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Biochemistry

Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene

(prostaglandin D_2/G protein-coupled receptor/gene cloning/mRNA distribution)

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ABSTRACT **Prostanoid receptors belong to the family of G protein-coupled receptors with seven transmembrane domains. By taking advantage of nucleotide sequence homology among the prostanoid receptors, we have isolated and identified a cDNA fragment and its gene encoding a mouse prostaglandin (PG) D receptor by reverse transcription polymerase chain reaction and gene cloning. This gene codes for a polypeptide of 357 amino acids, with a calculated molecular weight of 40,012. The deduced amino acid sequence has a high degree of similarity with the mouse PGI receptor and the EP_2 subtype of the PGE receptor, which together form a subgroup of the prostanoid receptors. Chinese hamster ovary cells stably expressing the gene showed a single class of binding sites for [^{3}H]PGD_2 with a K_d of 40 nM. This binding was displaced by unlabeled ligands in the following order: PGD_2 > BW 245C (a PGD agonist) > BW A868C (a PGD antagonist) > STA_2 (a thromboxane A_2 agonist). PGE_2, $PGF_{2\alpha}$, and iloprost showed little displacement activity at concentrations up to 10 μM. PGD_2 and BW 245C also increased cAMP levels in Chinese hamster ovary cells expressing the receptor, in a concentration-dependent manner. BW A868C showed a partial agonist activity in the cAMP assay. Northern blotting analysis with mouse poly (A)$^+$ RNA identified a major mRNA species of 3.5 kb that was most abundantly expressed in the ileum, followed by lung, stomach, and uterus.**

Prostanoids such as the prostaglandins (PGs) and thromboxane (TX) are cyclooxygenase metabolites of arachidonic acid (1) and exert a variety of biological activities to maintain local homeostasis in the body (2). Each of these compounds acts through its own specific receptor, which is coupled to the regulatory G protein(s) (3). At least seven pharmacologically distinct prostanoid receptors have been identified (4): the TXA_2 receptor, the EP_1, EP_2, and EP_3 subtypes of the PGE receptor, the PGF receptor, the PGI receptor, and the PGD receptor. Of these, the EP_2 subtype of the PGE receptor, the PGI receptor, and the PGD receptor have been classified as relaxant prostanoid receptors (4); they preferentially activate adenylate cyclase in their second messenger pathways and produce relaxation in smooth muscle preparations. PGD_2 is formed in a number of tissues and cells and produces a variety of biological responses (4, 5). For example, PGD_2 acts during platelet aggregation as a negative-feedback regulator (5), produces relaxation of vascular and nonvascular smooth muscle (6–8), and modifies autonomic as well as sensory nerve functions (9, 10). It is also released by mast cells and cells of the macrophage lineage to play a role during immunologic reactions (11–13). Finally, PGD_2, synthesized in the brain, has been proposed to be a physiological regulator of sleep (14).

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Recently, we isolated cDNAs for the TXA_2 receptor (15, 16) and the EP_1 (17), the EP_2 (18), and the EP_3 (19, 20) subtypes of the PGE receptors by homology screening. These studies revealed that prostanoid receptors constitute a subfamily within the rhodopsin-type, G protein-coupled receptor superfamily (21), and they enabled us to identify highly conserved amino acid sequences in these receptors. To clone the cDNAs for other prostanoid receptors, we have carried out reverse transcription polymerase chain reaction (RT-PCR) studies based on such motifs and have successfully amplified three cDNAs encoding sequences homologous to prostanoid receptors. Of these three, one has been identified as being part of the PGF receptor cDNA (22) and another as being part of the PGI receptor cDNA (23). The third, unidentified, cDNA showed a high degree of sequence similarity to the PGI receptor (23) and to the EP_2 subtype of the PGE receptor (18). We postulated that the cDNA may code for a member of the relaxant prostanoid receptor family. Here, we report the primary structure of this mouse cDNA and its gene.† We also describe the identification of the gene product as a mouse PGD receptor, based on its ligand binding characteristics and second messenger responses. The tissue distribution of its mRNA expression is also shown.

