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Prostanoid Receptors: Structures, Properties, and Functions

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Narumiya, Shuh, Yukihiko Sugimoto, and Fumitaka Ushikubi. Prostanoid Receptors: Structures, Properties, and Functions. *Physiol. Rev.* 79: 1193–1226, 1999.—Prostanoids are the cyclooxygenase metabolites of arachidonic acid and include prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxne A_2. They are synthesized and released upon cell stimulation and act on cells in the vicinity of their synthesis to exert their actions. Receptors mediating the actions of prostanoids were recently identified and cloned. They are G protein-coupled receptors with seven transmembrane domains. There are eight types and subtypes of prostanoid receptors that are encoded by different genes but as a whole constitute a subfamily in the superfamily of the rhodopsin-type receptors. Each of the receptors was expressed in cultured cells, and its ligand-binding properties and signal transduction pathways were characterized. Moreover, domains and amino acid residues conferring the specificities of ligand binding and signal transduction are being clarified. Information also is accumulating as to the distribution of these receptors in the body. It is also becoming clear for some types of receptors how expression of their genes is regulated. Furthermore, the gene for each of the eight types of prostanoid receptor has been disrupted, and mice deficient in each type of receptor are being examined to identify and assess the roles played by each receptor under various physiological and pathophysiological conditions. In this article, we summarize these findings and attempt to give an overview of the current status of research on the prostanoid receptors.

I. INTRODUCTION

Prostanoids that consist of the prostaglandins (PG) and the thromboxanes (Tx) are cyclooxygenase products derived from C-20 unsaturated fatty acids (Fig. 1). Prostaglandins contain a cyclopentane ring and two side chains named α and ω attached to the ring. According to the modifications of this cyclopentane ring, they are classified into types A to I, in which types A, B, and C are believed not to occur naturally but are produced only

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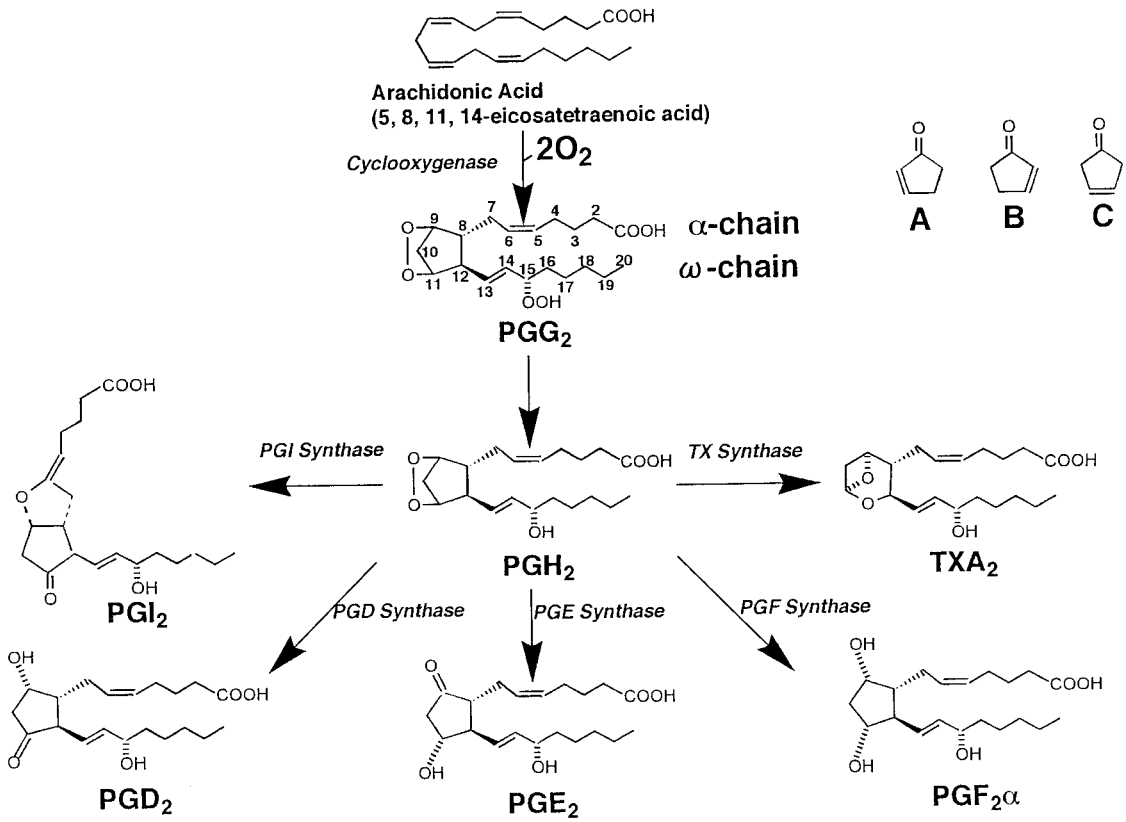

FIG. 1. Biosynthetic pathways of prostanoids. Formation of series 2 prostaglandins (PG), PGD_2, PGE_2, $PGF_{2\alpha}$, PGG_2, PGH_2, and PGI_2, and a thromoboxane (Tx), TxA_2, from arachidonic acid is shown. The first 2 steps of pathway, i.e., conversion of arachidonic acid to PGG_2 and then to PGH_2, are catalyzed by cyclooxygenase, and subsequent conversion of PGH_2 to each PG is catalyzed by respective synthase as shown. Ring structures of A, B, and C types of PG are shown separately.

artificially during extraction procedures. Prostaglandins G and H share the same ring structure but differ at C-15, having a hydroperoxy and hydroxy group, respectively. Another cyclooxygenase product, TxA, has an oxane ring instead of the cyclopentane ring. Prostanoids are further classified into three series (1, 2, and 3) based on the number of double bonds in their side chains; the series 1 prostanoids contain a 13-*trans* double bond, the series 2 prostanoids have 5-*cis* and 13-*trans* double bonds, and the series 3 prostanoids have 5-*cis*, 13-*trans*, and 17-*cis* double bonds. The prostanoids in series 1, 2, and 3 are synthesized from γ-homolinolenic acid (8,11,14-eicosatrienoic acid), arachidonic acid (5,8,11,14-eicosatetraenoic acid), and 5,8,11,14,17-eicosapentaenoic acid, respectively. Because arachidonic acid is the most abundant among these precursor fatty acids in most mammals, including humans, the series 2 prostanoids are predominantly formed in their bodies. The above fatty acids are liberated from membrane phospholipids in response to various physiological and pathological stimuli by the action of phospholipase A_2 and are converted to various prostanoids by the sequential actions of cyclooxygenase and the respective synthases. Prostanoids thus formed are released outside of the cells immediately after synthesis. Prostaglandin G, PGH, PGI, and TxA are chemically unstable and are degraded into inactive products under physiological conditions, with a half-life of 30 s to a few minutes. Other PG, although chemically stable, are metabolized quickly. For example, they are inactivated during a single passage through the lung. It is believed therefore that prostanoids work locally, acting only in the vicinity of the site of their production.

Prostanoids exert a variety of actions in various tissues and cells. The most typical actions are the relaxation and contraction of various types of smooth muscles. They also modulate neuronal activity by either inhibiting or stimulating neurotransmitter release, sensitizing sensory fibers to noxious stimuli, or inducing central actions such as fever generation and sleep induction. Prostaglandins also regulate secretion and motility in the gastrointestinal tract as well as transport of ions and water in the kidney. They are involved in apoptosis, cell differentiation, and oncogenesis. Prostanoids also regulate the activity of blood platelets both positively and negatively and are involved in vascular homeostasis and hemostasis. Because prostanoids are produced from fatty acids and

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hence are generally regarded as hydrophobic compounds, it was thought in earlier times that they were incorporated into the cell membrane and exerted their action by perturbing lipid fluidity. The concept of prostanoid action via prostanoid receptors gradually appeared. This arose from several different lines of studies. First, prostanoids are not as hydrophobic as they were once thought to be and do not incorporate into or permeate the cell membrane (18). Second, each prostanoid has a unique activity profile not exactly overlapping with others, indicating that each prostanoid has a specific site of action. This became apparent by comparing the potencies of various prostanoids and their synthetic analogs on various tissues by bioassay. Studies along this line led to the suggestion of the presence of multiple types of prostanoid receptors in different tissues and cells such as the lung and platelets (7, 66, 179) and culminated in a proposal to classify the prostanoid receptors in 1982 (107). Moreover, various synthetic TxA_2 agonists and antagonists were developed in the late 1970s to early 1980, and using these compounds, a receptor for TxA_2 was identified pharmacologically as the site of competition of TxA_2 agonists and antagonists (for example, see Ref. 95).

The presence of a receptor(s) for prostanoids had also been suggested biochemically. It had been repeatedly reported that the actions of prostanoid were associated with changes in the level of second messengers. Already in the mid 1960s, some prostanoid actions had been noticed to be associated with changes in cAMP levels (see, for example, Ref. 31). Later, the association of the actions of these prostanoids with changes in phosphatidylinositol (PI) turnover and free Ca^{2+} concentrations in the cell were reported. In addition, with the availability of radiolabeled derivatives of prostanoids, it was found in the early 1970s that many tissues and cells contain specific high-affinity binding sites for the prostanoids (117, 183, 184). Although the binding sites identified in earlier studies may not have represented functional receptors, functional correlates of the binding activities to the bioactivity or to the second messenger systems were examined in later studies. Coleman et al. (44) integrated the information obtained by these approaches to propose a comprehensive classification of the prostanoid receptors. They proposed the presence of receptors specific for Tx, PGI, PGE, PGF, and PGD and named them the TP, IP, EP, FP, and DP receptors, respectively. They further classified the EP receptor into three subtypes: EP_1, EP_2, and EP_3, all of which respond to the naturally occurring agonist PGE_2 but differ in their actions and in their responses to various analogs. They later reported a fourth subtype, the EP_4 receptor, which, like the EP_2 receptor, is positively coupled to adenylate cyclase but differs in its response to certain ligands (43). However, none of the receptors had been isolated and cloned until the TxA_2 receptor was purified from human blood platelets in 1989 (239) and its cDNA cloned in 1991 (79). These studies revealed that the TxA_2 receptor was a G protein-coupled rhodopsin-type receptor with seven transmembrane domains. Homology screening in mouse cDNA libraries subsequently identified the structures of all of the eight types and subtypes of the prostanoid receptors. These receptors have been expressed, and their ligand binding properties and signal transductions have been examined. In addition, the tissue and cell distribution of the receptors was studied by Northern blot and by in situ hybridization analyses of their mRNA expression. Correlation of such knowledge with findings accumulated by pharmacological studies using cyclooxygenase inhibitors and using various prostanoid analogs having agonistic and antagonistic activities helps to define the actions of each type of receptor. They also help to reveal novel actions of these receptors. Recently, knockout mice deficient in each receptor have been generated by gene targeting, and implications and significances of prostanoid actions in various physiological and pathophysiological processes are being examined and assessed. This review summarizes current information obtained by these studies. The correlattion of these studies to previous pharmacological works is emphasized to give an overview of the physiological and pathophysiological roles of the prostanoid receptors.

II. STRUCTURES OF PROSTANOID RECEPTORS AND THEIR GENES

A. Prostanoid Receptors as Rhodopsin-Type Receptors

The structure of the human TxA_2 receptor is shown in Figure 2 as a representative of the prostanoid receptors. It is a protein composed of 343 amino acids and is a G protein-coupled rhodopsin-type receptor with 7 putative transmembrane domains. Since the cloning of this receptor in 1991 by Hirata et al. (79), homology screening based on its sequence was performed in various species, and all of the eight types and subtypes of the prostanoid receptors previously defined pharmacolgically were identified. They include the human and mouse PGD receptor (DP) (21, 80); the mouse, rat, and human PGE receptor EP_1 subtype (22, 63, 244); the mouse and human PGE receptor EP_2 subtype (101, 187); the mouse, human, rat, rabbit, and bovine PGE receptor EP_3 subtype (3, 27, 154, 186, 217, 226, 250); the mouse, human, and rat PGE receptor EP_4 subtype (originally reported as the EP_2 subtype; see below) (6, 13, 84, 193); the mouse, human, bovine, rat, and sheep PGF receptor (FP) (2, 70, 110, 191, 216); the mouse, human, and rat PGI receptor (IP) (20, 103, 149, 152, 197); and the mouse, rat, and bovine TxA receptor (TP) (1, 146, 153). Initially, two species of cloned receptors were reported as EP_2; one was originally cloned

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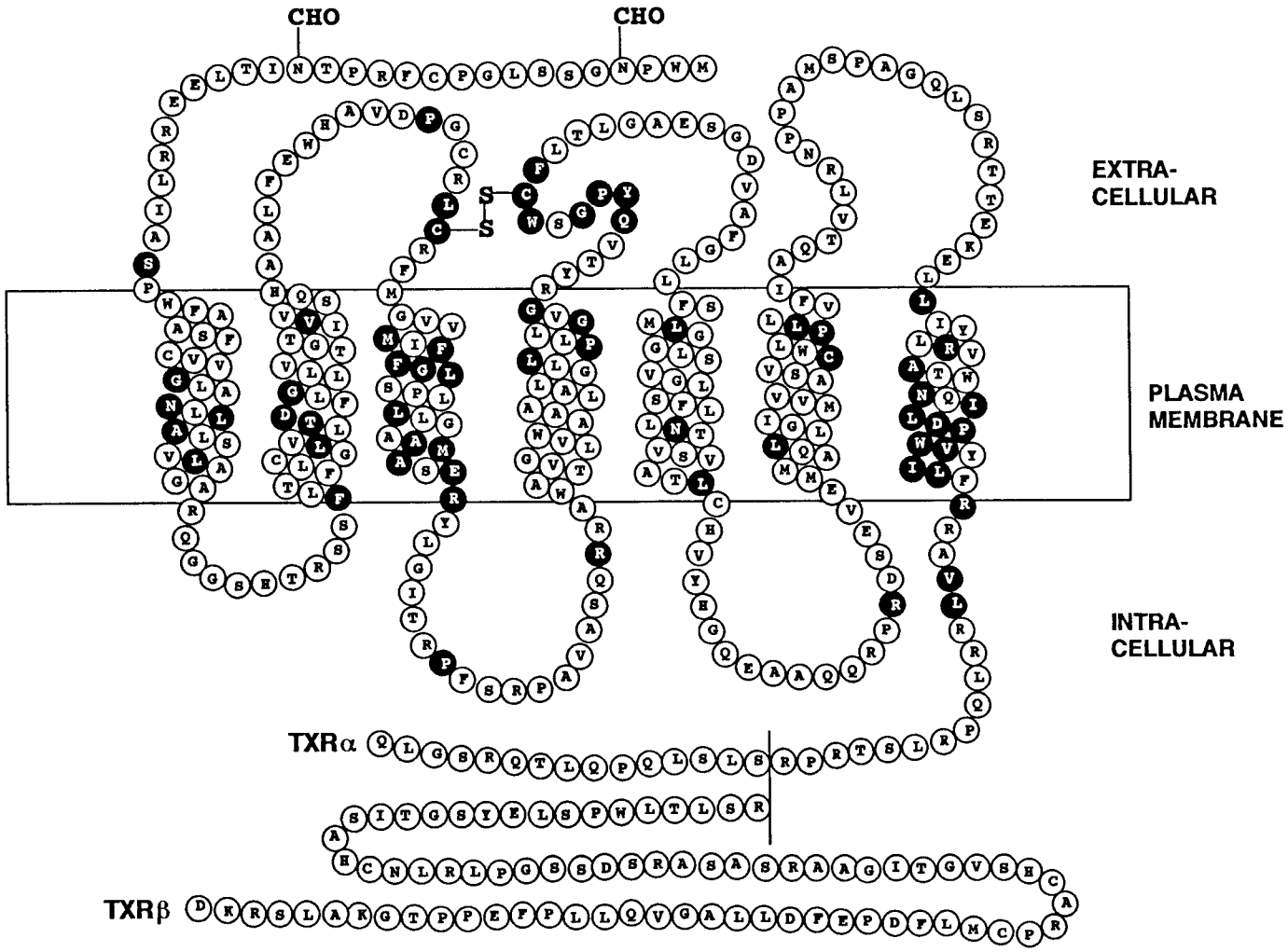

