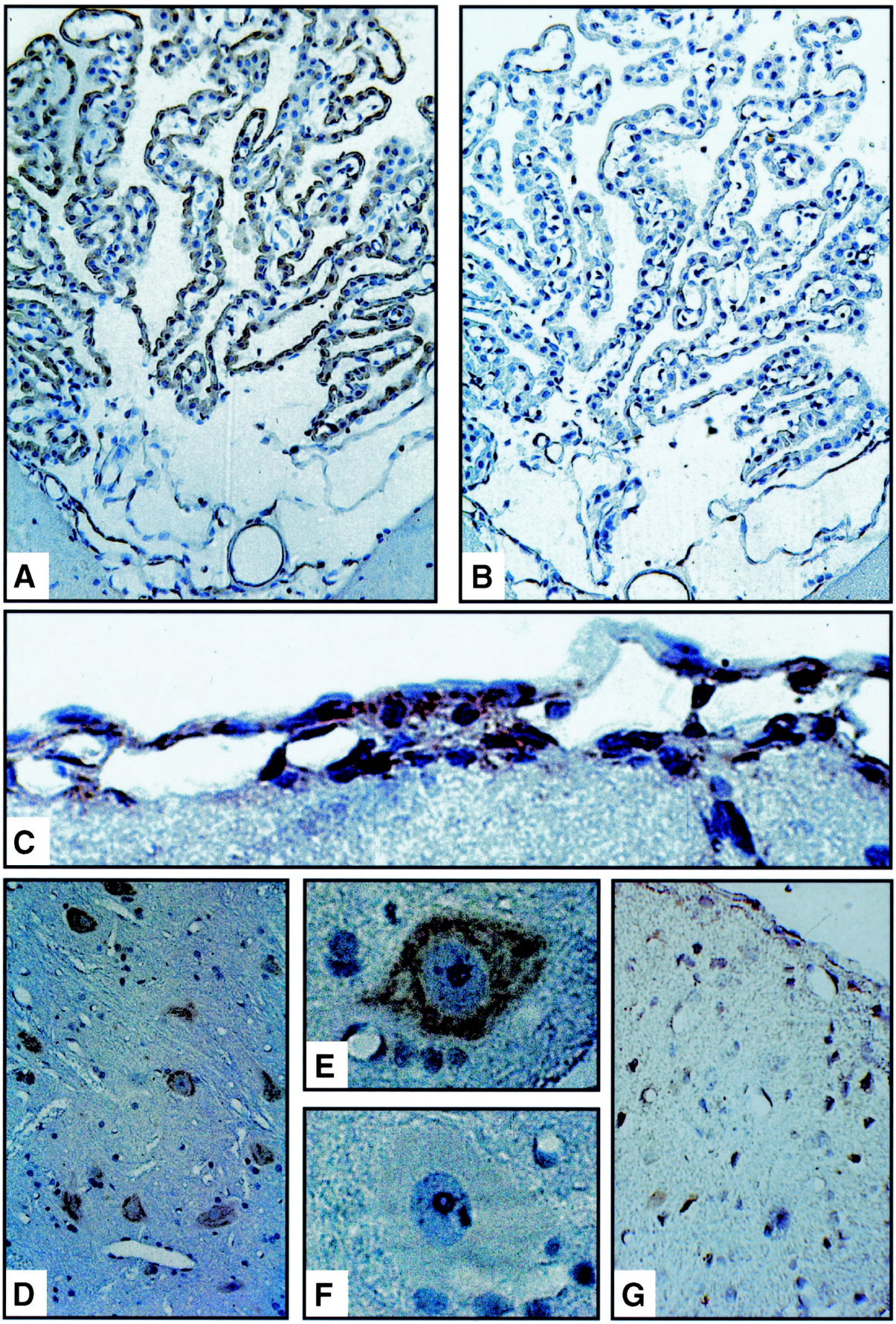
[a] Maximal stimulation was defined as the cAMP response produced with 1 μM prostaglandin D_2.
[b] Maximal responses could not be calculated for prostaglandin E_2, prostaglandin $F_{2\alpha}$, iloprost and U46619 because the concentration–response curves were not complete.