MATERIALS AND METHODS

Ligands. PGD_2, PGE_2, $PGF_{2\alpha}$, and STA_2 (9,11-epithio-11,12-methano-TXA_2) were kindly provided by Ono Pharmaceuticals (Osaka). BW 245C [5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin] and BW A868C [3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin] were generous gifts from Wellcome. Iloprost was purchased from Amersham, and [5,6,8,9,12, 14,15(N)-^{3}H]PGD_2 (4255 GBq/mmol) was obtained from New England Nuclear.

RT-PCR Screening. A pair of degenerate oligonucleotides (5′-GG(N)AC(N)TGGTG(C/T)TT(C/T)(A/T)T-3′ and 5′-A(T/G)(A/G)(A/T)A(A/G/T)ATCCA(N)GG(A/G)TC-3′) were synthesized which corresponded to the peptides GT-WCF (I/L) in the second extracellular loop and DPWI (Y/F)(I/L) in the seventh transmembrane domain, respectively. DNA amplification of first strand cDNA from mouse thymus was performed with a cycling profile of 95°C for 60 sec, 38°C for 60 sec, and 70°C for 60 sec, repeated over 35 cycles. One of the amplified fragments, PG25, contained a sequence highly homologous to the fifth and sixth transmembrane domains of the prostanoid receptors.

Mouse Genomic Library Screening. A mouse (strain 129Sv) genomic library in Lambda FIX II (Stratagene) was screened

Abbreviations: PG, prostaglandin; TX, thromboxane; RT-PCR, reverse transcription polymerase chain reaction; CHO, Chinese hamster ovary; dhfr, dihydrofolate reductase.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. D29764 and D29765).

with the ^{32}P-labeled PG25 fragment. Four positive, overlapping clones were isolated from 2×10^5 recombinant plaques originally screened. One of these clones contained a 13-kb insert. Southern hybridization and restriction analysis of the insert identified two *Xba* I fragments (3.3 kb and 6 kb) that hybridized to the probe. These *Xba* I restriction fragments were subcloned and further analyzed.

Construction of an Open Reading Frame and Its Expression in Chinese Hamster Ovary (CHO) Cells. PGc1, an 824-bp cDNA fragment which spans both exons of the PG25 gene, was amplified by PCR from mouse lung cDNA using a pair of oligonucleotides (5′-GGAGTGCTGGCTGTCTCT-3′ and 5′-CTTCAGTGCTGATCCCTC-3′). The nucleotide sequence of PGc1 was confirmed to be identical to the corresponding exons from the genomic clone. PGc1 was digested by *Nar* I, and the 3′ restriction fragment was then ligated to the *Pst* I–*Nar* I fragment of the genomic clone at the *Nar* I site. This resulted in a 1.2-kb fragment, PGc9, which contains the putative open reading frame. PGc9 was subcloned into the eukaryotic expression vector pdKCR-dhfr, which contains a mouse dihydrofolate reductase (dhfr) gene (24). The resulting plasmid, pdKCR-dhfr-PGc9, was transfected into dhfr-deficient CHO (CHO-dhfr$^-$) cells (25) as previously described (26). Several transformant CHO cell clones were isolated by selecting cultures with nucleoside-depleted medium. One of these clones, named CHO-J, was used in subsequent experiments.