FIG. 2. Structure of human TxA_2 receptor. Membrane topology model of 2 isoforms of human TxA_2 receptor is shown. Amino acid residues are indicated by single letter code. Two isoforms are different only in carboxy-terminal tail (see sect. II*B* and Fig. 4). Amino acid residues conserved by most of prostanoid receptors are shown by solid circles with white letters. *N*-glycosylation at Asn^4 and Asn^{16} is indicated by -CHO, and putative disulfide bond between first and second extracellular loops is shown.

by Honda et al. (84) and subsequently by other people (6, 13, 193) and one was later cloned by Regan et al. (187). Although Honda et al. (84) reported their cloned PGE receptor as EP_2 on the basis of its positive coupling to adenylate cyclase, this receptor is insensitive to butaprost, a synthetic PGE derivative, which is inconsistent with the pharmacolgically defined EP_2 receptor (44). On the other hand, the receptor cloned by Regan et al. (187) is sensitive to butaprost. Later, the presence of another PGE receptor subtype with positive coupling to adenylate cyclase was suggested by pharmacological methods (43). The subsequent characterization of the "EP_2" receptor of Honda et al. (84) revealed that it is sensitive to an EP_4-specific ligand, AH23848B (163). Moreover, a mouse homolog of the receptor cloned by Regan et al. (187) was cloned and shown to have properties consistent with the pharmacologically defined EP_2 (101). These results suggest that the receptors cloned originally by Honda et al. (84) in the mouse and subsequently by other groups in other species represent the EP_4, and that cloned by Regan et al. (187) represents the EP_2 subtype.

The amino acid sequence alignment of the eight types and subtypes of cloned mouse prostanoid receptors is shown in Figure 3*A*. They are aligned based on the homology of the putative seven transmembrane domains of these receptors. There are a total of 28 amino acid residues conserved in and close to these regions. Among them, eight residues are shared also by other families of rhodopsin-type receptors, and they are believed to be involved in the maintenance of structure and/or function of these types of receptors in general. For example, Asp in the second transmembrane domain has been shown in other receptors to be involved in activation of the receptors, by coupling ligand binding to the activation of G

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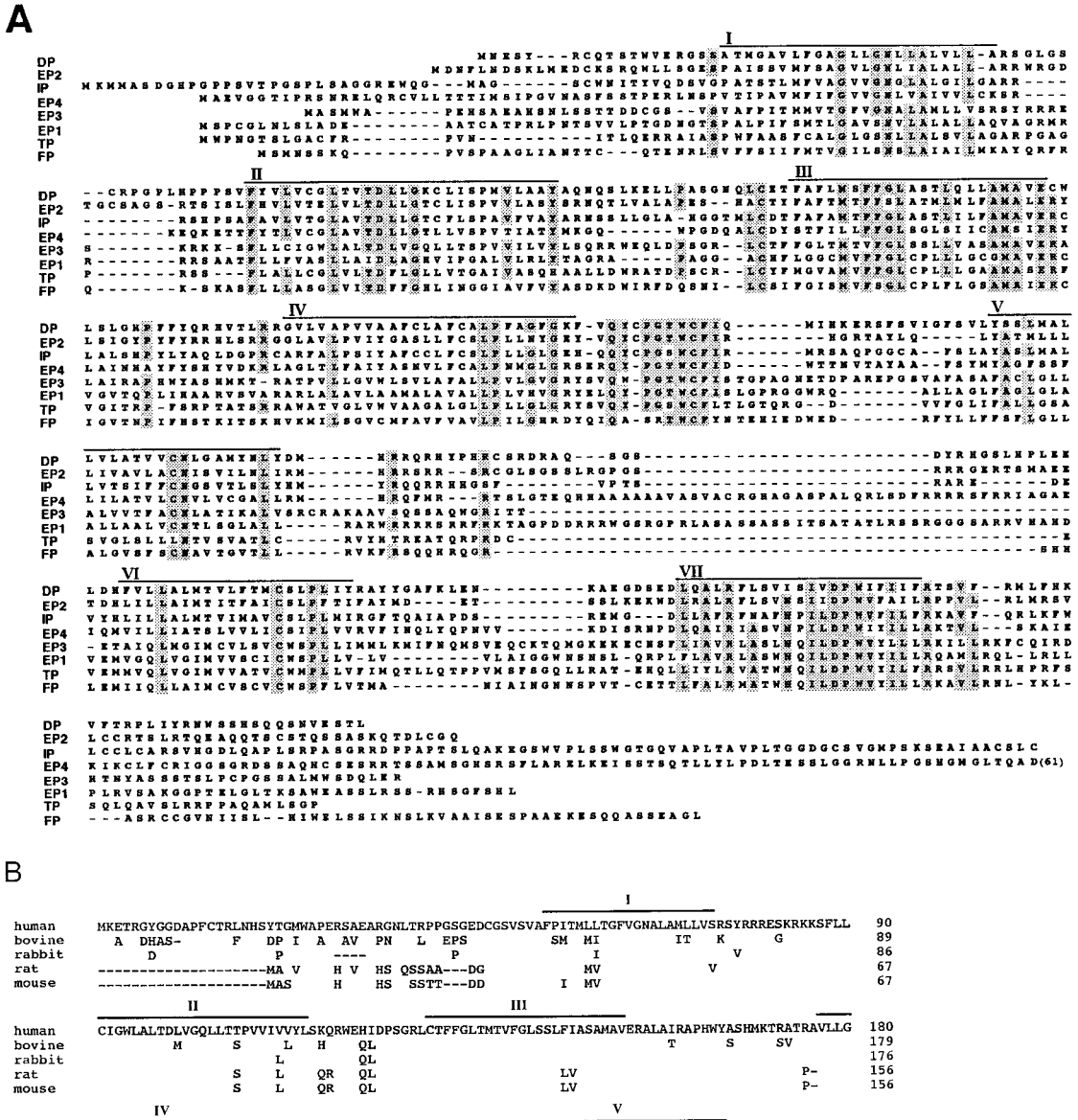

FIG. 3. *A*: amino acid sequence alignment of 8 types and subtypes of prostanoid receptors. Amino acid sequences of 8 types and subtypes of mouse prostanoid receptors are aligned. Amino acid residues shared by >5 receptors are shadowed. Putative transmembrane domains are indicated by lines above sequence and numbered. Most carboxy-terminal 64-amino acid residues of EP_4 receptor are not shown. *B*: amino acid sequence alignment of EP_3 receptors from different species. Amino acid sequences of corresponding isoform of EP_3 receptor from human, bovine, rabbit, rat, and mouse are aligned. Only amino acid residues different from corresponding residues of human receptor are shown for other species. Note that amino-terminal portions of rat and mouse receptors are ~20 amino acids shorter than receptors from other species.

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proteins (198). Two Cys residues, one in the first and the other in the second extracellular loop, are also conserved. These residues are believed to form a disulfide bond critical for stabilization of receptor conformation and for ligand binding. This function of these residues has been examined in the prostanoid receptors (see section III*C*). In addition to these conserved residues, several features common in the rhodopsin-type receptors are also found in the prostanoid receptors. First, they have one or more consensus sequences for *N*-glycosylation of asparagine residue(s), Asn-X-Ser/Thr, in the amino-terminal extracellular portion. This motif is found, for example, at Asn-4 and Asn-16 in the amino-terminal portion of the human TxA_2 receptor. Although the TxA_2 receptor protein purified from the platelet membrane was ~57 kDa, the molecular mass calculated from the primary structure is 37 kDa. This difference appears to be accounted for by glycosylation of the receptor. The analysis of the amino-terminal sequence of the purified protein showed that both of these sites were modified (79), and the treatment of the purified protein with *N*-glycanase reduced the molecular mass of the protein to ~37 kDa (126). Chiang and Tai (37) reported that deletion of the carbohydrate moieties of the human TP receptor by adding tunicamycin during infection of Sf21 cells or mutation of both Asn-4 and Asn-16 abolished the ligand binding to the receptor and suggested that *N*-glycosylation is crucial for its binding function. Similar potential *N*-glycosylation sites are also seen in the first extracellular loop of the DP, IP, and EP_2 receptors and in the second extracellular loop of the EP_3 and EP_4 receptors. In addition, as in many other rhodopsin-type receptors, serine and threonine residues, which comprise putative phosphorylation sites, are widely distributed in the cytoplasmic portion of the prostanoid receptors. Phosphorylation of these residues is thought to participate in the desensitization of these receptors, as noted in other rhodopsin-type receptors (77). In fact, agonist-induced phosphorylation (71, 175) and phosphorylation by protein kinase A (PKA) and protein kinase C (PKC) (108) of the human TP receptor are reported to be involved in receptor desensitization. On the other hand, although some rhodopsin-type receptors are palmitoylated at the Cys residue in the carboxy tail and form an additional intracellular loop (24), the consensus sequence for this modification, Leu-X-Cys-(X)n-Arg/Lys- in which the Cys residue is located 11–16 residues distal to the end of the seventh transmembrane domain, is not found in the prostanoid receptors, although IP, EP_2, and EP_4 receptors have a Cys residue at the appropriate position.

In addition to the features common to other rhodopsin-type receptors, particular motifs specifically conserved among the prostanoid receptors are found in several regions (Fig. 3*A*). For example, L-X-A-X-R-X-A-S/T-X-N-Q-I-L-D-P-W-V-Y-I-L or homologous motifs are found in the seventh transmembrane domain of most of the prostanoid receptors. Two other sequences, G-R-Y-X-X-Q-X-P-G-T/S-W-C-F and M-X-F-F-G-L-X-X-L-L-X-X-X-A-M-A-X-E-R, are found in the second extracellular loop and in the third transmembrane domains, respectively. Because these highly conserved regions are shared by prostanoid receptors from various species (Fig. 3*B*), they may participate in the construction of binding domains for structures common to prostanoid molecules. The arginine in the seventh transmembrane domain, which is conserved in all of the prostanoid receptors, was proposed to be the binding site of the carboxyl group of prostanoid molecules by analogy to the retinal binding site, Lys-296, of rhodopsin (79, 155), and has been subjected to extensive mutational analyses (see sect. III*C*). In comparison with the sequences of the transmembrane domains, the alignment of the sequences of intracellular structures is difficult because of their diversity both in composition and in length. However, the conservation of several residues has been found. For example, an Arg or other basic residue is found in the first intracellular loop of all of the prostanoid receptors at analogous positions. A spontaneous mutation of this arginine residue has been found in the TP receptor of patients with a hereditary bleeding disorder and identified as the cause of this disease (see sect. III*C*).

Despite the presence of these conserved sequences, overall homology among the prostanoid receptors is quite limited, ranging from ~20 to 30%. It is worth noting that there is only this limited extent of homology even among the four subtypes of PGE receptors, which makes it difficult to get any insight into the areas determining the ligand binding specificity of each receptor, simply by comparing the sequence of one receptor with those of the receptors for other prostanoids. On the other hand, the homology of a given type or subtype of receptor among various species is considerably higher. For example, the sequence homology between human and mouse IP, TP, EP_1, EP_3, EP_4, and FP receptors is 79, 76, 84, 84, 88, and 89%, respectively, and the homology among human, bovine, rabbit, rat, and mouse EP_3 receptors ranges from 84 to 97% (Fig. 3*B*). There are differences in the translation initiation sites of some receptor types and subtypes in different species, which affect the length of the amino-terminal extracellular domain of the receptor. For example, this portion of the human, bovine, and rabbit EP_3 receptor is 20 amino acids longer than that of the rat and mouse receptors. Conversely, this portion of the human IP receptor is 30 amino acids shorter than that of the rat and mouse receptors. Differences in the actions of prostanoids between species are well known. For example, EP-157, a PGI analog, acts on human and horse platelets as an agonist, whereas it is an antagonist when examined in pig and rat platelets (10). Rabbit platelets are known to differ from human, cat, and canine platelets in their response to CTA_2 and PTA_2, which are both Tx analogs (30).

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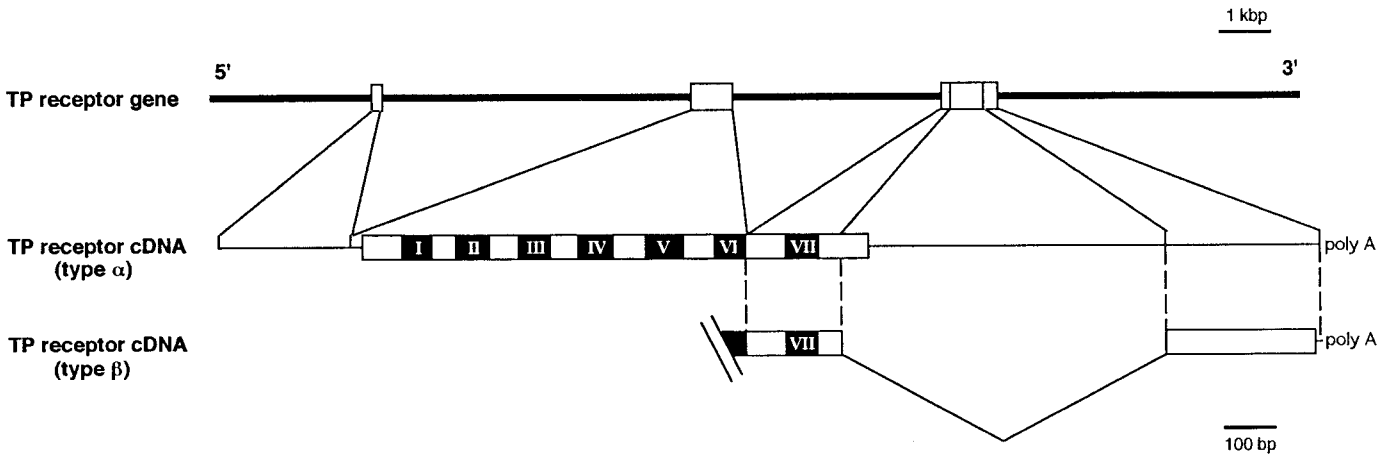

FIG. 4. Organization of human TxA_2 receptor gene and generation of receptor isoforms by alternative splicing of its mRNA. Three exons of TP receptor gene are indicated by white boxes (*top*). Amino acid coding regions of cDNA are shown by boxes (*middle* and *bottom*), and those corresponding to putative transmembrane domains are indicated by solid boxes and numbers. Note that alternative usage of third exon generates 2 isoforms of receptor, TPα and -β that differ in their carboxy-terminal tails.