Equilibrium competition binding assays employing [^{3}H]prostaglandin D_2 were performed to determine the affinities of prostanoids and related analogues at both rat and human DP receptors (Table 1). Prostaglandin D_2 and BW 245C had K_i values of 25 and 15 nM, respectively, at the FLAG-rat DP receptor. The FLAG-rat DP receptor had lower affinity for L-644,698, however, with a K_i value of 173 nM, while prostaglandin E_2, prostaglandin $F_{2\alpha}$, iloprost and U46619 all had K_i values greater than 1 μM. Comparable results were obtained upon challenge of the non-epitope tagged rat DP receptor (data not shown). In contrast, prostaglandin D_2 and its analogue BW 245C bound to the FLAG-human DP receptor with K_i values of 2.8 and 3.8 nM, respectively. In particular, the DP receptor-specific agonist L-644,698 had a 350-fold higher affinity for the FLAG-human DP receptor as compared with the FLAG-rat DP receptor. Again, prostaglandin E_2, prostaglandin $F_{2\alpha}$, iloprost and U46619 all demonstrated K_i values greater than 1 μM at the FLAG-human DP receptor. There was no binding of [^{3}H]prostaglandin D_2 to membranes generated from non-transfected HEK 293(EBNA) wild type cells (data not shown).

3.3.2. Functional assays — cAMP generation

Second messenger assays monitoring the predominant signaling pathway for the DP receptor, the stimulation of cAMP production by adenylate cyclase, were performed on both the FLAG-rat DP and FLAG-human DP receptors (Fig. 3 and Table 2). In general, the rank order of efficacies followed the rank order of affinities determined in the competition radioligand binding assays. Prostaglandin J_2, a metabolite of prostaglandin D_2, was also tested since it has previously demonstrated affinity and efficacy equal to the parent compound at the human DP receptor (Wright et al., 1998). The DP receptor-specific ligand BW 245C had the highest efficacy at the FLAG-rat DP receptor with an EC_{50} value of 1.9 nM. Prostaglandin D_2 and prostaglandin J_2 were equipotent with EC_{50} values of 4.8 and 6.9 nM. Once again, L-644,698 had reduced efficacy compared to the other DP receptor-specific agonists at this receptor, with an EC_{50} value of 32 nM. Of the other ligands tested, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were markedly less potent while iloprost and U46619 were silent at this receptor (Fig. 3A,B and Table 2). Similar results were obtained upon challenge of cells expressing the non-epitope tagged rat DP receptor (data not shown). In comparison, the profile of efficacy at the FLAG-human DP receptor was as expected, with the DP receptor-specific ligands prostaglandin D_2, prostaglandin J_2, BW 245C, and in particular L-644,698, all demonstrating marked efficacies with EC_{50} values below 10 nM. Again, the other ligands tested (prostaglandin E_2, prostaglandin $F_{2\alpha}$, iloprost and U46619) were less efficient at this receptor (Fig. 3C,D and Table 2). There was no cAMP accumulation above the level obtained with vehicle in non-transfected HEK 293(EBNA) wild type cells or vector-transfected HEK 293(EBNA) cells (data not shown).