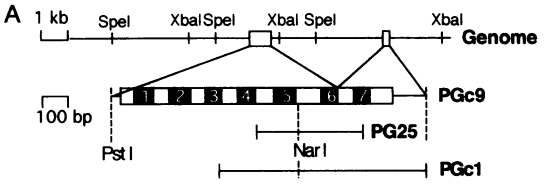
[^{3}H]PGD$_2$ Binding Analysis. CHO-J cells were maintained in α-MEM(−) (MEM lacking ribo- and deoxyribonucleosides; Sigma) supplemented with 10% fetal calf serum and penicillin (100 units/ml). The cells were washed once with phosphate buffered saline (137 mM NaCl/2.7 mM KCl/4.3 mM Na_2HPO_4/1.4 mM KH_2PO_4), scraped from the culture plates, and collected by centrifugation for 15 min at $800 \times g$. The cell pellet was suspended in 10 vol of homogenization buffer (25 mM Hepes–NaOH, pH 7.4 at 20°C/1 mM EDTA/5 mM $MgCl_2$/250 mM D-mannitol/2 mM 2-mercaptoethanol/1 mM phenylmethylsulfonyl fluoride/1 mM benzamidine·HCl) and homogenized by sonication. The homogenate was then incubated in the presence of 10 nM [^{3}H]PGD$_2$, with or without unlabeled ligands, at 4°C for 60 min. After incubation, the reaction was terminated by the addition of ice-cold washing buffer (25 mM Hepes–NaOH, pH 7.4/1 mM EDTA/5 mM $MgCl_2$/140 mM NaCl/5 mM KCl) and the mixture was passed through a GF/C glass fiber filter (Whatman). The filter was then washed four times with ice-cold washing buffer and the bound radioactivity was measured by liquid scintillation counting. Nonspecific binding was defined as the radioactivity bound to the filter in the presence of a 200-fold excess of unlabeled PGD$_2$. Incubation of the reaction mixture at 37°C resulted in a high nonspecific binding as noted previously (27).

cAMP Assay. CHO-J cells were seeded at 5×10^4 cells per well in 24-well plates and were cultured in αMEM(−) with 10% fetal calf serum for 48 h. The cellular cAMP levels were measured by radioimmunoassay as previously described (23), using a cAMP radioimmunoassay kit (Amersham) after stimulation with PGD$_2$, BW 245C, or BW A868C in the presence of 1 mM 3-isobutyl-1-methylxanthine.

Northern Blotting. Total RNA was isolated from several organs of 5-week-old mice, and enriched for poly(A)$^+$ RNA with OligoTex dT30 (Takara, Kyoto). After electrophoresis and transfer of the RNA to a nylon membrane, the membrane was prehybridized in 5× SSPE (1× SSPE = 0.15 M sodium chloride/10 mM sodium phosphate/1 mM EDTA, pH 7.4), 5× Denhardt's solution (1× Denhardt's solution = 0.02% Ficoll 400/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), 1% SDS, yeast RNA at 100 μg/ml, then hybridized to the ^{32}P-labeled PG25 fragment in the same solution for 14 h at 65°C. The membrane was finally washed in 0.2× SSC (1× SSC = 0.15M sodium chloride/0.015 M sodium citrate, pH 7), 0.1% SDS at 65°C and exposed to x-ray film for 7 days.

RESULTS

RT-PCR based on the highly conserved residues in the prostanoid receptors amplified a cDNA fragment from mouse thymus. This fragment, called PG25, is 425 bp long and codes for an open reading frame that shows sequence homology to the fifth (45%), sixth (35%), and seventh (76%) transmembrane domains of the EP$_2$ subtype of the mouse PGE receptor (18). Initial attempts to identify the cells or tissues expressing PG25 by RNA blotting analysis were unsuccessful, as was using PG25 as a probe to screen cDNA libraries generated



B

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                                                CTGCAGCCTTTCTTTCCAGTCAGACGGGACAAGGGCT   -1
ATGAACGAGTCCTATCGCTGTCAGACATCCACCTGGGTGGAAAGGGGCTCCTCGGCGACG    60
 M  N  E  S  Y  R  C  Q  T  S  T  W  V  E  R  G  S  S  A  T    20
                                                   I
ATGGGCGCTGTGCTCTTCGGTGCGGGGCTTCTGGGCAATCTTCTGGCGCTGGTGCTGCTG   120
 M  G  A  V  L  F  G  A  G  L  L  G  N  L  L  A  L  V  L  L    40

GCGCGCTCGGGACTGGGGTCTTGCCGGCCAGGGCCACTACACCCGCCGCCCTCGGTCTTT   180
 A  R  S  G  L  G  S  C  R  P  G  P  L  H  P  P  P  S  V  F    60
                                                            II
TATGTGCTCGTGTGTGGCTTGACGGTCACCGACTTGCTGGGCAAGTGTCTGATCAGCCCG   240
 Y  V  L  V  C  G  L  T  V  T  D  L  L  G  K  C  L  I  S  P    80