The potency of ONO-11120, an antagonist of the TP receptor, is two orders of magnitude lower in rabbit platelets than in human platelets (156). These species differences may be due to differences in the structure of the respective receptors, despite high sequence homology among receptor homologs from various species. The molecular basis for the difference in sensitivity of the rat and human TP receptor to I-BOP, a TP agonist, has been examined in the rat/human chimeric TP receptor (see sect. III*C*).

B. Gene Structures and Isoform Generation of Prostanoid Receptors

Chromosomal localizations of the genes encoding the mouse and human prostanoid receptors have been determined. The genes encoding the mouse DP, EP_1, EP_3, EP_4, FP, IP, and TP receptors were mapped to chromosomes 14, 8, 3, 15, 3, 7, and 10, respectively (91, 225). The genes encoding the human EP_1, EP_3, EP_4, FP, IP, and TP receptors were mapped to chromosome bands 19p13.1, 1p31.2, 5p13.1, 1p31.1, 19q13.3, and 19p13.3, respectively (59, 165). The gene encoding the human DP receptor and those encoding the mouse and human EP_2 receptors have not yet been mapped. These studies showed that the EP_1, EP_4, IP, and TP receptor genes are localized in chromosomal segments of each animal previously found homologous between the mouse and the human (28, 50, 229). Furthermore, it is notable that the loci of the EP_3 and FP receptor genes are in close proximity in both the mouse and human chromosome, suggesting that the distal mouse chromosome 3 is the homologous segment of the short arm of human chromosome 1 and that the EP_3 and FP receptor genes evolved by gene duplication.

The structure of a prostanoid receptor gene was first clarified for the human TP receptor, which contains three exons separated by two introns, one in the 5′-noncoding region and the other at the end of the sixth transmembrane domain (165) (Fig. 4). This exon-intron relationship appears to be conserved in other types of prostanoid receptors and across various species, such as in the mouse and the human DP receptor (21, 80), the mouse EP_1 receptor (15), the mouse EP_2 receptor (104), the human EP_3 receptor (186), the human and mouse EP_4 receptor (8, 62), the mouse FP receptor (75), and the human IP receptor (167). The first intron is located upstream of the ATG start codon in the reported prostanoid receptor genes, except in the mouse EP_4 receptor gene, in which it is located 16 bp downstream of the translational start site (8). There are additional exons encoding carboxy-terminal tails in some of the prostanoid receptors, and alternative splicing of these exons further creates several isoforms. This was observed in the mouse, rat, bovine, rabbit, and human EP_3 receptors (3, 27, 90, 115, 154, 161, 186, 219, 226), the human TP receptor (185), and the ovine FP receptor (180). This alternative splicing occurs in the carboxy-terminal region after the seventh transmembrane domain and creates various receptor isoforms that differ only in their carboxy tails. The isoforms of the EP_3, FP, and TP receptors have almost identical ligand-binding specificities among each receptor. However, isoforms of the bovine EP_3 and human TP receptors are coupled to different G proteins and induce different signaling pathways (82, 154), and those of the mouse EP_3 receptor are different in their efficacy of G protein coupling (219), in their sensitivity to agonist-induced desensitization (161), and in their extent of constitutive activity (74, 157). The differences in constitutive activity have also

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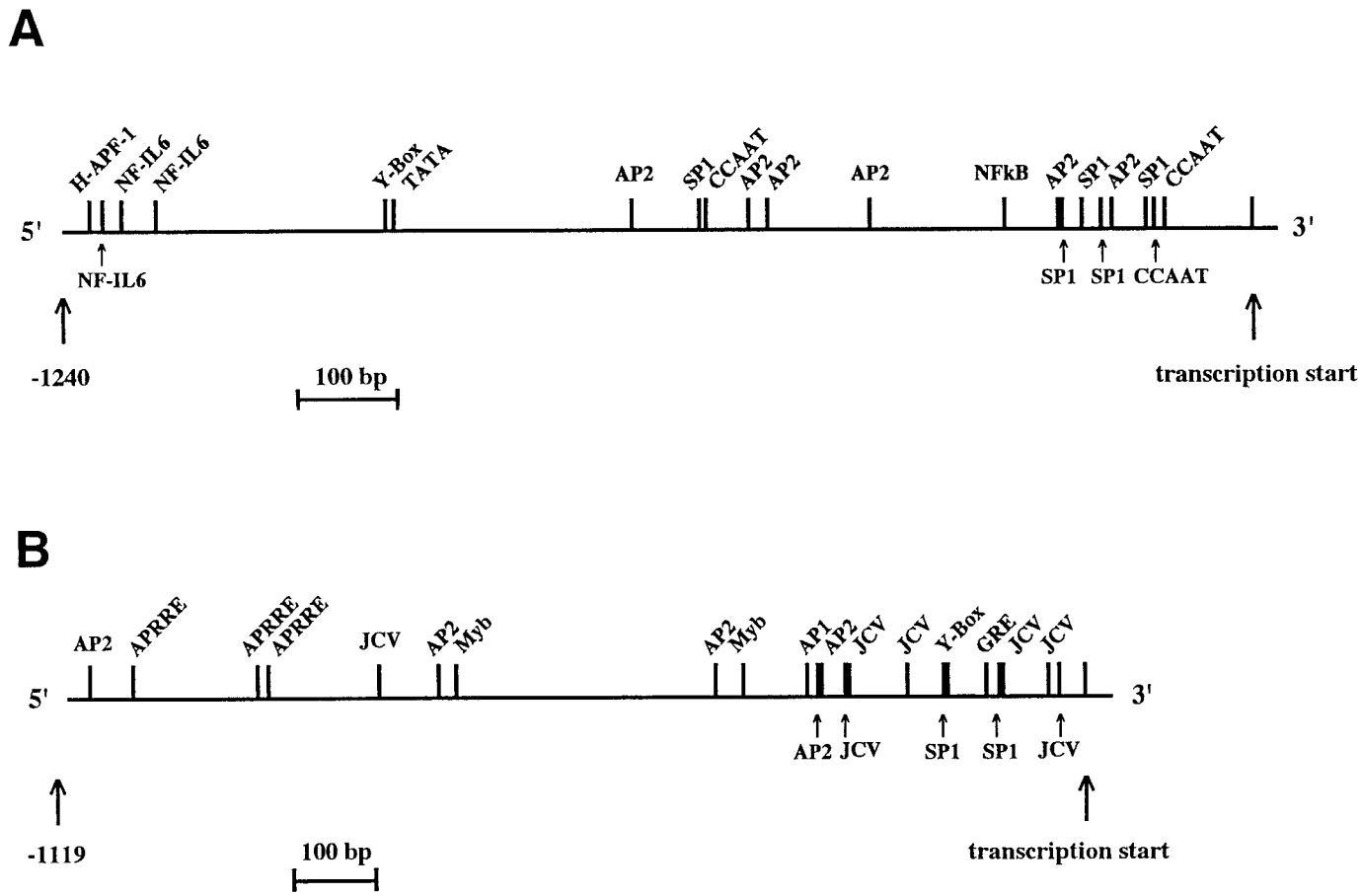

FIG. 5. Transcriptional *cis*-elements in promotor regions of prostanoid receptor genes. Promotor regions of human EP_4 receptor gene (*A*) (62) and human IP receptor gene (*B*) (165) are shown.

been reported for isoforms of the human EP_3 and ovine FP receptors (94, 180). Because the carboxy-terminal domains of some EP_3 receptor isoforms are similar between species whereas others do not show such homology, the possible existence of at least seven EP_3 receptor isoforms in any given species has been proposed (186). In fact, eight human EP_3 receptor isoforms have recently been reported (115). These results may suggest the presence of other isoform(s) of the TP receptor, since there is no homology in the carboxy-terminal domains of the two human TP receptor isoforms and the mouse TP receptor. Another variant receptor has been reported in the rat EP_1 receptor (174). This variant receptor was generated by failure of splicing in the sixth transmembrane domain in exon 2 and is suggested to have a seventh transmembrane domain that is not homologous to the seventh transmembrane domain highly conserved among all members of the prostanoid receptors. When expressed in cultured cells, this receptor shows similar ligand binding specificity with the EP_1 receptor but is defective in signal transduction and suppresses signaling through other PGE receptors. The result that splice variants are found only in EP_1, EP_3, FP, and TP and not in DP, EP_2, EP_4, and IP coincides with the results of phylogenetic analyses of the prostanoid receptors, which show that the former and latter groups of receptors form different branches in receptor evolution (see sect. II*C*).

Analyses of the 5′-flanking region and the first intron of prostanoid receptor genes have revealed several consensus sequences in the *cis*-acting regulatory elements (Fig. 5). Basal promotor motifs for transcription such as the TATA box and CCAAT box have been identified in the 5′-flanking region of the transcription initiation site of some of the prostanoid receptor genes. The promotor regions of the mouse EP_4, the human EP_3, and the human EP_4 receptor genes have a TATA box, a TATA-like box, and two CCAAT boxes, respectively. On the other hand, the human TP, the human IP, the mouse FP, and the mouse EP_1 receptor genes lack these conventional motifs. A variety of other regulatory elements have also been found in the promotor region of the prostanoid receptor genes. The human TP receptor gene contains SP-1 binding sites, AP-2 consensus sequences, a phorbol ester response element (TRE), acute-phase reactant regulatory elements (APRRE), a c-*myc* binding motif, and a glucocorticoid response element (165). The human IP recep-

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tor gene shows AP-2 consensus sequences, APRRE, human polyoma virus JC promotor elements (JCV), c-*myb* binding motifs (c-Myb), an AP-1 binding site, SP-1 binding sites, and a glucocorticoid response element (GRE) (167). There is an AP-1 site and an AP-2 site in the mouse EP_1 receptor gene (15) and a *sis*-inducible factor (SIF) binding element, E boxes, AP-2 sites, an interferon (IFN)-γ responsive element (γ-IRE), a c-Myb, and a GC box in the human EP_3 receptor gene (115). The human EP_4 receptor gene contains several responsive motifs for proinflammatory agents such as NF-IL6, NFκB, and H-apf-1 in addition to a Y box, AP-1 sites, and AP-2 sites (62). The mouse EP_4 receptor gene contains AP-1 sites, AP-2 sites, SP-1 sites, an NFκB element, an E box (MyoD), an NF-IL6 element, a GRE, and Pit-1 sequences (8). The mouse EP_2 receptor gene contains NF-IL6 and NFκB elements as well as a progesterone response element (PRE) (104). These motifs are well correlated with the result that the EP_2 gene is regulated by both proinflammatory and hormonal stimuli. Interestingly, the transcriptional initiation sites of the EP_2 gene are different between the macrophage and the uterus, suggesting alternative promotor usage in these tissues (104).

The above findings suggest that the expression of prostanoid receptors is regulated by several factors through action on *cis*-acting regulatory elements of the respective receptor genes. However, only limited information is presently available regarding this regulation. As noted above, the promotor region of the TP receptor gene has a TRE (165). In accordance with this result, treatment with phorbol esters induced TP receptor expression in human erythroleukemia (HEL) cells, a megakaryocyte-like cell line (108, 150), and in CHRF-288 megakaryoblastic cells (57). On the other hand, Kinsella et al. (108) reported that glucocorticoids and inducers of the acute-phase response, such as interleukin (IL)-1, IL-6, lipopolysaccharide (LPS), C-reactive protein, and tumor necrosis factor (TNF), could not induce this gene expression in HEL cells, despite the presence of a GRE and APRRE in its promotor region. However, in contrast to HEL cells, glucocorticoids and IL-6 induced TP receptor expression in rat cultured vascular smooth muscle cells (224). These differences in the regulation of gene expression of the TP receptor may result from differences in species or cell types. Remarkably, Halushka and colleagues reported that testosterone induced gene expression of the TP receptor in HEL cells (129) and increased platelet thromboxane A_2 receptor density and the aggregation response in humans (4). Gene expression of the prostanoid receptors induced by phorbol esters was examined in cultured cell lines of monocytoid and lymphoid lineage (141). When these cells were stimulated by phorbol esters, EP_4 receptor gene expression was upregulated in THP-1 and U 937 cells (monocytoid cell lines), and Raji cells (B-cell line), and was downregulated in MOLT-4 and Jurkat cells (T-cell lines). This effect of phorbol esters on THP-1 cells was specific to the expression of the EP_4 receptor gene, and expression of the other prostanoid receptors, such as the EP_1, EP_2, EP_3, IP, and DP receptors, was not upregulated. EP_4 receptor expression was also upregulated in NIH 3T3 fibloblast and RAW 264.7 macrophage cell lines after stimulation with serum or bacterial LPS, respectively (8). Although EP_2 receptor expression was also upregulated in RAW 264.7 cells, that of the IP receptor was downregulated in both NIH 3T3 and RAW264.7 cell lines. Katsuyama et al. (100) showed that in the J774.1 macrophage cell line, LPS treatment augumented the expression of both EP_2 and EP_4, but the increase in EP_2 expression was much more drastic. Furthermore, the simultaneous addition of IFN-γ only inhibited LPS-induced expression of EP_2, but not that of EP_4. These results suggest a possibility that gene expression of prostanoid receptors is regulated in the body under various physiological and pathopysiological conditions. However, such in vivo regulation of receptor expression has not yet been demonstrated.