Fig. 4. Histochemical localization of DP receptor mRNA in the central nervous system of the rat by in situ hybridization. Reactions within the choroid plexus (200 × magnification) of the rat brain demonstrate a signal with application of the antisense probe (Panel A) which was absent with application of the exact complementary sense probe (Panel B), used as a negative control. A positive signal was also obtained in the leptomeninges (600 × magnification) of the rat brain (Panel C). In the rat spinal cord, abundant staining was detected within the ventral horn (Panels D and E; 200 × and 600 × magnifications, respectively) which was completely absent following application of the sense control probes (Panel F, 600 × magnification). Staining of lesser intensity was present in the superficial dorsal horn (Panel G, 200 × magnification), which was also completely absent following application of the sense control probes. Data were confirmed by at least two independent non-overlapping anti-sense riboprobes with respective complementary sense riboprobes used as negative controls.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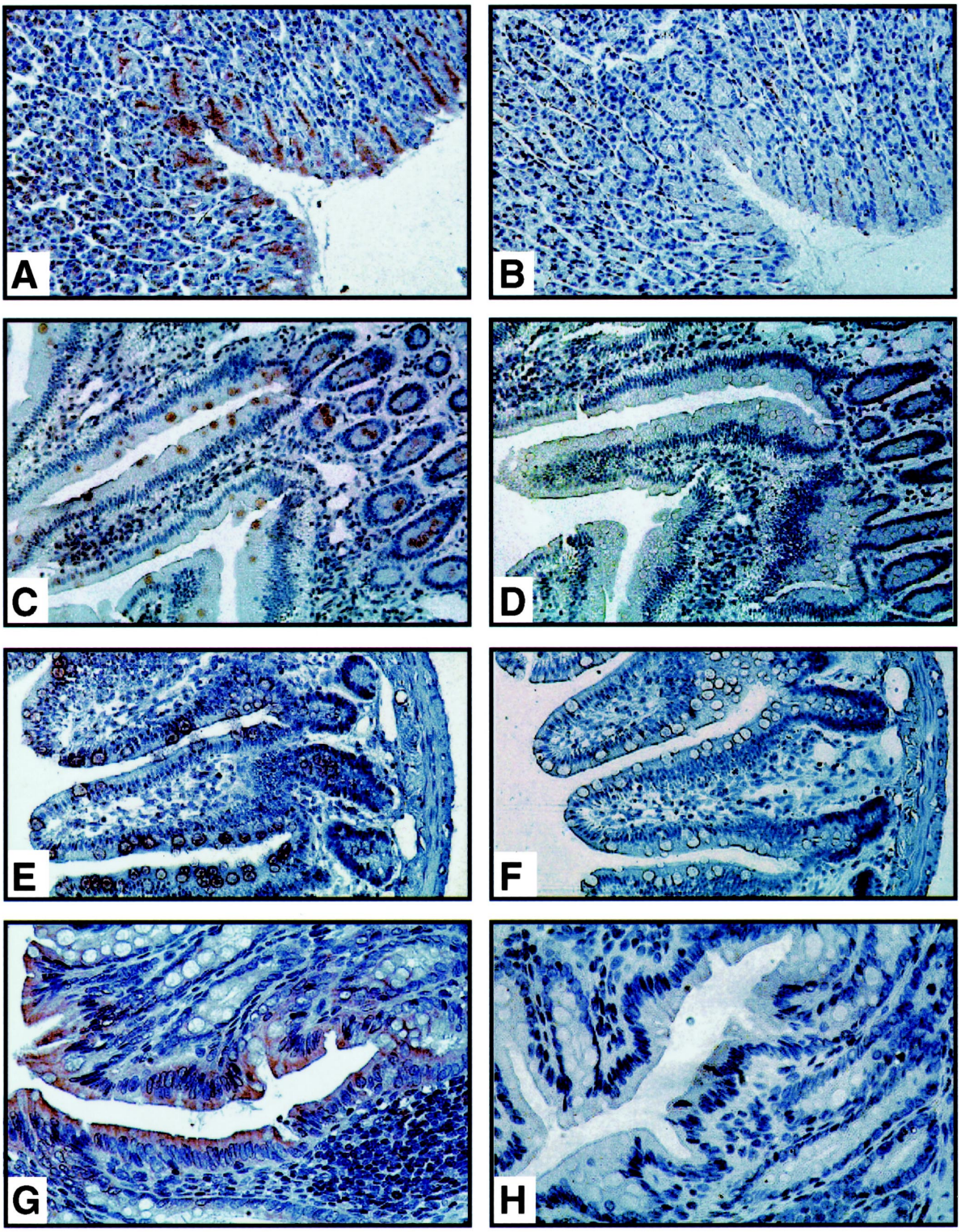

Fig. 5. Histochemical localization of DP receptor mRNA in the gastrointestinal tract of the rat by in situ hybridization. Reactions results (200× magnification) are presented from rat stomach (Panels A,B), duodenum (Panels C,D), ileum (Panels E,F), and colon (Panels G,H). Panels A,C and E illustrate, in each case, a strong, specific, positive signal obtained within the mucous-producing goblet cells upon application of the anti-sense probe which was not present with application of the exact complementary sense probe (Panels B,D and F), used as a negative control. In the colon, a specific signal was detected instead within the cuboidal epithelium (Panel G) which lies adjacent to the goblet cells. No signal was present in the sense-probed negative control tissues (Panel H). Data were confirmed by at least two independent non-overlapping anti-sense riboprobes with respective complementary sense riboprobes used as negative controls.