ATGGTCCTGGCTGCCTACGCGCAAAACCAGAGCCTAAAGGAACTGCTGCCTGCCTCAGGC   300
 M  V  L  A  A  Y  A  Q  N  Q  S  L  K  E  L  L  P  A  S  G   100
                          Δ
AATCAGTTATGCGAAACGTTCGCCTTCCTGATGTCCTTCTTTGGGCTAGCCTCGACCTTA   360
 N  Q  L  C  E  T  F  A  F  L  M  S  F  F  G  L  A  S  T  L   120
                   III
CAGCTGTTGGCTATGGCGGTGGAGTGCTGGCTGTCTCTGGGACACCCCTTCTTCTACCAA   420
 Q  L  L  A  M  A  V  E  C  W  L  S  L  G  H  P  F  F  Y  Q   140

AGGCACGTCACCTTGCGCCGGGGAGTGCTGGTGGCACCGGTCGTGGCCGCCTTCTGCTTG   480
 R  H  V  T  L  R  R  G  V  L  V  A  P  V  V  A  A  F  C  L   160
                                  IV
GCTTTCTGTGCGCTCCCCTTTGCTGGTTTTGGGAAGTTCGTGCAGTACTGTCCAGGCACC   540
 A  F  C  A  L  P  F  A  G  F  G  K  F  V  Q  Y  C  P  G  T   180

TGGTGTTTCATCCAGATGATCCACAAGGAGCGTTCATTTTCGGTAATAGGCTTCTCTGTG   600
 W  C  F  I  Q  M  I  H  K  E  R  S  F  S  V  I  G  F  S  V   200
                                                   V
CTCTACTCCAGCCTCATGGCGCTGCTGGTCCTCGCAACCGTGGTGTGCAACCTGGGTGCC   660
 L  Y  S  S  L  M  A  L  L  V  L  A  T  V  V  C  N  L  G  A   220

ATGTACAACCTCTATGACATGCACAGGCGCCAGAGGCACTATCCTCACCGCTGCTCCAGG   720
 M  Y  N  L  Y  D  M  H  R  R  Q  R  H  Y  P  H  R  C  S  R   240

GACCGCGCCCAGTCAGGCTCAGACTACAGGCACGGGTCCCTGCATCCTTTGGAGGAGCTG   780
 D  R  A  Q  S  G  S  D  Y  R  H  G  S  L  H  P  L  E  E  L   260

GACCACTTTGTGCTGCTGGCTCTCATGACAGTGCTCTTCACCATGTGTTCCCTGCCTTTA   840
 D  H  F  V  L  L  A  L  M  T  V  L  F  T  M  C  S  L  P  L   280
                VI
ATT GTGAGTTGCTGGGCAA ---------- 4kbp----------TTTTCCTCCCCAA
 I
CAGTATCGTGCGTACTATGGAGCCTTTAAACTTGAGAACAAAGCTGAAGGAGACTCAGAA   900
   Y  R  A  Y  Y  G  A  F  K  L  E  N  K  A  E  G  D  S  E   300

GACCTCCAAGCCTTGCGTTTCCTGTCTGTGATTTCCATAGTGGACCCCTGGATCTTCATC   960
 D  L  Q  A  L  R  F  L  S  V  I  S  I  V  D  P  W  I  F  I   320
                VII
ATCTTCAGGACTTCAGTATTCCGGATGTTATTTCACAAGGTTTTCACAAGACCTCTGATC  1020
 I  F  R  T  S  V  F  R  M  L  F  H  K  V  F  T  R  P  L  I   340