C. Molecular Evolution of the Prostanoid Receptors

The prostanoid receptors thus consist of eight types, each encoded by different genes. These receptors can be grouped into three categories on the basis of their signal transduction and action: the relaxant receptors, the contractile receptors, and the inhibitory receptors. The relaxant receptors, which mediate increases in cAMP and induce smooth muscle relaxation, consist of the IP, DP, EP_2, and EP_4 receptors. The contractile receptors consist of the TP, FP, and EP_1 receptors, which mediate Ca^{2+} mobilization and induce smooth muscle contraction. The EP_3 receptor is an inhibitory receptor that mediates decreases in cAMP and inhibits smooth muscle relaxation (for detailed discussion, see sect. II*B*). Sequence homology among these functionally related receptors is higher than those between the receptors from the three separate groups. The overall homology among the relaxant receptors is between 32 and 44%, and the homology in the transmembrane domains among contractile receptors is ~50%. In contrast, the homology of the EP_3 receptor with any receptor from the other two groups remains below 30%. Toh et al. (233) used a computer-assisted method and performed a more detailed sequence comparison of the prostanoid receptors. They also included receptors for other types of lipid mediators, namely, human and guinea pig platelet-activating factor (PAF) receptor (85, 151) and human lipoxin A receptor (61) in their analysis and constructed a phylogenetic tree to infer the evolutional relationship among the lipid mediators. They found that the prostanoid receptors constitute a distinct cluster

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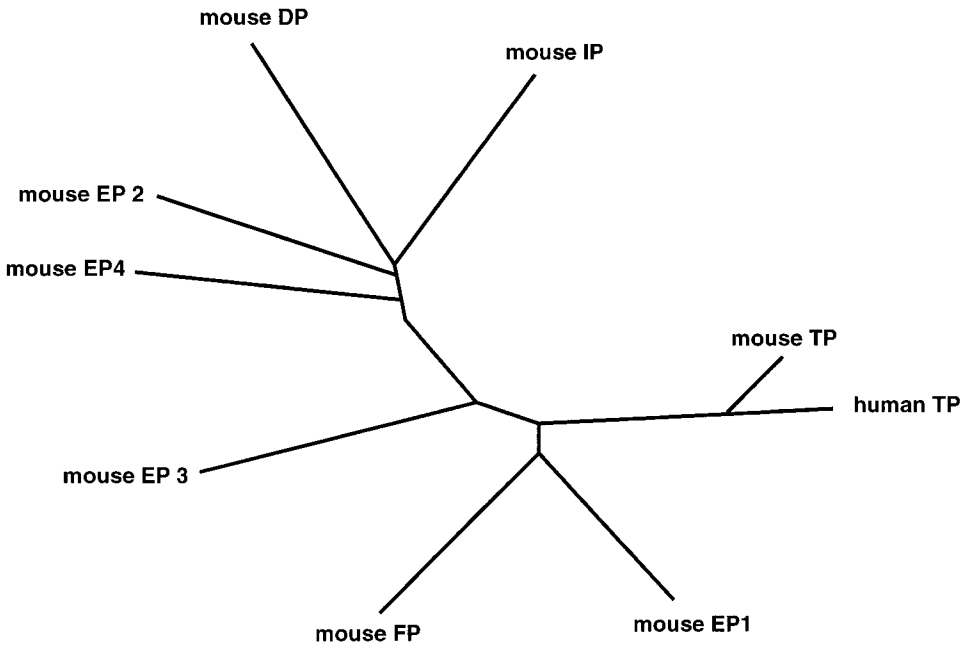

FIG. 6. Molecular evolution of prostanoid receptors. Phylogenetic tree of prostanoid receptors was constructed by sequence comparison of prostanoid receptors from various species.

within the rhodopsin-type receptors, while PAF and lipoxin receptors belong to another cluster shared by peptide receptors such as those for tachykinin, bradykinin, and endothelin. The prostanoid receptor cluster was further divided into three subclusters: cluster 1 consists of the relaxant receptors, EP_2, EP_4, IP and DP; cluster 2 consists of the contractile receptors EP_1, FP, and TP; and cluster 3 consists of the inhibitory receptor EP_3 (Fig. 6). A similar phylogenetic tree was reported by Reagan et al. (187) and Boie et al. (21). These results suggest that the cyclooxygenase pathway may have been initiated as a system composed of PGE and its receptor, where the subtypes of the PGE receptor then evolved from this primitive PGE receptor to mediate different signal transduction pathways and that receptors for other PG and Tx subsequently evolved from functionally related PGE receptor subtypes by gene duplication. The results also suggest that the prostanoid receptors evolved differently from receptors for other lipid mediators. Recently, Yokomizo et al. (252) reported the identification of the leukotriene B_4 receptor. This receptor is another G protein-coupled rhodopsin-type receptor that was previously isolated as an orphan chemoattractant receptor. It shows significant homology not only to the lipoxin A receptor but also, like the lipoxin receptor, to peptide receptors such as somatostatin receptor type 3 and the IL-8 receptor, but not to the prostanoid receptors. These findings suggest that the cyclooxygenase pathway and the lipoxygenase pathway may have evolved independently and then integrated into the arachidonate cascade.

III. PROPERTIES OF PROSTANOID RECEPTORS

A. Ligand-Binding Properties

Each of the eight types and subtypes of prostanoid receptors shows selective ligand-binding specificity that distinguishes it from the others. In previous studies, this specificity was mostly characterized by the selective responses of tissues to prostanoids and their analogs in a group of bioassay systems. Indeed, each of the prostanoid receptors was initially identified by its preferential responsiveness to a particular type of naturally occuring prostanoid and was subsequently characterized by various synthetic prostanoid analogs that have been synthesized in an attempt to selectively mimic or inhibit particular prostanoid actions. The chemical structures of PG analogs frequently used in such analyses are shown in Figure 7. For example, the EP receptor was initially characterized in the guinea pig ileum and fundus, dog fundus, chick ileum, and cat trachea as a site at which PGE_2 showed the most potent agonistic activity among the prostanoids (107). This receptor was then subdivided into two on the basis of its sensitivity to antagonism by SC-19220, one designated as the EP_1 receptor, exerting its action in the guinea pig ileum and the dog and guinea pig fundus, and the other in the cat trachea and chick ileum. The receptor in the cat trachea and chick ileum was then divided into EP_2 and EP_3 on the basis of their different sensitivities to AY23626 (11-deoxy-PGE_0) and sulprostone (45). The fourth receptor, EP_4, was later discovered as a receptor with smooth muscle relaxant effects similar to

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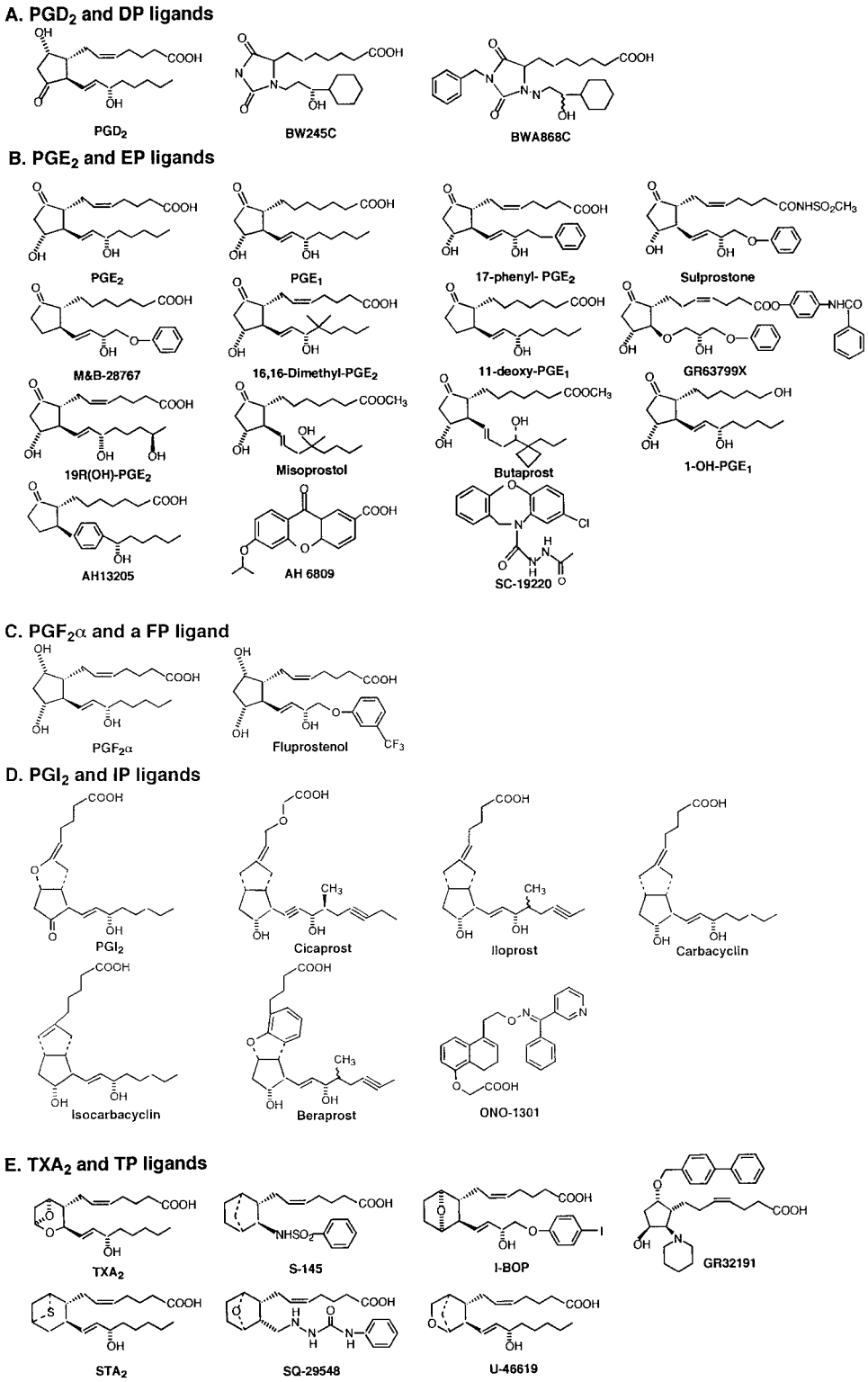

FIG. 7. Structures of synthetic prostanoid analogs. *A*: PGD_2 and DP ligands. *B*: PGE_2 and EP ligands. *C*: $PGF_{2\alpha}$ and a FP ligand. *D*: PGI_2 and IP ligands. *E*: TxA_2 and TP ligands. Binding affinities of these compounds to 8 types of mouse prostanoid receptors are shown in Table 1.

EP_2 but different from EP_2 on the basis of the weak potency of AH13205 and its antagonism by AH23848 (43). This approach of receptor characterization has thus been quite valid and successful. However, these studies only provided qualitative rather than quantitative information as to the ligand-binding properties of the receptors, firstly because most bioassay tissues contain more than one type of prostanoid receptor, and the compounds tested show summed action on various receptors, and also because the efficacy of action is different in different bioas-

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TABLE 1. *K_i values of various prostanoids and their analogs for the eight types of mouse prostanoid receptors expressed in cultured Chinese hamster ovary cells*

Ligands	DP	IP	TP	FP	EP_1	EP_2	EP_3	EP_4
PGD_2	21			47			280	
BW245C	250			1,700				
BWA868C	220							
Cicaprost		10			1,300		170	
Iloprost		11			21	1,600	27	2,300
Carbacyclin		110		1,200		1,600	31	2,300
Isocarbacyclin		15				1,000	31	
Beraprost		16					110	
ONO-1301		47					740	
S-145			0.7					
I-BOP			0.6	100		220	100	
GR-32191			13					
STA2	1,600		14	100		220	23	350
SQ-29548			13					
U-46619			67	1,000				
$PGF_{2\alpha}$				3	1,300		75	
Fluoprtostenol				4				
PGE_2				100	20	12	1	2
PGE_1		33			36	10	1	2
17-Phenyl-PGE_2				60	14		4	1,000
Sulprostone				580	21		0.6	
M&B-28767			1,300	124	120		0.7	500
16,16-Dimethyl-PGE_2				350		17	2	43
11-Deoxy-PGE_1					600	45	2	23
GR-63799X							2	480
19*R*(OH)-PGE_2				1,000				
Misoprostol					120	250	67	67
Butaprost						110		
1-OH-PGE_1							330	190
AH-13205						240	82	
AH-6809						350		
SC-19220								

Inhibitory constant (K_i) values are expressed in nM. When no value is given, K_i values were >3.3 μM. [Modified from Kiriyama et al. (109).]

say systems. Different degrees of responsiveness of the same receptor type in different species were also noted. Cloning of the prostanoid receptors has enabled the homogeneous expression of each type of receptor from the same species and made the evaluation of ligand-binding characteristics of each receptor as well as the cross-reactivity of prostanoid compounds over several types of receptors possible. Kiriyama et al. (109) used cultured cells expressing each of the eight types of mouse prostanoid receptors to examine the binding affinities of 33 prostanoids and their analogs to each receptor by determining the inhibition constants (K_i) values for the specific radioligand binding to the receptor. Such systematic analyses are not available for the receptors from other species. However, similar K_i values for radioligand receptor binding have been shown or can be calculated for some of the human receptors. These results are summarized below and are shown in Table 1.

1. DP receptor and DP ligands

The mouse DP receptor showed an affinity for ligands in the order of PGD_2 > BWA868C, BW245C > STA_2. Their K_i values were 21, 220, 250, and 1,600 nM, respectively. This order of affinity differs from the properties reported for the cloned human DP receptor, which showed almost equal ligand binding affinities for PGD_2, BW245C, and BWA868C at 1.1, 0.9, and 1.7 nM, respectively (21). On the contrary to this difference in binding affinities between the two species, agonist potencies of BW245C and PGD_2 are almost the same. They both act as full agonists for the DP receptor, and their EC_{50} values for cAMP elevation were 0.54 and 6.8 nM, respectively, for the mouse receptor and 0.7 and 6 nM, respectively, for the human receptor. A presumed DP receptor antagonist, BWA868C, can evoke a limited response, indicating that this compound is a partial agonist. Consistent with its K_i value for the human receptor, BW868C showed a pK_B of 8.7 for BW245C-induced relaxation in the rabbit jugular vein (67). Thus the DP receptor only binds its own putative ligands with high affinity. The binding affinities of other prostanoids and their analogs are at least more than two orders of magnitude lower than these compounds. On the contrary, PGD_2 bound to the mouse FP receptor with an affinity comparable to that for the mouse DP receptor,

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a K_i value of 47 nM (Table 1), indicating that PGD_2 may act on the FP receptor. In fact, PGD_2-induced bronchoconstriction in the anesthetized dog has been suggested to be mediated by the FP receptor (42).

2. EP_1 receptor and EP ligands

The rank order of affinity for the mouse EP_1 receptor was 17-phenyl-PGE_2, PGE_2, sulprostone, iloprost > PGE_1 > misoprostol, M&B-28767 > 11-deoxy-PGE_1 > $PGF_{2\alpha}$. Their K_i values were 14, 20, 21, 21, 36, 120, 120, 600, and 1,300 nM, respectively (Table 1). Some EP agonists such as 16,16-dimethyl-PGE_2, GR-63799X, butaprost, 1-OH-PGE_1, and AH13205 did not show any significant binding to this receptor. SC-19220 and AH-6809, known as antagonists for the EP_1 receptor (44), showed no affinity for the mouse EP_1 receptor. The human EP_1 receptor bound PGE_2 with a dissociation constant (K_d) value of 1 nM, and bound other PG in the rank order of PGE_2 > PGE_1 > $PGF_{2\alpha}$ >> PGD_2 (63). The human receptor also bound AH6809 with a calculated K_i of 333 nM. Weak affinities were also noted for SC19920 and butaprost; the respective K_i values were calculated at 4.5 and 33 μM. This species difference is important as these compounds are frequently used to determine if a action of PGE is mediated by the EP_1 receptor. It should also be mentioned that SC-19920 has a procainelike local anesthetic action (181). It is also noteworthy that 17-phenyl-PGE_2, which is considered to be a relatively specific agonist for the EP_1 receptor, bound to the mouse EP_3 receptor with a higher affinity than to the EP_1 receptor (Table 1).

3. EP_2 receptor and EP ligands

The rank order of affinity of the EP ligands for the mouse EP_2 receptor was PGE_1, PGE_2 > 16,16-dimethyl-PGE_2 > 11-deoxy-PGE_1 > butaprost > AH13205, misoprostol > AH-6809. Their K_i values were 10, 12, 17, 45, 110, 240, 250, and 350, respectively (Table 1). In addition, this receptor bound with low affinity to two TP ligands, I-BOP and STA_2, and one IP ligand, isocarbacyclin, with low affinity; their K_i values were 220, 220, and 1,000 nM, respectively. 19*R*(OH)-PGE_2 was reported to be a specific agonist for the EP_2 receptor (248). However, this ligand showed no affinity for the mouse EP_2 receptor and had only a weak affinity for the FP receptor. Butaprost showed affinity only to the EP_2 receptor, indicating its high selectivity for this receptor. No significant binding was observed with other EP agonists such as 17-phenyl-PGE_2, sulprostone, M&B-28767, GR63799X, or 1-OH-PGE_1. The human EP_2 receptor binds PGE_2 and PGE analogs similarly to the mouse receptor, with a rank order of PGE_2 > PGE_1 > 16,16-dimethyl-PGE_2 > 11-deoxy-PGE_1 > butaprost > AH13205, 19*R*(OH)-PGE_2>1-OH-PGE_1, M&B-28767 > sulprostone = 0. Prostaglandin E_2, 1-OH-PGE_1, AH13205, and butaprost work as full agonists of the human receptor with EC_{50} values of 43, 2,000, 3,100, and 5,800 nM, respectively.