Maximal responses for each ligand (the cAMP produced at a concentration of 1 μM) at the FLAG-rat DP and FLAG-human DP receptors were normalized to those elicited by 1 μM prostaglandin D_2 (Table 2). The DP receptor specific ligands (prostaglandin D_2, prostaglandin J_2, L-644,698 and BW 245C) responded as full agonists at the rat DP receptor, with normalized maximal responses all above 80%. In fact BW 245C was a very efficient agonist with a maximal response of 130% compared to prostaglandin D_2. Similar results were obtained for the FLAG-human DP receptor except that the maximal stimulation with BW 245C was comparable to prostaglandin D_2. The maximal production of cAMP produced with 1μM of prostaglandin D_2 was 30–40 pmol/well for the FLAG-rat DP receptor and 80 pmol/well for the FLAG-human DP receptor.

3.4. In situ hybridization of rat tissues with rat DP receptor-specific probes

In situ hybridization reactions were carried out to determine the cell-specific localization of rat DP receptor mRNA transcripts using digoxigenin-labelled rat DP receptor-specific cRNA riboprobes (Figs. 4 and 5). Each positive result was corroborated by analogous experiments performed using at least one other non-overlapping rat DP receptor-specific antisense riboprobe. Negative controls were performed employing exact complementary sense riboprobes. In order to validate the in situ hybridization conditions, initial experiments used brain tissue (Fig. 4A–C), which has previously been reported to contain DP receptor mRNA (Oida et al., 1997). A positive signal was detected discontinuously around the periphery of the brain, in the area defined as the leptomeninges (Fig. 4C). A specific signal also appeared in the choroid plexus (Fig. 4A,B).

The presence of rat DP receptor-specific transcripts was also investigated in spinal cord tissue sections (Fig. 4D–F). Cervical, thoracic and lumbar spinal cord sections were evaluated and only the latter was found to contain a positive signal. The signal was most abundantly detected in neurons located in the ventral horn of the transversely-sectioned lumbar spinal cord (Fig. 4D,E). The relatively large size of these cell bodies and their ventral location suggests that these cells are motor neurons (Fig. 4E). In addition, less abundant staining was detected in the dorsal horn of the lumbar spinal cord (Fig. 4G) suggesting the presence of the rat DP receptor transcripts in the interneurons.

The existence of rat DP receptor-specific transcripts in the stomach, duodenum, ileum, and colon of the rat gastrointestinal tract was also investigated (Fig. 5). Positive staining identified with the anti-sense probes appeared in all tissues (Fig. 5A,C,E,G). Staining was judged specific because it was not detected in tissues probed with sense control probes (Fig. 5B,D,F,H). In the stomach (Fig. 5A,B), the mucous-secreting goblet cells which lie within the epithelium of the rugae were stained very intensely. Limited staining of the columnar epithelium which lies next to the lumen was also apparent. The duodenum (Fig. 5C,D) also demonstrated staining of the goblet cells. Less intense staining was also present in some of the connective tissue cells of the villi. The ileum (Fig. 5E,F) demonstrated intense, positive staining within both the goblet cells and the columnar epithelium. Some of the connective tissue cells within the body of the villi also stained positively. No staining of the goblet cells was evident in the colon (Fig. 5G,H), however, a positive signal was observed within the cuboidal epithelium. Some of the connective tissue cells also stained positive, though this staining was much less pronounced than observed within other portions of the gastrointestinal tract. Confirmation of the presence and localization of goblet cells within all gastrointestinal tract tissue preparations was confirmed by staining with Alcian Blue (data not shown) which is known to stain acidic mucopolysaccharides in tissue sections (Steedman, 1950).