TACAGAAACTGGAGCAGCCATTCCCAGCAAAGTAACGTGGAATCCACTTTGTGA       1074
 Y  R  N  W  S  S  H  S  Q  Q  S  N  V  E  S  T  L  *        357
GGGCTTTGCGTGTTCTGATAAGCTGAAAATACAGCACACATTCAAAGAGCCGTGCTTGGA  1134
AAAGCCTTACAATTTAAATCCTTAAAAACTATCTCCCATGAAGAGATGGAGAGAGGGATC  1194
AGCACTGAAG                                                    1204
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FIG. 1. Nucleotide and deduced amino acid sequences of the mouse PGD receptor gene. (*A*) The relationship between the genome, the RT-PCR products PGc1 and PG25, and the synthetic cDNA PGc9 is shown. The exons in the genomic clone are indicated by open boxes, and the proposed open reading frame for PGc9 is boxed. The transmembrane domains are indicated by black boxes and are numbered in white Arabic numerals. (*B*) Nucleotide and deduced amino acid sequences of the mouse PGD receptor gene. The nucleotides in PGc9 are numbered according to the first methionine of the open reading frame. The nucleotides in the intron but not in PGc9 are italicized. The deduced amino acids are shown under the nucleotide sequence. The position of the translation stop codon is marked with an asterisk and the positions of the putative transmembrane domains are underlined. N-glycosylation sites (△) and potential protein kinase C phosphorylation sites (◆) are indicated below the amino acids. The nucleotide sequence corresponding to PG25 is boxed.

from several mouse tissues. Instead of expanding the screening process to isolate the full-length cDNA, we decided to isolate the gene encoding this prostanoid receptor-like sequence and to construct an open reading frame from a genomic clone for functional expression studies. Using a ^{32}P-labeled PG25 fragment as a probe, we screened a mouse genomic library and isolated four partially overlapping clones from 2×10^5 recombinants. One of these clones had a 13-kb insert with 3.3-kb and 6-kb *Xba* I restriction fragments that hybridized to the probe. Analysis of these *Xba* I restriction fragments revealed that the 309-bp sequence corresponding to the 5′ segment of PG25 was present in the 3.3-kb fragment. The rest of the PG25 sequence was located in the 6-kb fragment approximately 4 kb away from the upstream sequence (Fig. 1*A*). There is a potential translation initiation codon (28) 534 bp upstream of the PG25 sequence in the 3.3-kb fragment. This ATG initiates an open reading frame that codes for an amino acid sequence homologous to the first six transmembrane domains of the prostanoid receptors. The amino acid sequence of the open reading frame in the downstream 6-kb *Xba* I fragment contains sequence homologous to the seventh transmembrane domain of the prostanoid receptor followed by a short tail sequence and an in-frame stop codon (Fig. 1*A*). The intron has donor and acceptor splice sites compatible with the consensus sequence (29) (Fig. 1*B*), and its relative position in the coding sequence is identical to that of the human TXA_2 receptor gene (30). Amplification by RT-PCR and Southern blotting analysis of the mouse genomic DNA revealed bands of the expected sizes on gels (data not shown).

A DNA fragment containing the open reading frame was constructed by connecting a genomic fragment to amplified cDNA. We performed RT-PCR with a pair of oligonucleotides: the 5′ oligonucleotide corresponding to a sequence in the upstream exon, and the 3′ oligonucleotide from a sequence 130 bp downstream of the putative in-frame stop codon in the 3′ exon. We amplified an 824-bp fragment (PGc1) and constructed a 1.2-kb synthetic cDNA (PGc9) by ligating the *Pst* I–*Nar* I restriction fragments of the genomic fragment and PGc1 at their *Nar* I sites. Fig. 1*A* summarizes the relationship between the genomic clone, PG25, PGc1, and PGc9. PGc9 contains an open reading frame of 1071 bp that codes for a polypeptide of 357 amino acids (Fig. 1*B*). The translated product has a calculated molecular weight of 40,012, and its hydrophobicity profile (31) reveals the presence of seven hydrophobic regions which likely represent transmembrane domains. There are two asparagine residues for putative N-glycosylation: Asn-2 in the N-terminus and Asn-89 in the first extracellular loop. There are also two potential protein kinase C phosphorylation sites (32), Ser-47 and Thr-144, in the first and the second cytoplasmic loops, respectively. In addition, there are eight serines and threonines in the carboxyl-terminal tail, some of which may be sites for phosphorylation by a receptor kinase (21).