4. EP_3 receptor and EP ligands

The mouse EP_3 receptor bound most of EP ligands with a rank order of affinity of sulprostone, M&B-28767, PGE_2, PGE_1, 11-deoxy-PGE_1, GR63799X, 16,16-dimethyl-PGE_2, 17-phenyl-PGE_2 > misoprostol, AH13205 > 1-OH-PGE_1. Their K_i values were 0.60, 0.68, 0.85, 1.1, 1.5, 1.9, 1.9, 3.7, 67, 82, and 330 nM, respectively (Table 1). In addition, the mouse EP_3 receptor bound three IP ligands, iloprost, carbacyclin, and isocarbacyclin, and one TP ligand, STA_2, with K_i values comparable to those for their respective receptors. This receptor also bound two other IP ligands, beraprost and cicaprost, with K_i values of 110 and 170 nM, respectively. Furthermore, this receptor bound $PGF_{2\alpha}$, I-BOP, and PGD_2 with K_i values of 75, 100, and 280 nM, respectively. These findings are in good agreement with the reported agonist order of potency of some of these compounds in rabbit cortical collecting tubule cells: PGE_2, PGE_1, 16,16-dimethyl-PGE_2 > carbacyclin, $PGF_{2\alpha}$ > PGD_2 (211). Although sulprostone, M&B-28767, 16,16-dimethyl-PGE_2, and 11-deoxy-PGE_1 also bound to other receptors, they showed the highest affinities for the EP_3 receptor (Table 1). Sulprostone showed affinities for both the EP_1 and FP receptors. M&B-28767, which is known as an EP_1 and EP_3 receptor agonist, also bound to the FP receptor with a K_i value of 124 nM. 16,16-Dimethyl-PGE_2 bound to the EP_2 and EP_4 receptors with the highest affinity out of all the PGE analogs. 11-Deoxy-PGE_1 showed affinities to the EP_2, EP_4, and FP receptors. Misoprostol, known as an EP_2 and EP_3 receptor agonist, showed K_i values of 118, 254, 66.8, and 66.8 nM for the EP_1, EP_2, EP_3, and EP_4 receptors, respectively (Table 1). AH13205, a known EP_2 agonist, also bound to the EP_3 receptor with a higher affinity. On the other hand, GR63799X showed high affinity only to the EP_3 receptor, indicating its high selectivity for this receptor. The human EP_3 receptor binds PGE_2 and M&B-28767 with K_d and K_i values of 0.7 and 0.2 nM, respectively (3). Curiously, this receptor also binds AH6809 with a calculated K_i of 1.3–4.7 nM. This is in contrast to the mouse EP_3 receptor that does not show binding affinity to AH6809.

5. EP_4 receptor and EP ligands

The rank order of affinity of the ligands for the mouse EP_4 receptor was PGE_2, PGE_1 > 11-deoxy-PGE_1, 16,16-dimethyl-PGE_2, misoprostol > 1-OH-PGE_1, GR63799X, M&B-28767 > 17-phenyl-PGE_2. Their K_i values were 1.9, 2.1, 23, 43, 67, 190, 480, 500, and 1,000 nM, respectively (Table 1). This rank order is in good agreement with previously reported findings. For example, the rank order of potency for the EP_4 receptor in the fetal rabbit ductus arteriosus was PGE_2 >> misoprostol > GR63799X >>

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AH13205, and the equieffective molar ratios of these ligands were 1, 145, 685, and >100,000, respectively (209). The values for the same ligands for the mouse EP_4 receptor were 1, 59, 294, and >2,000, respectively (Table 1). In addition to these EP ligands, STA_2 bound to this receptor with a K_i value of 350 nM. As for the human EP_4 receptor, only qualitative information based on a radioligand displacement experiment is available (13). This experiment showed the equal binding affinities of the human EP_4 receptor to PGE_2 and PGE_1 and a relatively high affinity to M&B-28767; their respective IC_{50} values were ~1 and 9 nM, respectively. This receptor showed only very weak affinity to butaprost and AH6809, with IC_{50} values of >10 μM.

6. FP receptor and FP ligands

The mouse FP receptor only bound to $PGF_{2\alpha}$ and fluprostenol with high affinity; their K_i values were 3.4 and 3.7 nM, respectively. Some prostanoids can cross-react with this receptor, but with at least a 10-fold lower affinity than the above two compounds, with a rank order of PGD_2, 17-phenyl-PGE_2 > STA_2, I-BOP, PGE_2, M&B-28767 > 16,16-dimethyl-PGE_2, sulprostone > U-46619, 19*R*(OH)-PGE_2. Their K_i values were 47, 60, 97, 100, 100, 124, 350, 580, 1,000, and 1,000 nM, respectively (Table 1). Fluprostenol could only bind to the FP receptor, indicating the high selectivity of this ligand. The result that a variety of non-FP ligands show relatively high binding affinities for this receptor indicates that the ligand-binding specificity of the FP receptor is broader than previously suspected. This is more marked in the human FP receptor than in the mouse and showed a similar rank of binding affinity of $PGF_{2\alpha}$, fluprostenol > PGD_2 > PGE_2 > U-46619 > iloprost, with respective calculated K_i values of 2.1, 2.7, 5.4, 65, 112, and 920 nM, respectively (2).

7. IP receptor and IP ligands

The rank order of affinity of the ligands for the mouse IP receptor was cicaprost, iloprost, isocarbacyclin > beraprost, PGE_1 > ONO1301 > carbacyclin > 11-deoxy-PGE_1. Their K_i values were 10, 11, 15, 16, 33, 47, 110, and 1000 nM, respectively (Table 1). A similar rank order of binding affinity was found in the human IP receptor, where iloprost >> carbacyclin > PGE_2 >> PGD_2, $PGF_{2\alpha}$, and U-46619 (20, 103). This is in good agreement with the reported rank order of potency of ligands, cicaprost, iloprost > carbacyclin, in platelets from several species (10). Isocarbacyclin, beraprost, and ONO-1301 also showed high affinities for the IP receptor, as previously reported (96, 228, 249). Interestingly, all of the IP ligands used in this study bound to the EP_3 receptor with K_i values ranging from 22 to 740 nM (Table 1). Among these ligands, iloprost, carbacyclin, and isocarbacyclin showed affinities comparable to those found for the IP receptor. This result suggests the possibility that IP ligands act on the EP_3 receptor. Cross-reaction of IP ligands on the EP_3 receptor was recently suggested in the presynaptic EP_3 receptor in guinea pig vas deferens (227). In fact, carbacyclin has been reported to act on the EP_3 receptor (211). Only iloprost could also bind to the EP_1 receptor (Table 1); the actions of this compound on the EP_1 receptor have already been reported (53).

8. TP receptor and TP ligands

The rank order of affinity of ligands for the mouse TP receptor was I-BOP, S-145 > GR32191, SQ29548, STA_2 > U-46619. Their K_i values were 0.56, 0.68, 13, 13, 14, and 67 nM, respectively (Table 1). This rank order and K_i values correspond well to previously reported results. For example, Morinelli et al. (145) reported a rank order of I-BOP > SQ29548 > STA_2 > U-46619, with respective IC_{50} values of 2.2, 4.7, 17, and 62 nM in ligand-binding competition experiments on human platelets. Other ligands known to act on other types of prostanoid receptors had no affinity for the TP receptor, except for M&B-28767; M&B-28767 bound to the receptor with a K_i value of 1,300 nM. It has been reported that PGD_2 and $PGF_{2\alpha}$-induced bronchoconstriction in humans is mediated by the TP receptor (46). It has also been reported that $PGF_{2\alpha}$ and PGE_2 contract the rat aortic ring via the TP receptor (56). However, PGD_2, $PGF_{2\alpha}$, and PGE_2 showed no affinity for the TP receptor in the mouse. Thus the TP receptor is quite specific for putative TP ligands. On the other hand, STA_2 bound to the EP_3, EP_2, and EP_4 receptors with K_i values of 23, 220, and 350 nM, respectively (Table 1), and I-BOP bound to the FP, EP_3, and EP_2 receptors with K_i values of 100, 100, and 220 nM, respectively (Table 1). Although there have been no reports stating that TP ligands act on these receptors, these results should be taken into consideration when performing experiments using these compounds.

B. Signal Transduction

Signal transduction pathways of prostanoid receptors have been studied by examining agonist-induced changes in the levels of second messengers (cAMP, free Ca^{2+}, and inositol phosphates), and by identifying G protein coupling by various methods. These results are summarized in Table 2. These studies, which combined the results from cultured cells expressing individual cloned prostanoid receptors and those obtained from native receptors in tissues, not only confirmed the previous biochemical findings in crude systems, but also revealed several novel characteristics of the prostanoid receptors.

Several species of G proteins have been reported to participate in signaling via the TP receptor. These proteins include G_q (204), G_q and an 85-kDa unidentified G

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TABLE 2. *Signal transduction of prostanoid receptors*

Type	Subtype	Isoform	G Protein	Second Messenger
DP			G_s	cAMP ↑
EP	EP_1		Unidentified	Ca^{2+} ↑
	EP_2		G_s	cAMP ↑
	EP_4		G_s	cAMP ↑
	EP_3	EP_{3A}	G_i	cAMP ↓
		EP_{3B}	G_s	cAMP ↑
		EP_{3C}	G_s	cAMP ↑
		EP_{3D}	G_i, G_s, G_q	cAMP ↓, cAMP ↑, PI response
FP			G_q	PI response
IP			G_s, G_q	cAMP ↑, PI response
TP		TPα	G_q, G_i	PI response, cAMP ↓
		TPβ	G_q, G_s	PI response, cAMP ↑

Data obtained from receptors of various species are summarized, and representative signal transduction of each receptor is shown. TP receptor isoforms were from humans, EP_3 receptor isoforms were from bovine, and other receptors were from mice. PI, phosphatidylinositol; ↑, increase; ↓, decrease.

protein (113), G_q and G_i2 (240), and G_{12} and G_{13} (166). There have been several pharmacological studies suggesting the heterogeneity of TP receptors, not only among various tissues (73, 168) but also within a single cell type, such as in the blood platelet (54, 223). The above observations, together with the existence of isoforms of the TP receptor described in the previous section, may explain the multiplicity of signal transduction pathways that are activated via this receptor. It is interesting in this respect that the recently identified TP receptor mutant in human platelets with a point mutation at Arg-60 (81) cannot induce aggregation but can induce shape change in platelets and the activation of phospholipase (PL) A_2 (65). Hirata et al. (82) examined the signal transduction pathways of the two splicing isoforms of TP. They found that both isoforms couple to PLC activation equally well, but couple oppositely to adenylate cyclase. One isoform, TPα, activates adenylate cyclase, whereas the other, TPβ, inhibits adenylate cyclase. The Arg60Leu mutation impairs PLC activation by both isoforms; it impairs adenylate cyclase activation by TPα but retains the ability of TPβ to inhibit the cyclase. On the basis of these findings, Hirata et al. (82) suggested that the pathway linked to adenylate cyclase inhibition, or other pathway(s) not affected by the above mutation of TP, may be involved in some of the TP-mediated platelet responses, such as shape change and PLA_2 activation. The cloned FP receptor is also coupled to the activation of PLC via G_q. Functional coupling of the FP receptor with G_q was shown by an experiment using anti-$G_q\alpha$ antibodies (92). In NIH 3T3 cells, $PGF_{2\alpha}$ induces DNA synthesis through this pathway (246). Although coupling was not observed with G_i or G_s in FP receptor-transfected Chinese hamster ovary (CHO) cells, $PGF_{2\alpha}$ has been shown to inhibit gonadotropin-stimulated cAMP formation in luteal cells (232).

The species of G protein to which EP_1 receptors are coupled remains unidentified. The EP_1 receptor mediates PGE_2-induced elevation of free Ca^{2+} concentration in CHO cells. This increase in free Ca^{2+} concentration was dependent on the availability of extracellular Ca^{2+} and accompanied by a barely detectable PI response (244). This observation suggests that the EP receptor may regulate Ca^{2+} channel gating via an unidentified G protein. The EP_2 and EP_4 receptors are coupled to G_s and mediate increases in cAMP concentration. The major signaling pathway of the EP_3 receptor is inhibition of adenylate cyclase via G_i. However, it is noteworthy that the splice variants of the EP_3 receptor described above are coupled to different signaling pathways and that one of these isoforms negatively regulates G protein activity. For example, the bovine EP_3A receptor is coupled to G_i and induces the inhibition of adenylate cyclase, whereas the EP_3B and EP_3C receptors are coupled to G_s and act to increase levels of cAMP. The EP_3D receptor is coupled to G_q, in addition to G_i and G_s, and evokes a pertussis toxin-insensitive PI response (154). Moreover, the bovine EP_3C receptor has been seen to demonstrate a novel type of receptor-G protein interaction, in addition to the conventional stimulation of G_s. When an agonist is bound to this receptor, the activity of G_o is directly inhibited due to an apparent increase in its affinity for GDP but not for GTP (160). The finding that the carboxy-terminal tail of a receptor has an important role in determining G protein coupling specificity explains the previously reported multiplicity of signal transduction pathways that reportedly operate via the EP_3 receptor (44, 69, 159). Such mechanisms where G protein specificity is determined by the carboxy-terminal tail may also work in the signal transduction of other rhodopsin-type receptors.

The IP receptor has been known to stimulate adenylate cyclase. However, expression studies revealed that it mediates not only a rise in cAMP levels but also PI responses (152). Prostaglandin I_2 has been reported to induce the elevation of free Ca^{2+} concentration in several lines of cultured cells (243, 247). IP receptor-induced PI responses in CHO cells were not inhibited by either pertussis toxin or cholera toxin, suggesting that the G_q family of G proteins is likely to be participating in this response (152). The DP receptor is coupled to G_s and mediates increases in cAMP concentrations. No PI response was observed in DP receptor signaling (21, 80), although the stimulation of the human DP receptor expressed in HEK 293 cells induced a transient increase in intracellular free Ca^{2+} concentration possibly via a cAMP system (21).

Curiously, in the expression systems of cloned receptors, some prostanoid-evoked responses occur at much lower ligand concentrations compared with their K_d values. For example, iloprost-stimulated elevations in cAMP levels, in IP receptor-transfected CHO cells, had an EC_{50} value of 100 pM (152). Similarly, PGE_2 decreases cAMP levels in EP_3 receptor-transfected CHO cells with an IC_{50}

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value of 100 pM (219). These values are 45- and 30-fold lower, respectively, than their K_d values for binding. The reason for this discrepancy is not clear, but it may reflect differences in the efficacy of postreceptor signal transduction mechanisms among various cells. In fact, variations in coupling efficacy of the IP receptor were observed in platelets of various species (10). In canine cortical collecting tubule cells, picomolar concentrations of PGE_2 antagonize vasopressin actions and induce a decrease in cAMP levels (177). Such differences in the efficacy of signaling are also seen between different signal transduction pathways via the same receptor. For example, the EC_{50} value of an iloprost-stimulated PI response in an expression system was 100 nM, which is three orders of magnitude higher than that of iloprost-stimulated elevations of cAMP levels.