4. Discussion

The rat homologue of the DP receptor is the latest member of the prostanoid receptor family to be cloned for this species. In addition to the cloning and characterization of the rat DP receptor, we present in situ hybridization results which provide evidence for the cell-specific localization of rat DP receptor mRNA transcripts in the central nervous system and the gastrointestinal tract. The latter data suggests a novel physiological mechanism for prostaglandin D_2.

In this study, the ORF differs at two positions from a previously published sequence (GenBank accession U92289) identified as the rat DP receptor (Gerashchenko et al., 1998). The current study identifies Asp instead of His at position 71 and Thr instead of Ala at position 338. The Asp for His substitution is of particular importance since Asp is conserved at this position in virtually all G-protein coupled receptors (Savarese and Fraser, 1992). This Asp appears to be vital for both the stimulation and attenuation of adenylate cyclase, as well as the activation of phospholipase C. Furthermore, this His for Asp substitution in the angiotensin II type I receptor impairs both G-protein coupling and downstream signal transduction (Hunyady et al., 1994). Gerashchenko et al. did not report functional expression of their receptor so we are unable to address this substitution directly. The sequence described here was, however, successfully functionally expressed.

Saturation analyses performed on the FLAG-rat DP receptor identified a single site of specific binding which, though equal in abundance, had ~4-fold lesser affinity for prostaglandin D_2 relative to the FLAG-human DP receptor. The expression of rat and human receptors in a rat cell line was not attempted. Therefore, a distinction between the effects of cellular milieu and inherent receptor affinity cannot be made. The B_{max} value for the FLAG-rat DP

receptor expressed in HEK 293(EBNA) cells is comparable to that found previously for the mouse DP receptor (Hirata et al., 1994) expressed in Chinese Hamster Ovary (CHO) cells. However, the affinity of prostaglandin D_2 for the mouse DP receptor in CHO cells is lower than for the FLAG-rat DP receptor (K_D values of 40 and 14 nM, respectively).

Competition for [^{3}H]prostaglandin D_2 binding identified a rank order of affinities of prostaglandin D_2 = BW 245C > L-644,698 for the FLAG-rat DP receptor and L-644,698 > prostaglandin D_2 = BW 245C for the FLAG-human DP receptor. Similar values were identified for the compounds at the recombinant human DP receptor without the epitope tag expressed on HEK 293(EBNA) cells (Wright et al., 1998), suggesting that the FLAG-epitope tag does not interfere with the receptor's ability to bind ligand. The prostanoids and related analogues demonstrated lower affinities for the FLAG-rat DP receptor relative to the FLAG-human DP receptor. The decrease in affinity is ligand-dependent, ranging from 4-fold for BW 245C to over 180-fold for L-644,698. Although inhibitor constants (K_i) are not directly calculated in the characterization of the mouse DP receptor, it appears from Fig. 3A within Hirata et al. (1994) that the K_i value for BW 245C is slightly less than 1 μM. The more recent values for the mouse DP receptor of Kiriyama et al. (1997) are in better agreement with the current data, where K_i values of 21 and 250 nM for prostaglandin D_2 and BW 245C, respectively, were determined.

The rank order of ligand potencies for receptor-mediated increases in intracellular cAMP at the FLAG-rat DP receptor expressed in HEK 293(EBNA) cells was BW 245C = prostaglandin D_2 = prostaglandin J_2 > L-644,698, whereas for the FLAG-human DP receptor expressed in HEK 293(EBNA) cells the rank order was BW 245C = prostaglandin D_2 = prostaglandin J_2 = L-644,698. EC_{50} values for these DP receptor ligands were from 2-fold to 20-fold lower when measured at the FLAG-rat DP receptor relative to the FLAG-human DP receptor. All DP receptor-specific ligands behaved as full agonists at both receptors. Although EC_{50} values are not presented for the mouse DP receptor (Hirata et al., 1994), it appears from Fig. 4B therein that the mouse DP receptor is of similar efficacy to the FLAG-rat DP receptor in the current study. In both cases, BW 245C was slightly more potent than prostaglandin D_2 and EC_{50} values were in the very low nanomolar range for both compounds.