Fig. 2 shows the alignment of the deduced amino acid sequence from PGc9 compared with the sequences from other mouse prostanoid receptors. This analysis revealed that the PGc9 product has 39% and 32% sequence identity over the entire amino acid sequence with the PGI receptor (23) and the EP_2 subtype of the PGE receptor (18), respectively, but showed less than 25% amino acid sequence identity with other prostanoid receptors. Thus, the receptor encoded by PGc9, the PGI receptor, and the EP_2 subtype of the PGE receptor form a distinct subgroup within the prostanoid receptor family.

FIG. 2. Comparison of the amino acid sequence of the receptor encoded by PGc9 with other prostanoid receptors. The amino acid sequences from PGc9 (DP), the mouse PGI receptor (IP), the EP_2, EP_3, and EP_1 subtypes of the PGE receptor, the TXA_2 receptor (TP), and the PGF receptor (FP) were aligned to achieve maximal sequence similarity. The shaded areas indicate amino acids that are conserved between the receptor encoded by PGc9 and other prostanoid receptors. Putative transmembrane domains are indicated by horizontal lines above the sequence.

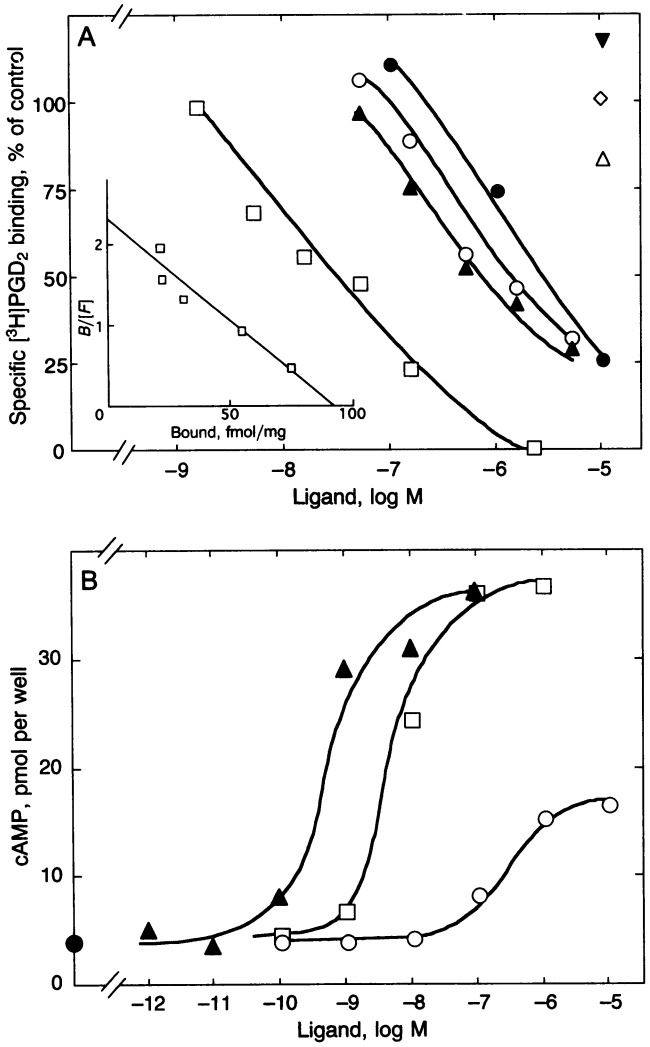

FIG. 3. Pharmacologic characteristics of the PGD receptor stably expressed in CHO cells. (*A*) Binding of [^{3}H]PGD_2 to CHO-J cells and its displacement by unlabeled ligands. Cell homogenate and [^{3}H]PDG_2 were incubated with and without unlabeled compounds at the indicated concentrations. The compounds used were: PGD_2 (□), BW 245C (▲), BW A868C (○), STA_2 (●), PGE_2 (△), iloprost (◇), and $PGF_{2\alpha}$ (▼). (*Inset*) Scatchard plot of the equilibrium binding of [^{3}H]PGD_2. *B*, bound [^{3}H]PGD_2; *F*, free [^{3}H]PGD_2. (*B*) cAMP levels in CHO-J cells treated with PGD_2 and analogues. CHO-J cells were incubated with 3-isobutyl-1-methylxanthine and the indicated concentrations of PGD_2 (□), BW 245C (▲), BW A868C (○), or solvent alone (●) and the cAMP content was determined as described in *Materials and Methods*.