C. Domains Involved in Ligand Binding and Signal Transduction

Domains and amino acid residues involved in ligand binding and signal transduction have been examined by creating mutant receptors by site-directed mutagenesis. Particular attention has been paid to the residues conserved in the prostanoid receptors and in the rhodopsin-type receptors in general. As descrived above, the seventh transmembrane region of the prostanoid receptors has a highly conserved motif, i.e., L-X-A-X-R-X-A-S/T-X-N-Q-I-L D-P-W-V-Y-I-L-X-R. Funk et al. (64) examined the role of the three amino acid residues, L291, R295 and W299, in this region of the human TP. The point mutants TP-W299L, R295Q, W299R and L291F all lost their binding to the antagonist [^{3}H]SQ-29548. In addition, three of the mutants, R295Q, W299R, and L291F, also failed to show binding to the agonist I-BOP, whereas W299L showed binding to I-BOP and another agonist, U-46619, with affinities indistiguishable from the wild-type receptor. These results demonstrated the importance of the seventh transmembrane domain in ligand binding to the thromboxane receptor and indicated that agonists and antagonists may be recognized differently. The importance of the arginine and other charged residues in the seventh transmembrane domain was examined also in the EP_3 receptor (11, 87). One study (11) showed that mutations of this arginine of rabbit EP_3, R329A and R329E, both abolished [^{3}H]PGE_2 binding to the receptor, whereas the mutation of an aspartic acid in this region, D338A, showed no alteration in ligand-binding properties. On the other hand, the D338A mutant was defective in signal transduction, showing no decreases in cAMP when activated with up to a 1 μM concentration of sulprostone, an EP_1/EP_3 agonist. The other study (87) showed that mutation of the corresponding arginine residue (R309) of mouse EP_3 to glutamate or valine also led to loss of the ligand binding, whereas mutation of this residue to lysine did produce the binding with higher affinity. These results indicate that the seventh transmembrane domain is involved in both ligand binding and transduction processes. The R295 of human TP, the R329 of rabbit EP_3, and the R309 of mouse EP_3 corrrespond to the arginine conserved by all the prostanoid receptors. Because modification of the carboxylic group of prostanoid molecules usually reduces the agonistic potencies of these compounds (see, for example, Ref. 201), discussion of these results led to the suggestion that this conserved arginine makes a charge-charge interaction with the carboxy moiety of the prostanoid ligand. However, the affinities of carboxy methyl esters of prostanoid molecules to their receptors differ from one receptor to another, and even in the same receptor from one species to another. For example, the K_i value of PGE_2 methyl ester for [^{3}H]PGE_2 binding to rabbit EP_3 is 1,600 nM, which is 1,000 times higher than that of PGE_2, 1.6 nM. On the other hand, its K_i value of the mouse $EP_{3\alpha}$ receptor is only 10 times higher than that of PGE_2 in the same receptor. These findings are difficult to interpret if the difference in the binding affinities between the free carboxylic acid and methyl ester is determined solely by interaction of the respective group with the arginine residue. This issue has been clarified by the work of Audoly and Breyer (12), in which they introduced point mutations into another conserved motif in the second extracellular loop of the rabbit EP_3 receptor and examined binding properties of the mutant receptors. When the first tryptophan (W199) or threonine residue (T202) in the sequence of Q^{198}-W-P-G-T-W-C-F was replaced by alanine, the affinity for carboxy methyl esters of PGE derivatives was increased by up to 128-fold, and the selectivity ratios comparing the K_i values of the methyl esters with the free carboxylic acids were reduced greatly from a few hundred times to ~10 times or less. The authors discussed these findings saying that the preference for free carboxylate derivatives is primarily determined by this region in the second extracellular loop, which may work itself as a part of a ligand-binding domain or as a filter or bait to the transmembrane binding pocket.

What then is the role of the conserved arginine in the seventh transmembrane region? Chang et al. (34, 35) pointed out that an arginine residue can form both ionic bonding and hydrogen bonding, acting as a hydrogen donor, and they evaluated the contribution of these two types of bonding by the conserved arginine, on ligand binding and transduction of the EP receptors. In one experiment, they examined the binding and potency of three compounds, PGE_2, PGE_2 methyl ester, and 1-OH-PGE_2 to the EP receptors. These compounds act as a negative charge, a hydrogen acceptor, and a hydrogen donor, respectively. They found that PGE_2 and the PGE_2 methyl ester showed almost the same potency to the four EP receptors, whereas the potency of 1-OH-PGE_2 was

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much lower, indicating that hydrogen bonding is enough for an agonist to exert its action. They also tested this hypothesis by mutating this charged arginine either to the noncharged but polar Gln or Asn, or to the nonpolar Leu, and examining the binding and potency of PGE_2 and its analogs to these mutant receptors. The affinity of PGE_2 was hardly affected by the mutations to Gln or Asn but decreased by ~40 times by the Leu substitution. These results suggest that, although the arginine can provide both ionic interactions and hydrogen bonding, the ionic interaction is not essential and the hydrogen bonding alone can support ligand binding. These results also indicate that some PG analogs with carboxy modifications interact with the receptors only via hydrogen bonding. Based on this hypothesis, Negishi et al. (158) compared the potencies of PGE_2 and sulprostone in the signal transduction of EP_3 to G_i and G_s. Although sulprostone showed equal potency to PGE_2 for G_i activation, its potency to activate G_s was more than 10 times weaker than PGE_2. Moreover, sulprostone showed the lower binding affinity to the G_s-coupled EP_3 receptor than to the G_i-coupled receptor, and PGE_2 failed to bind to the G_s-coupled EP_3D-R332Q mutant receptor and to activate its pathway. Although these authors indicate from these findings that the hydrogen bonding interaction may not be enough for signal transduction to G_s and suggest that the ligand-binding properties of the prostanoid receptors can be different depending on the species of G protein coupled to the receptor, the difference of the reactivity between PGE_2 and sulprostone to the G_s-coupled EP_3 receptor may be caused by interactions other than the C_1 moieties of these molecules and the arginine residue in the seventh transmembrane domain, because the sulfonamide of the C_1 moiety of sulprostone can be ionized at neutral pH.

Another question raised by several studies is whether the cysteine residue in the second extracellular loop forms a disulfide bond important for receptor structure. Audoly and Breyer (12) substituted alanine for Cys-204 in this region of rabbit EP_3 and found no change in the receptor's binding affinities for PGE_2 and its analogs. This is in contrast to the findings reported for TP (36, 47). In these studies, serine was substituted for different cysteine residues in the human TP receptor. Among the substitutions, substitution of Cys-105 in the first extracellular loop and Cys-183 in the second extracellular loop (which is at an analogous position to Cys-204 in rabbit EP_3) completely abolished ligand-binding activity. Because Cys residues at analogous positions are proposed to make a disulfide bond in other rhodopsin-type receptors (52) and because the TP receptor loses ligand-binding activity after reduction with dithiothreitol or sulfhydryl alkylation (55), these authors suggested that Cys-105 and Cys-183 make an essential disulfide bond. The authors also found that mutation of Cys-102 also affected the ligand-binding activity. In addition, D'Angelo et al. (47) further found that the Cys223Ser substitution retained ligand binding but abolished agonist-induced Ca^{2+} mobilization activity. Because this cysteine residue is not conserved by most of the other prostanoid receptors, the implication of this binding in the G protein coupling of other receptors remains obscure. On the other hand, the involvement of a single conserved amino acid residue in the G protein coupling of a prostanoid receptor has become apparent from a study on an inherited disorder. Hirata et al. (81) analyzed a hereditary bleeding disorder and found that it was associated with the Arg-60 to Leu mutation in the first intracellular loop of the human TP receptor. The receptor with this mutation showed normal binding properties but was defective in PI turnover. A subsequent study by the same authors (82) revealed that this mutation affected the PI turnover mediated by G_q in both TP receptor isoforms but did not inhibit the G_i-mediated decrease of adenylate cyclase in the β-isoform of this receptor, suggesting that this region is involved in coupling with G_q but not with G_i. This arginine is conserved at analogous positions in all of the prostanoid receptors. Which type of G protein coupling this arginine residue is involved with in each receptor is not known at present.

As described, each type and subtype of the prostanoid receptors shows specific ligand-binding properties, and the same receptor from different species sometimes shows different binding properties. Domains conferring ligand-binding specificity have been examined in chimeric receptors composed of two receptors with different selectivities. As described above, IP shows high-affinity binding to prostacyclin analogs such as iloprost and carbacyclin as well as PGE_1, but not to PGE_2 or other types of prostanoids, whereas DP shows selective binding to the type D PG. Kobayashi et al. (114) constructed chimeric DP/IP receptors and examined the domains conferring specificity to each receptor. The binding specificity of IP was found to be determined by recognition of both the ring structure and the side chain configuration and that the latter is primarily recognized by the sixth and seventh transmembrane regions, whereas the domain that recognizes the former seems to be located elsewhere and can accomodate the rings not only of PGI and PGE, but also of PGD. On the other hand, selective binding of DP appears to be determined by the first three transmembrane regions. A similar line of study was published recently by Kedzie et al. (105). These authors also made use of a high degree of homology between the relaxant group of prostanoid receptors. They introduced point mutations to amino acid residues conserved in the EP_2 and EP_4 receptor but not in the IP receptor and examined the residues conferring responsiveness to IP ligands. They found that a Leu304Tyr mutation in the seventh transmembrane region of the EP_2 receptor enhanced the potency of iloprost ~100-fold, almost equal to that of PGE_1. This may be consistent with the above proposal by Kobayashi et al.

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(114) that the sixth and seventh transmembrane regions are responsible for accomodation of the α-chain of IP ligands. A question still remains, however, as to the mechanism of the selectivity of the IP receptor, because IP can bind both iloprost and PGE_1 but not PGE_2, while their mutant receptor binds PGE_2 more preferentially than iloprost and PGE_1. Chimeric receptors have also been used to examine the amino acid residues responsible for ligand-binding difference among the receptors from different species. The rat TP binds the agonist I-BOP with about a 10-fold higher affinty than human TP. Dorn et al. (58) constructed chimeric rat/human TP receptors and examined the domain determining this property. They found that the portion from the amino terminus to an area in the first transmembrane region of the rat TP is enough to give this binding affinity. Because the amino acid sequence of this area is almost identical between the two receptors, differing in just 10 residues, they further examined which residues were responsible for the higher affinity of rat TP and found that the three residues in the first transmembrane region, Val-36, Val-37, and Ala40, are involved in this specificity.

IV. DISTRIBUTION OF PROSTANOID RECEPTORS

Previous pharmacological and biochemical studies have indicated that prostanoid receptors are expressed in many tissues in the body. The exact distribution of each receptor and the identities of cells expressing each receptor, however, remain mostly unknown because of the relatively low expression levels of these receptors and the expression of multiple receptors in a single tissue. Molecular biology has provided a new approach to overcome this problem. Techniques such as Northern blot analysis and in situ hybridization have provided detailed information about prostanoid receptor distribution. These analyses have shown that each receptor is specifically distributed in the body and that expression levels are variable among tissues.

A. DP Distribution

Among the prostanoid receptors, DP is the least abundant. In mice, it was expressed moderately only in the ileum and very weakly in the lung, stomach, and uterus (80). Consistently, only low levels of expresssion were detected in humans (21); on Northern blot analysis, its expression was detected only in the small intestine. Only low levels of expression of DP were detected in the brain of both species, in spite of the central actions of PGD_2 such as the induction of sleep (see sect. v, *A–C*). These results indicated that DP, if present, is expressed in limited areas or in specific cells in the brain. Oida et al. (169) examined this issue by performing an in situ hybridization study of DP mRNA expression in the mouse brain. Significant hybridization signals were only detected in the leptomeniges. No signal was detected in neurons or glia in the brain parenchyma. This was confirmed by Northern blot analysis showing that the 3.5-kb transcript of DP was significantly enriched in the leptomeninges, but not detected in the brain parenchyma. Thus their results suggest that DP is present in the leptomeninges and much less, if present at all, in the brain. Functional activity of the DP receptor in the leptomeninges was recently confirmed by Scammell et al. (200). They infused PGD_2 into the subarachnoid space below the basal forebrain, which is a procedure known to efficiently evoke sleep (see below), and identified cells activated by PGD_2 by staining Fos by immunohistochemistry. Intense Fos immunoreactivity was detected in the leptomeninges below the hypothalamus. Their findings suggested that the DP receptor indeed mediates a signal in the leptomeninges. Interestingly, Urade et al. (237) recently examined the localization of brain-specific PGD synthase by both immunohistochemistry and in situ hybridization and found that this enzyme is expressed abundantly in the leptomeninges and the choroid plexus. Thus the enzyme responsible for PGD_2 synthesis and the receptor for this PG are both expressed in the leptomeninges. These findings suggest that PGD_2 produced by cells in the leptomeninges acts in an autocrine or paracrine manner on cells in the leptomeninges. Interestingly, Matsumura et al. (130) examined the site of the sleep-inducing action of PGD_2 by microinjecting PGD_2 into various parts of the brain and found that it induces sleep more effectively when injected into the subarachnoid space than when injected into the brain parenchyma. From these studies, they concluded that PGD_2 acts on the ventral surface of the rostral basal forebrain. However, no expression of DP receptor mRNA was detected in the parenchyma of the brain in this area. On the other hand, the leptomeninges is markedly thick in this area, with high signal levels for DP receptor mRNA. It is possible, therefore, that PGD_2 injected into the subarachnoid space of this region works on the DP receptors in the leptomeninges to induce sleep. If this is the case, these observations suggest a new mode of regulation of brain function in which a humoral factor released by the leptomeninges is delivered to the brain and affects its function. The leptomeninges is located in a strategic position between the peripheral circulation and the brain and may mediate the transmission of blood-borne signals such as cytokines to the brain by this mode of regulation.

B. EP Distribution

Among the prostanoid receptors, the EP_3 and EP_4 receptors are widely distributed throughout the body, and

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their mRNA have been found to be expressed in almost all mouse tissues examined (84, 217). In contrast, the distribution of EP_1 mRNA is restricted to several organs, such as the kidney, lung, and stomach (244), and EP_2 is the least abundant among the EP receptors. However, EP_2 is effectively induced in response to stimuli. As described in section II*B*, EP_2 expression was upregulated by LPS in a macrophage cell line, and this augumentation was completely inhibited by the simultaneous administration of IFN-γ (100). As described below, EP_2 mRNA is also induced by gonadotropins in luminal epithelial cells in the uterus (102, 122).