This report also describes the distribution of the rat DP receptor in the central nervous system (CNS) and gastrointestinal tract by in situ hybridization. Within the CNS a positive signal was observed in the leptomeninges. A similar distribution was recently reported for both the mouse DP receptor (Oida et al., 1997) and the rat DP receptor (Gerashchenko et al., 1998). As mentioned previously (Oida et al., 1997), the discontinuous nature of the signal observed around the leptomeninges may be an artifact introduced during tissue preparation rather than a genuine characteristic of rat DP receptor distribution, although the lack of signal observed with the sense probes (negative controls) in this study argues against this possibility.

Positive signals were also located within the choroid plexus. Previous in situ studies have not reported the DP receptor within the choroid plexus, probably due to the differing sensitivities of the techniques used. Earlier reports addressing the distribution within rat brain of the DP receptor (Yamashita et al., 1983; Watanabe et al., 1986) by ligand binding and prostaglandin D synthase by in situ hybridization (Urade et al., 1993) support the present findings. The co-localization of DP receptors and prostaglandin D synthase in the leptomeninges and choroid plexus suggests an autocrine/paracrine relationship, a common aspect of prostanoid-mediated bioactivity (Pierce et al., 1995). This includes the third ventricle that lies directly adjacent to a prostaglandin D_2-sensitive zone within the rostral forebrain shown to modulate sleep (Matsumura et al., 1994) and brain temperature (Hayaishi, 1988). The question remains as to what function is served by the DP receptors localized within the subarachnoid space but outside the sleep-sensitive/brain temperature zone.

In situ hybridization was also performed using rat spinal cord sections since there are reports describing prostaglandin D_2-mediated inhibition of allodynia (discomfort and pain evoked by innocuous tactile stimuli) invoked by prostaglandin E_2 (Minami et al., 1996) and nociceptin (Minami et al., 1997), as well as prostaglandin D_2-mediated hypoalgesia (lowered sensitivity to painful stimuli) (Horiguchi et al., 1986). Within the dorsal horn, signals are confined to the substantia gelatinosa, a spinal cord region involved in the processing of nociceptive stimuli. This is consistent with previous experiments in porcine (Watanabe et al., 1986) and chick (Vesin et al., 1995) spinal cord where prostaglandin D_2-binding proteins and prostaglandin D synthase, respectively, were localized. Recently, murine prostaglandin D synthase has been similarly localized and prostaglandin E_2-mediated allodynia has been shown to be facilitated by prostaglandin D_2 in femtogram to picogram amounts and inhibited by prostaglandin D_2 in picogram to nanogram amounts (Eguchi et al., 1999). Hyperalgesia (increased sensitivity to painful stimuli) has also been demonstrated following the administration of prostaglandin D_2 into mouse spinal cord (Uda et al., 1990) and Substance P antagonists can block the hyperalgesia induced by prostaglandin D_2. Thus, within the substantia gelatinosa of the dorsal horn, prostaglandin D_2 may modulate hyperalgesia and allodynia through different populations of DP receptor-expressing cells.

The localization of DP receptor mRNA transcripts to the ventral horn of the lumbar spinal cord is consistent with the localization of prostaglandin D synthase in chick spinal cord (Vesin et al., 1995). Previously, prostaglandin

E_2-sensitive EP_2 receptors were identified in the same regions of both the ventral and dorsal horn of the rat spinal cord (Kawamura et al., 1997) where a role for EP_2 in monosynaptic reflexes was suggested (Vesin et al., 1991). Co-localization of prostaglandin D synthase and EP_2 suggests an efferent role for prostaglandin D_2 which may include modulation of monosynaptic reflexes. Prostaglandin D_2 and prostaglandin E_2 have opposing functions on the regulation of sleep, where the former promotes sleep and the latter wakefulness (Hayaishi, 1988), and in the regulation of body temperature, where prostaglandin D_2 lowers it and prostaglandin E_2 raises it (Ito et al., 1989). It is possible, therefore, that prostaglandin D_2 and prostaglandin E_2 also demonstrate opposing functions in the modulation of monosynaptic reflexes. The activity opposing prostaglandin D_2 may be mediated by EP_1 in the spinal cord. A prostaglandin E_2-mediated calcium-dependent mechanism of allodynia has recently been reported (Sakai et al., 1998) which is sensitive to EP_1 (but not EP_3) agonists and can be blocked by prostaglandin D_2.