The PGI receptor, the EP_2 subtype of the PGE receptor, and the PGD receptor are classified pharmacologically as relaxant prostanoid receptors (4). Since PGc9 codes for a receptor that is closely related to two of these receptors, it is possible that PGc9 encodes a PGD receptor. This was tested by expressing PGc9 in CHO cells. CHO-J cells expressing PGc9 displayed a saturable, high-affinity binding of [^{3}H]PGD_2, and Scatchard analysis revealed a single class of binding sites with a K_d of 40 nM and a B_{max} of 93 fmol/mg of homogenate protein (Fig. 3*A Inset*). Displacement studies showed that the PGD agonist BW 245C (5, 33) and the antagonist BW A868C (34–36) competitively displaced [^{3}H]PGD_2 binding at a concentration range of 10 nM to 5 μM (Fig. 3*A*). PGE_2, $PGF_{2\alpha}$, and iloprost showed little displacement at 10 μM, whereas the TXA_2 agonist STA_2 displaced the binding in a concentration-dependent manner from 100 nM. Parental CHO-dhfr$^-$ cells showed no specific [^{3}H]PGD_2 binding (data not shown). As shown in Fig. 3*B*, PGD_2 raised cAMP levels in CHO-J cells in a concentration-dependent manner with an EC_{50} of 6.8 nM. BW 245C was about 10 times more potent than PGD_2, with an EC_{50} of 0.54 nM. BW A868C also increased cAMP levels at concentrations greater than 100 nM; however, the increase reached a plateau at 10 μM, with the efficacy being about 35% of the maximal stimulation obtained with PGD_2. These results suggest that BW A868C is a partial agonist on a mouse PGD receptor. PGD_2, BW 245C, and BW A868C had no effect on cAMP levels in the parental CHO-dhfr$^-$ cells (data not shown). PGD_2 did not induce significant inositolphospholipid breakdown in CHO-J cells (data not shown). These properties are compatible with those of the pharmacologically defined PGD receptor (4, 5), and we therefore conclude that PGc9 codes for a functional PGD receptor.

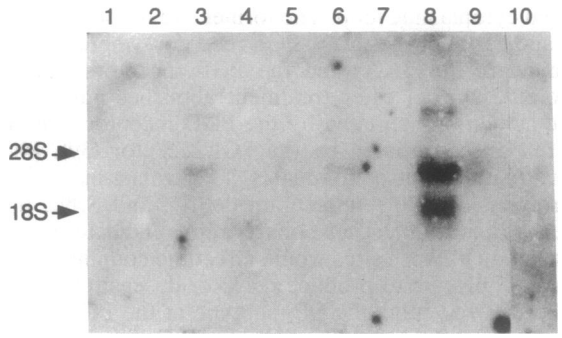

FIG. 4. Northern blotting analysis of RNA isolated from various mouse tissues. Twenty micrograms of poly(A)$^+$ RNA isolated from the tissues was applied to each lane. Hybridization was performed with ^{32}P-labeled PG25 fragment as a probe. Lane 1, brain; lane 2, thymus; lane 3, lung; lane 4, heart; lane 5, spleen; lane 6, stomach; lane 7, liver; lane 8, ileum; lane 9, kidney; and lane 10, uterus. The positions of 28S and 18S rRNA on the gel are given by arrows at the left side of the figure.