In situ hybridization studies of PGE receptors in the kidney (25, 218) have revealed that the EP_3 receptor is expressed in the tubular epithelium in the outer medulla and possibly also in the thick ascending limb and cortical collecting ducts, the EP_1 receptor is expressed in the papillary collecting ducts, and the EP_4 receptor is expressed in the glomerulus. These distribution patterns appear to correspond with the PGE_2-mediated regulation of ion transport, water reabsorption, and glomerular filtration, respectively. However, there was no expression of EP_2 mRNA. A recent study revealed a similar distribution pattern of EP subtypes in the human kidney (26). Northern blot analysis showed that EP_3 mRNA was most abundantly expressed in the brain (217). In situ hybridization analysis revealed that EP_3 mRNA is widely distributed over the central nervous system (220). For example, EP_3 mRNA is expressed in the neurons of the cortex, hippocampus, thalamus, hypothalamus, midbrain, and lower brain stem. In the hypothalamus, EP_3 mRNA is expressed in the neurons surrounding the organum vasculosa lamina terminalis (OVLT). The OVLT has been regarded as a structure poor in the blood-brain barrier. Indeed, cyclooxygenase-2 (COX-2) is induced in this region in response to the peripheral administration of LPS (32). The EP_3 receptor in this region may be involved in fever generation. EP_3 mRNA is expressed in monoaminergic neurons in the brain stem, such as in the locus ceruleus (adrenergic), raphe nuclei (serotonergic), and substantia nigra (dopaminergic). EP_3 expressed in these neurons may function to modulate the autoregulation of monoaminergic neurons. Indeed, Momiyama et al. (140) found that EP_3 agonists depolarize the membrane by a cationic conductance, leading to the excitation of dorsal raphe neurons. In contrast, EP_4 mRNA is found only in neurons of the hypothalamus and lower brain stem, and EP_1 mRNA is found in neurons of the thalamus (Y. Sugimoto, unpublished data). The functions of these receptors in the central nervous system should be examined. On the other hand, EP_1, EP_3, and EP_4 mRNA are expressed in neurons of the dorsal root ganglion (DRG) (170, 220). EP_3 mRNA is expressed in one-half of the DRG neurons, which are largely small in size, suggesting the involvement of this receptor in PGE_2-mediated hyperalgesia. However, pain modulation by prostanoids is also closely associated with IP expression in DRG neurons, as described in sections IV*D* and V*B*.

The expression of EP receptor subtypes was also examined in the mouse gastrointestinal tract (144). EP_1 mRNA is expressed moderately in the muscularis mucosae layer of the stomach. It is also expressed by this layer in the esophagus and intestine, but the signal is much weaker. No signal was found in the smooth muscle proper layer, indicating that EP_1 may be involved in the local movement and folding of the mucosa. EP_3 mRNA is expressed by smooth muscle cells in longitudinal muscle throughout the gastrointestinal tract, but not in the circular muscle layer. In addition, high expression of EP_3 mRNA was found in neurons of the myenteric ganglia. These results indicate that EP_3 may regulate smooth muscle contraction both directly and indirectly through modulation of the enteric nervous system. The expression of EP_3 mRNA was also found in fundic gland epithelial cells, both in parietal cells and in chief cells. EP_3 has been suggested to be involved in acid secretion, and its localization in parietal cells is consistent with this proposal. On the other hand, EP_4 mRNA is highly expressed in the gland of the gastric antrum, indicating that this subtype is involved in PGE_2-mediated mucus secretion. In addition, the transcript of this receptor is also present in epithelial cells, especially those in the upper part of the villi, throughout the intestine. Because EP_4 is coupled to an increase in cAMP, and PGE_2 stimulates chloride secretion and inhibits NaCl absorption in the intestine through the production of cAMP (29), the above findings suggest that EP_4 is involved in these processes and consequently in PGE_2-induced diarrhea. This study did not detect EP_2 receptor mRNA in the gastrointestinal tract, suggesting that EP_2 is not expressed, or expressed at very low levels, in the digestive system.

In situ hybridization was also used to examine the cellular localization of mRNA for the EP receptor subtypes in the mouse uterus (102). The abundance and localization of mRNA for EP_2, EP_3, and EP_4 were found to change considerably when mice undergo pseudopregnancy by treatment with pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). EP_2 mRNA, which is hardly detectable before stimulation, is expressed considerably in luminal epithelial cells upon stimulation. This expression peaks at *day 5* of pregnancy and disappears quickly thereafter. Because this induction occurs in parallel with that of COX-2 in this tissue, and coincides with the time of blastocyst implantation, which is sensitive to indomethacin treatment, the authors concluded that EP_2 may be involved in the implantation process. EP_4 mRNA expression sharply increases on *day 3* of pseudopregnancy and is maintained at a high level after *day 5*. Its expression is limited to luminal epithelial cells at *day 0* but is also expressed in endometrial stromal

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cells and the glandular epithelium after induction. Prostaglandin E_2 has been reported to cause a phenotypic change in decidualization via elevation of cAMP levels. This may be mediated by EP_4 receptors induced in stromal cells. In contrast to the EP_2 and EP_4 receptors that are expressed in the endometrium, EP_3 receptor mRNA is expressed in smooth muscle layers. Its expression level is low on *day 1*, increases up to *day 5*, and then declines. The cellular localization of EP_3 mRNA changes during pseudopregnancy; it is expressed in the longitudinal muscle layer before stimulation, but in circular smooth muscles on *day 5*. Thus studying the uterine expression of EP receptors provides an interesting example of prostanoid receptor induction under physiological conditions. Both the amount and localization of receptors change during pregnancy, which may correspond to changes in uterine contraction during fertilization and implantation.

C. FP Distribution

As suggested by earlier ligand-binding studies (183, 184), the organ most abundantly expressing FP mRNA is the corpus luteum. The expression pattern of FP mRNA in luteal cells was examined in gonadotropin-primed pseudopregnant mice (76). No expression of FP mRNA was found in the ovaries from immature female mice treated with PMSG for 48 h. However, once ovulation was induced by hCG treatment, the granulosa cells in the ruptured follicles began to express FP mRNA. Interestingly, this expression level increased until the luteal cells underwent apoptosis. Thus the expression of FP mRNA in the copora lutea is variable during the estrous cycle, indicating a close relationship between FP gene expression and luteolysis. The mouse FP mRNA is also expressed in the kidney, heart, lung, and stomach, but expression in these tissues does not vary during the estrous cycle. Because $PGF_{2\alpha}$ is a physiological inducer of luteolysis in pregnancy as described in section v*D*, the mechanisms underlying luteal expression of FP mRNA are important key factors. Hasumoto et al. (75) recently focused on this issue; they generated transgenic mice with the potential promoter region of the FP gene (up to 7.3 kb upstream from the ATG) connected to the *lacZ* reporter gene. This region worked as a promoter for kidney and stomach expression, but not for luteal expression. These results suggest that a separate control mechanism exists for FP expression in the ovary, distinct from expression control in other tissues. This study also showed that FP mRNA is uniquely expressed in the cortical tubules of the kidney and in stomach glands. FP has been identified in fibroblastic cell lines such as NIH 3T3. In these cells, $PGF_{2\alpha}$ can act as a mitogen, possibly through the G_q-PLC pathway (246). Indeed, Arakawa et al. (8) recently found significant levels of FP mRNA expression in NIH 3T3 cells.

D. IP Distribution

The expression of IP mRNA has been examined by in situ hybridization in various mouse organs (170). IP mRNA is most abundantly expressed in neurons of the DRG, in which it was colocalized with the mRNA for preprotachykinin A, a precursor of substance P, indicating that IP may be involved in the mediation of pain. Interestingly, in some neurons, IP mRNA is coexpressed with the mRNA of EP receptor subtypes. This may suggest that EP and IP play overlapping or different roles in transmission of pain sensation. IP mRNA is also highly expressed in megakaryocytes and the smooth muscles of arteries, which is consistent with the action of PGI_2 in the cardiovascular system. No expression is found in the veins. In the kidney, it is also expressed by afferent arterioles of the glomerulus, indicating its role in regulation of the glomerular filtration rate. In the thymus and spleen, it is expressed by mature thymocytes and splenic lymphocyes. However, the function of IP in lymphocytes remains unknown.

E. TP Distribution

Northern blot analysis of mRNA expression in various mouse tissues showed that TP mRNA is expressed abundantly not only in tissues rich in vasculatures such as the lung, kidney, and heart, but also in immune-related organs such as the thymus and spleen (153). Ushikubi et al. (238) used the radioligand binding assay to examine cells expressing TP in the thymus. They found that immature thymocytes such as CD4−8− and CD4+8+ express TP at a density as high as that in platelets. Receptor levels decreased during T-cell maturation, but peripheral T cells still showed significant levels of TP expression. They moreover showed that a TP receptor agonist induced apoptotic cell death of immature thymocytes in a receptor-dependent manner. These observations suggest that the TP receptor may play a role in thymocyte differentiation and development, in addition to its well-known roles in the cardiovascular and respiratory systems.

V. KNOCKOUT MOUSE STUDIES OF PROSTANOID RECEPTORS

Prostanoids are presumed to play many important roles in a variety of physiological and pathophysiological processes in the body. The roles of prostaglandins have been suggested both by examining the effects of aspirin-like drugs that inhibit prostanoid production and by analyzing the in vitro and in vivo actions of each prostanoid added exogenously. However, it is not necessarily clear as to which type of prostanoid and which type of prostanoid receptor is involved in each process. Neither is it clear as

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TABLE 3. *Major phenotypes of mice deficient in prostanoid receptors*

Disrupted Gene	Major Phenotypes of Knockout Mice	Reference Nos.
DP	Not reported	
EP_1	Not reported	
EP_2	Impaired ovulation and fertilization	83, 106
	Salt-sensitive hypertension	
EP_3	Impaired febrile response to pyrogens	241
EP_4	Patent ductus arteriosus	162, 202
	Decreased inflammatory bone resorption	192, 221a
FP	Loss of parturition	221
IP	Thrombotic tendency, decreased inflammatory swelling	147
	Decreased acetic acid writhing	
TP	Bleeding tendency	231
		Murata et al., unpublished data

to how critical the actions of prostanoids are in each process. Mice deficient in each prostanoid receptor have been generated recently by the disruption of each receptor gene by homologous recombination, and initial analyses of IP-, FP-, EP_4-, EP_1-, EP_2-, EP_3-, and TP-deficient mice have been reported (Table 3). In this section, we summarize the reported findings of these knockout mice and discuss their significances. The potential usefulness of these knockout mice in further analyses is also suggested.

A. Central Nervous System Actions

1. Fever

Fever is a representative component of the acute-phase response to immunological challenge and is elicited by cellular components of infectious organisms, such as LPS, as well as by noninfectious inflammatory insults. Both exogenous pyrogens and noninfectious insults stimulate the production of cytokines that work as endogenous pyrogens. These cytokines, including IL-1, IL-6, TNF-α, INF-α, and INF-γ act on the preoptic area (POA), which then stimulates the neural pathways that raise body temperature (112, 195). Fever thus generated can be suppressed by nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin. Because these drugs share the ability to inhibit PG biosynthesis (242), it is assumed that PG are important in fever generation. There has been much debate on the identity of the PG that mediates fever. Milton and Wendlandt (136) were the first to suggest PGE_2 as a central mediator of fever. Indeed, PG of the E types are a powerful fever inducer when injected into the brain, and the level of PGE_2 increased in the POA during LPS-induced fever, and indomethacin completely abolished both LPS-induced fever and increased levels of PGE_2 in the POA (112, 136, 203, 206, 213). On the other hand, Mitchel et al. (137) argued against the role of PGE, based on the findings that some PGE antagonists did not inhibit cytokine-induced fever. Although studies on the involvement of PG in fever have thus focused on PGE_2, some investigators suggested the participation of other prostanoids in fever generation. Several brain regions in rabbits responded to microinjections of small doses of $PGF_{2\alpha}$, as well as PGE_2, by producing fever (143). Fever induced by the intracerebroventricular injection of $PGF_{2\alpha}$ differed from that induced by PGE_2 in several points (41, 143, 190), suggesting that $PGF_{2\alpha}$ may stimulate thermogenesis via different mechanisms from that of PGE_2. The intracerebroventricular administration of PGI_2 induces variable febrile responses among various species (5, 38, 68, 97, 98, 116, 205), whereas PGD_2 and TxA_2 do not evoke fever production (68, 206).

There has also been confusion as to the receptor type mediating the febrile response of PGE_2. For example, Oka and Hori (173) injected 17-phenyl-PGE_2, an EP_1 and EP_3 receptor agonist, into the lateral cerebroventricle (LCV) of the rat and found a rapid and dose-dependent rise in colonic temperature. In this experiment, no fever was elicited by injection of butaprost (an EP_2 receptor agonist), 11-deoxy-PGE_2 (an EP_2, EP_3, and EP_4 receptor agonist), and M&B-28767 (an EP_3 receptor agonist). Moreover, LCV injection with SC-19220, an EP_1 antagonist, blocked PGE_2-induced hyperthermia. On the basis of these results, they suggested that PGE_2-induced fever in the rat is mediated through the EP_1 receptor. On the other hand, in the prepubertal pig, 11-deoxy-PGE_2, butaprost, and GR63799 (an EP_3 receptor agonist) raised core temperature, whereas 17-phenyl-PGE_2 had no significant effect (176), suggesting that PGE_2-induced fever is mediated via either the EP_2, EP_3, or EP_4 receptor in the pig. These discrepancies may reflect species differences or may result from the cross-reactivities of the PGE analogs used in the experiments over several types of PGE receptors (see sect. III*A*).

Ushikubi et al. (241) used mice lacking each subtype of the PGE receptor to address the above issues. In this study, mice lacking each of the four subtypes of PGE receptor, EP_1, EP_2, EP_3, and EP_4, were generated by homologous recombination, and fever responses to PGE_2, IL-1β, and LPS were examined. They found that only the EP_3 receptor-deficient mice failed to show febrile responses to all of these stimuli. This study has thus clearly demonstrated that PGE_2 mediates fever generation in response to both exogenous and endogenous pyrogens by acting on the EP_3 receptor. Where in the brain then is PGE_2 formed, and where does it act? Because electrolytic lesions placed within the OVLT altered the febrile response to endogenous pyrogens, involvement of the OVLT, a circumventricular organ, in fever production was

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suggested (212, 213). The OVLT is located in the midline of the POA and lacks a blood-brain barrier; thus it is suitable as the site for circulating cytokines to act to produce PGE_2 for fever production. This is also consistent with the findings that the OVLT is the most sensitive area of the brain in producing fever in response to microinjection of PGE_2 (199, 214) and that neurons in the OVLT are sensitive to thermal and PGE_2 stimuli (128). Matsumura and co-workers (131, 133) used quantitative autoradiography to determine [^{3}H]PGE_2 binding sites in the rat preoptic-hypothalamic area and in the whole rat brain. They found the highest density of binding in the regions of the anterior wall of the third ventricle surrounding the OVLT and the nucleus tractus solitarius (NTS). Two thalamic nuclei (the paraventricular and anteroventral nuclei) and the dorsal parabrachial nucleus also contained a high density of [^{3}H]PGE_2 binding sites. These studies, however, could not determine which subtypes of the PGE receptor mediate this effect. Sugimoto et al. (220) clarified this point by performing in situ hybridization analyses on expression of PGE receptor subtypes in the mouse brain (see also sect. IV*B*). They found that although the mRNA for the EP_3 receptor was widely distributed in the brain, it was particularly abundant in the regions surrounding the OVLT. Analogous examination of the EP_1 receptor mRNA showed abundant signals in the paraventricular nucleus of the hypothalamus (PVH) and supraoptic nucleus (15).

Scammell et al. (199) studied the neuronal pathways activated in association with PGE_2-mediated fever by examining Fos induction after the microinjection of PGE_2 into the ventromedial preoptic area (VMPO). They found Fos induction in the VMPO and the autonomic regulatory and corticotropin-releasing hormone (CRH)-producing subdivisions of the PVH. The PVH projects directly to preganglionic sympathetic and parasympathetic neurons, as well as to sympathetic premotor sites in the parabrachial nucleus, ventrolateral medulla (VLM), and NTS. Thus it was suggested that the VMPO, through its connections with the PVH, contributes to increased sympathetic activity and redistributes the blood flow required for the production of fever.