The absence of analogous signals from the dorsal and ventral horns of both the cervical and thoracic spinal cord is suggestive of a discrete contribution to pain transmission by the DP receptor in the neuromodulation of a particular subset of neurons, rather than a more general neuromodulatory role.

DP receptor mRNA was also identified in the stomach, duodenum, ileum and colon. Signals were localized to the mucous-secreting goblet cells, and within the columnar epithelium in some cases. Components of the mucosal connective tissue were also stained, though much less intensely. These results confirm previous reports of DP receptor mRNA expression in human small intestine (Boie et al., 1995) and mouse ileum (Hirata et al., 1994). Previously, both contractile and relaxant roles for prostaglandin D_2 had been reported in the gastrointestinal tract, but other reports failed to identify prostaglandin D_2 effects in the gastrointestinal tract (Giles and Leff, 1988). Prostaglandin D_2 administration has been correlated with contraction in the rat fundus, rabbit jejunum (Horton and Jones, 1974) and guinea pig (longitudinal) ileum (Bennett et al., 1980), as well as relaxation of guinea pig ileum (Bennett and Sanger, 1978) and rabbit stomach (Whittle et al., 1979). In the human colon and stomach, however, prostaglandin D_2 was without effect (Sanger et al., 1982). The current report is the first indication that the DP receptor may mediate mucous secretion and cytoprotection.

Interestingly, prostaglandin E_1 has previously been shown to increase both cAMP levels and mucin and water secretion in human intestine (Neutra and Forstner, 1987, and references within; McCool et al., 1990). In addition, 16,16-dimethyl prostaglandin E_2 was shown to increase cell-proliferation rates and mucous granule secretion from the gastrointestinal mucosa of a human colon-derived (HT29) cell line (Phillips et al., 1993). More recently, in an ex vivo isolated vascularly perfused rat colon preparation, 16,16-dimethyl prostaglandin E_2 was further demonstrated to be a potent mucin secretagogue (Plaisancie et al., 1997). Stimulation of the DP receptor on epithelial cells could induce mucin secretion directly through increases in intracellular cAMP or indirectly by facilitating the vectorial transport of chloride ions which has been shown to stimulate mucous secretion from goblet cells (Cozens et al., 1992 and references within). Experiments addressing the possibility of DP receptor-mediated mucous secretion and gastrointestinal cytoprotection are currently being undertaken.

In conclusion, the rat homologue of the DP receptor has been cloned. The current sequence differs from that previously presented by Gerashchenko et al. (1998) at two residues, one of them (Asp^{71}) being highly conserved amongst the family of G-protein coupled receptors where, as other studies have suggested, it probably plays a vital role in G-protein coupling and signal transduction. The fidelity of the current sequence allowed for successful functional expression of the rat DP receptor, as assessed by radioligand binding and cAMP accumulation studies. In addition, the cell-specific localization of DP receptor mRNA transcripts has been studied in the CNS and the gastrointestinal tract. DP receptor mRNA was confirmed within the leptomeninges and the choroid plexus of the brain, as well as in the motor neurons and interneurons of the lumbar spinal cord. Within the stomach, duodenum and ileum of the gastrointestinal tract, DP receptor mRNA was repeatedly detected in the mucous-secreting goblet cells and, to a lesser extent, within the columnar epithelium. In the colon, DP receptor mRNA was absent from the goblet cells but was present in the cuboidal epithelium. These data suggest the intriguing possibility of a role for the DP receptor in mucous regulation and cytoprotection.

Acknowledgements

The authors would like to thank Dr. Mark Abramovitz and Angela Northey for useful discussions, as well as Laboratory Animal Resources at Merck Frosst and Kevin Clark for technical assistance. Financial assistance was provided by a Medical Research Council, Pharmaceutical Manufacturers Association of Canada Health Program Award.

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