Northern blotting analysis of various mouse tissues with the PG25 fragment revealed a major hybridization signal at 3.5 kb (Fig. 4). This species of mRNA was most abundant in the ileum, followed by the lung, the stomach and the uterus. In the lung and ileum lanes, a band of about 7 kb was also apparent. In addition, a band at around 2 kb in ileal RNA was present.

DISCUSSION

With the PGD receptor described herein, we have now reported the primary structures of all seven pharmacologically defined prostanoid receptors. There are two motifs that are highly conserved in these receptors. The first conserved motif is the QYCPG(T/S)WCF sequence (amino acids 175–183 of the PGD receptor) in the second extracellular loop. Although the cysteine residue in this motif (Cys-182) is present in the majority of the G protein-coupled receptors, and is believed to form a disulfide bond with another conserved cysteine residue in the first extracellular loop (Cys-104) (37), the above motif can be found only in the prostanoid receptors. The second conserved motif, RXX(S/T)X(N/I)-XI(V/L)DPW(I/V)(F/Y)(I/L) (amino acids 306–320 of the PGD receptor) is found in the seventh transmembrane domain and contains two charged amino acids, Arg-306 and Asp-315. These conserved residues may interact with the common structure of the ligands for these receptors. Recently, it has been shown that a mutation in this arginine residue in the human TXA_2 receptor results in the loss of ligand binding activity (38).

There are two potential phosphorylation sites for protein kinase C: one in the first cytoplasmic loop and one in the

second cytoplasmic loop. The former site is conserved in the EP_2 and EP_3 subtypes of the PGE receptor, whereas the latter is found in the TXA_2 and the PGF receptors. In human platelets, phorbol ester treatment abolishes adenylate cyclase activation mediated by the PGD receptor, but spares the response mediated by the PGI receptor (39). Phorbol ester treatment also attenuates TX agonist-induced GTP hydrolysis in platelet membranes (F.U. and S.N., unpublished observation). These observations indicate that phosphorylation at these sites would affect the coupling of receptor and G protein to produce receptor desensitization.

The PGI receptor, the EP_2 subtype of the PGE receptor, and the PGD receptor have all been classified as relaxant prostanoid receptors (4). In agreement with this functional classification, their primary structures are more closely related to each other than to the other four prostanoid receptors. In contrast, the TXA_2 receptor, the EP_1 subtype of the PGE receptor, and the PGF receptor all preferentially raise intracellular calcium and can be functionally classified as contractile prostanoid receptors. They are also more closely related to each other in their primary structure than they are to any of the others (22). Thus, within the prostanoid receptor family, there are two groups of functionally and structurally related receptors. The genomic organization for these receptors may have been conserved during their evolution, because both the human TXA_2 receptor gene (30) and the mouse PGD receptor gene contain an open reading frame consisting of two exons separated by an intron at a homologous position.

Northern blotting for the PGD receptor identified a 3.5-kb mRNA in the lung, the gastrointestinal tract, and the uterus. The intestine is one tissue where high PGD synthase activity has been detected (40). It has also been reported that treatment with PGD_2 modifies the chloride transport across the intestinal mucosa (10, 41). In addition to the tissues identified by RNA blotting, RT-PCR detected the PGD receptor expression in the thymus and brain (M.H. and H. Oida, unpublished observations), suggesting that the PGD receptor expression in these organs may be confined to small region(s) or specific cell(s). PGD_2 is one of the major PGs formed in the brain and has been suggested to be a physiological regulator of sleep (14). The brain-type PGD synthase mRNA has been found to be abundantly expressed in the leptomeninges (42). PGD_2 is formed in the lung under allergic conditions and produces bronchoconstriction (5, 43). Our results have demonstrated that the PGD receptor preferentially mediates adenylate cyclase activation but not inositolphospholipid breakdown. The contractile responses to PGD_2 may therefore be a result from its action on contractile prostanoid receptors, such as the TXA_2 receptor (5, 6, 44).

In summary, we have identified the gene and a cDNA for a mouse PGD receptor. Availability of the nucleotide and amino acid sequences of the PGD receptor will permit the identification of cells expressing this receptor and allow elucidation of the role for PGD_2 in physiological and pathological conditions.

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