Although PGE_2 has a central role in the developement of fever, IL-8 and the macrophage inflammatory protein-1β have been reported to induce fever through prostaglandin-independent pathways (9, 254). However, the physiological roles of these cytokines in fever production have not yet been fully chracterized. Recently, a role for nitric oxide (NO) in fever production was reported (188). Lipopolysaccharide- or IL-1β-induced fever was blocked by NO synthase inhibitors preinjected into the OVLT, and intra-OVLT injection of NO donors induced a febrile response, which was inhibited by indomethacin pretreatment. However, NO synthase inhibitors could not block PGE_2-induced fever (124, 125). These results suggest that NO produced by pyrogens acts by inducing the production of PGE_2.

2. Hypothalamic-pituitary-adrenal axis

In addition to fever, stimulation of the hypothalamic-pituitary-adrenal (HPA) axis is another component of the acute-phase response (118). The immune system and the HPA axis reciprocally interact with each other. Thus cytokines produced from the immune system regulate the HPA axis, and corticosteroids produced from the adrenal gland in response modulate the function of the immune system (14). Among the cytokines, IL-1 has been shown to induce secretion of ACTH (17). Although the mechanism of the ACTH response induced by IL-1 is still largely unknown, it is sensitive to treatement with aspirin-like drugs (99), and the corticotropin-releasing factor (CRF) is involved in the mediation of this response (16, 196, 234). It has been reported that intrahypothalamic injection of PGE_2 stimulates the secretion of ACTH (142) and that this ACTH response is significantly suppressed by pretreatment with anti-CRF antibodies (245). It has also been reported that the OVLT, which is thought to be the site of action of pyrogenic cytokines for the production of PGE_2, was involved in IL-1β-induced ACTH release, which was mediated by the production of PGE_2 (99). These results suggest that, as in the case for fever generation, PGE_2 formed in the OVLT in response to IL-1 is involved in ACTH release and that CRF further mediates this action of PGE_2. However, the subtype(s) of the EP receptor mediating this action has not yet been clarified.

3. Sleep

Prostaglandin D_2 has been proposed as one of the endogenous sleep-promoting substances in rats and other mammals, including humans (78). As discussed in section IV*A*, DP is present in the leptomeninges but not in the brain (169), and PGD synthase is expressed abundantly in the leptomeninges and the choroid plexus (237). The leptomeninges is located in a strategic position between the peripheral circulation and the brain, and the PGD_2-DP system there may mediate the transmission of blood-borne signals such as cytokines to the brain. DP-deficient mice may help to answer under which conditions this system is activated to evoke PGD_2-induced sleep.

B. Inflammation, Pain, and Immunity

Local reddening, heat generation, swelling, and pain are classic signs of acute inflammation, of which the former three are caused by increased blood flow and vascular permeability with resultant edema. Previous studies suggested that PG are primarily involved in vasodilation in the inflammatory process and synergize with

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other mediators such as histamine and bradykinin to cause an increase in vascular permeability and edema. These studies also showed that PGE_2 and PGI_2 are most powerful in this action and that both PG are present at high concentrations at inflammation sites (reviewed by Davies et al., Ref. 49). Murata et al. (147) used IP-deficient mice to test the role of PGI_2 in inflammatory swelling. They employed a carageenan-induced paw swelling as a model. In this model, swelling increased in a time-dependent manner up to 6 h after injection. Indomethacin treatment decreased the swelling by ~50%. IP-deficient mice developed swelling only to levels comparable to those observed in indomethacin-treated wild-type mice, and indomethacin treatment of IP-deficient animals did not induce a further decrease in swelling. On the other hand, PGE_2 injected intradermally could synergize with bradykinin to induce increased vascular permeability in both wild-type and IP-deficient mice. These results indicate that PGI_2 and the IP receptor work as the principal PG system mediating vascular changes in this model of inflammation. Whether PGI_2 and the IP receptor play a dominant role in any type of inflammation remains to be seen. An alternative and more likely possibility is that this system and the PGE_2 and EP receptor system are utilized in a context-dependent manner, i.e., depending on the stimulus, site, and time of inflammation. This point will be clarified by comparing responses in IP-deficient mice with those in mice deficient in each subtype of the EP receptors in various inflammation models.

The role of prostaglandins in inflammatory pain is also well accepted. This is partly due to the antinociceptive effects of aspirin-like drugs, and also because of documentation in various model systems that PG added exogenously are able to induce hyperalgesic responses or increase sensitivity to touch. These studies using exogenous PG showed that PGE_2, PGE_1, and PGI_2 exert stronger effects than the other types of PG, indicating the involvement of the EP or IP receptor in inducing inflammatory pain (reviewed in Ref. 19). The main site of prostanoid action lies in the periphery, in which prostaglandins are believed to sensitize the free end of sensory neurons. However, recent studies demonstrated that PG have additional sites of action both in the spinal cord and in the brain to elicit hyperalgesia. Malmberg and Yaksh (127) showed that the spinal injection of nonsteroidal anti-inflammatory drugs into rats inhibits thermal hyperalgesia induced by the activation of spinal glutamate and substance P receptors. Oka et al. (172) also reported that the intracerebroventricular injection of PGE_2 induces thermal hyperalgesia in rats. The primary sensory afferents have their cell bodies in the DRG, and as described in section IV, several types of prostanoid receptor mRNA, including those of IP, EP_1, EP_3, and EP_4, were found in neurons in the ganglion (166, 220), suggesting their possible involvement. Notably, some IP-expressing neurons also express preprotachykinin A mRNA, indicating a role of IP in nociception via substance P-containing afferents (170). Murata et al. (147) used IP receptor-deficient mice to address this issue. The IP-deficient mice did not show any alteration in their nociceptive reflexes examined by hot plate and tail flick tests, indicating that PGI_2 is not involved in nociceptive neurotransmission at the spinal and supraspinal levels. On the other hand, when these mice were subjected to the acetic acid-induced writhing test, they showed markedly decreased responses compared with control wild-type mice, and their responses were as low as those observed in control mice treated with indomethacin. Additionally, both PGE_2 and PGI_2 injected intraperitoneally induced modest writhing responses in wild-type mice, whereas IP-deficient mice showed responses only to PGE_2. These results indicate that the hyperalgesic response in this model is evoked by endogenous PGI_2 acting on the IP receptor in the peripheral end of nociceptive afferents. This study, together with other reports that PGI_2 or its agonists are more effective in eliciting nociception in several model systems, has led to the proposal that IP has a definitive role in the facilitation of pain sensation (19). However, the involvement of EP receptors in this process cannot be overlooked. A recent study using a selective, neutralizing monoclonal antibody against PGE_2 showed that it inhibits phenylbenzoquinone-induced writhing in mice and carageenan-induced paw hyperalgesia in rats to the same extent as indomethacin (139, 182). Whether PGE_2 also elicits hyperalgesic actions by acting on peripheral EP receptors under diffrent conditions, or conveys other sensory signals such as allodynia, should be tested using knockout mice deficient in each of the EP receptor subtype. It should also be tested whether any of the EP and IP receptors expressed in dorsal ganglion neurons has a modulatory role in sensory neurotransmission in the spinal cord. Indeed, an autoradiographic study detected a high density of $[^3H]PGE_2$ binding sites in the dorsal horn, which was abolished by dorsal rhizotomy (132). Finally, the applicability of the knowledge obtained from studies in mice to humans awaits the availability of selective antagonists to EP and IP receptors and examination of the effects of the specific inhibition of each receptor in various clinical situations.

In addition to acute inflammation, PG are likely to play physiological roles in the regulation of immunity and allergy. As described, TP and IP receptors were found to be expressed highly by immature and mature thymocytes, respectively (170, 238). Furthermore, a TxA_2 mimetic was shown to induce apoptosis of immature thymocytes in vitro, leading to the suggestion that the TxA_2 and TP system may have an antigen-dependent immunomodulatory role (238). In this respect, it would be interesting to see whether any abnormality is found in the immunity of TP- and IP-deficient mice. Compared with TxA_2 and PGI_2,

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the type E PG have long attracted attention of immunologists because of its potent immunosuppressive actions. For example, it induces apoptosis of thymocytes and inhibits some T-cell functions such as production of IL-2 (134). Recently, however, PGE_2 has been shown to activate the Th-2 subset of T cells, while suppressing the Th-1 subset (178). Moreover, it has been reported that PGE_2 acts on the EP_2 and/or EP_4 receptor of B cells and synergizes with LPS or a Th-2 cytokine, IL-4, to facilitate IgE production (60). These findings led to the proposal that PGE_2 may work as a switch for Th2-mediated allergic responses. Whether such a mechanism operates under physiological conditions will be tested in mice deficient in each of the EP receptors. Another PG likely to play a role in allergic reactions is PGD_2. One of the major sources of PGD_2 in the body is mast cells, and they release a large amount of PGD_2 upon immunologic challenge (120). However, whether it facilitates, modifies, or downregulates allergic reactions remains obscure, and studies on DP-deficient mice should help to clarify this point.

C. Vascular Homeostasis

1. Thrombosis and hemostasis

Most PG elicit contractile and/or relaxing activities on vascular smooth muscles in vitro and in vivo. In particular, PGI_2 and TxA_2, produced abundantly by vascular endothelial cells and platelets, respectively, are a potent vasodilator and vasoconstrictor, respectively. It is therefore interesting to study how these PG contribute to the regulation of the cardiovascular system. Murata et al. (147) created mice the IP receptor gene of which was disrupted by homologous recombination and found that while IP-deficient mice lack the hypotensive response to the synthetic IP agonist cicaprost, their basal blood pressure and heart rate were not different from those of control animals. This is in contrast to what was observed in mice lacking another endothelium-derived vasorelaxant, NO (88). Mice deficient in the endothelial type of NO synthase showed elevated basal blood pressure. These results indicate that the PGI_2 and IP system does not work constitutively in regulation of the systemic circulation, and more likely works on demand in response to local stimuli. Prostaglandin I_2 and TxA_2 also act on platelets to inhibit or induce, respectively, platelet activation and aggregation. Because of their opposite actions on blood vessels and platelets, it has been proposed that the balance of the PGI_2 and TxA_2 systems is important for maintaining vascular homeostasis, i.e., to prevent thrombosis and vasospasm while performing efficient hemostasis. A study on IP-deficient mice showed that they develop and age normally. No increased occurrence of vascular accidents was observed. This suggests that in the absence of other predisposing factors, mice can survive safely without the action of PGI_2. However, an enhanced thrombotic tendency was observed in IP-deficient mice when endothelial damage was evoked. These findings confirmed the long proposed role of PGI_2 as an endogenous antithrombotic agent and suggest that this antithrombotic system is activated in response to vascular injury to minimize its effects. Contrary to PGI_2, TxA_2 has been implicated in thrombosis and hemostasis on the basis of its proaggregatory and vasocontractile activities. Recently, the analysis of TP-deficient mice has been reported. TP-deficient mice showed increased bleeding tendency and were resistant to cardiovascular shock induced by intravenous infusion of a TP agonist, U-46619, and arachidonic acid (231) (T. Murata, F. Ushikubi, and S. Narumiya, unpublished data). The increased bleeding tendency was also noted in patients with a TP receptor abnormality (81). This abnormality is caused by a mutation of Arg→Leu in the first cytoplasmic loop of the receptor, which impairs coupling of the receptor to the G_q protein but does not affect its coupling to G_i (see sect. III*B*; Ref. 82). Platelets from patients homozygous in this mutation showed no aggregation in response to TxA_2. These results suggest that the TxA_2 and TP system indeed plays a physiological role in hemostasis.

2. Hypertension

Prostaglandin E_2 also elicits contractile and/or relaxant responses of vascular smooth muscles in vitro. Kennedy et al. (106) administered PGE_2 and PGE analogs intravenously into wild-type and $EP_2-/-$ mice and examined the response in vivo. They observed that infusion of PGE_2 or an EP_2 agonist, butaprost, induces a transient hypotension in wild-type mice, whereas injection of an $EP_{1/3}$ agonist, sulprostone, resulted in an increase in mean arterial pressue. The hypotensive response to butaprost was not observed, and the hypertensive effects of sulprostone persisted in $EP_2-/-$ mice, and, surprisingly, PGE_2 evoked considerable hypertension. The authors discussed that the absence of the EP_2 receptor abolishes the ability of the mouse vasculature to vasodilate in response to PGE_2 and unmasks the contractile response via the vasoconstrictor EP receptor(s). Interestingly, when fed on a high-salt diet, the $EP_2-/-$ mice develop significant hypertension with concomitant increase in urinary excretion of PGE_2. These results indicate that PGE_2 is produced in the body in response to a high-salt diet and work to negatively regulate the blood pressure via the relaxant EP_2 receptor and that the dysfunction of this pathway may be involved in elicitation of salt-sensitive hypertension.

3. Ductus arteriosus

At birth, mammals including humans undergo a dramatic change in their circulation with the commencement of respiration, i.e., from the fetal circulation system that shunts the blood flow from the main pulmonary artery directly to the aorta via the ductus arteriosus, to the

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pulmonary circulation system in the neonate. This adaptive change is caused by the closure of the ductus. The patency of the ductus during the fetal period is supposed to be maintained principally by the dilator effects of a prostaglandin, and its closure is induced by withdrawal of the dilator prostaglandins as well as active contraction exerted by increased oxygen tension (208). This is supported by the fact that administration of aspirin-like drugs to a mother induced the premature contraction of the ductus in the fetus of various animals and that aspirin-like drugs or vasodilator PG such as PGE_1 are used, respectively, to suppress or to maintain the patency of the ductus in neonates with patent ductus arteriosus (40, 208). Vasodilator prostaglandins responsible for the patency of the ductus were examined by comparing the relaxing potencies of various prostaglandins on isolated preparations of the ductus arteriosus from various species. Some of these studies reported that PGE_2 is 1,000 times more potent than PGI_2 in dilating the lamb ductus precontracted with indomethacin, suggesting involvement of an EP receptor (39, 40). On the other hand, other studies showed that PGI_2 is the main arachidonate product of the ductus (230) and that cicaprost, a stable and selective PGI_2 mimetic, is only 20 times less potent than PGE_2, exerting a maximal relaxation greater than that attained by PGE_2, suggesting a possible role of IP in this process (209). A study using various synthetic PG analogs suggested that both IP and EP_4 are present in the ductus and work in the dilation of this vessel (209). The presence of the EP_4 receptor was confirmed by in situ hybridization of its mRNA in the mouse (162, 202). Disruption of the mouse IP gene did not appear to cause any abnormality of the ductus (147). On the other hand, the disruption of the EP_4 receptor gene resulted in the death of most homozygous $EP_4-/-$ neonates within 3 days after birth, due to marked pulmonary congestion and heart failure (162, 202). Contrary to our expectations, these animals did not show premature closure but showed full patency of the ductus after birth. These results suggest a critical role of EP_4 in the ductus and can be interpreted that the compensatory mechanism in its absence maintains ductus its patency not only in the fetal period but also after birth.

D. Reproduction

The F and E types of PG are implicated in many aspects of reproductive functions. These include not only the peripheral reproductive processes but also gonadotropin secretion in the central nervous system. To date, it has been accepted that luteinizing hormone-releasing hormone is secreted by the hypothalamus in response to PGE_2 (171). In the ovary, the PG content of follicles increases as the follicles mature. Indomethacin abolishes luteinizing hormone-induced ovulation, and this effect is reversed by treatment with PGE_2 or $PGF_{2\alpha}$ (148). Prostaglandin $F_{2\alpha}$ has been accepted as an inducer of luteolysis in the estrous cycle. Luteolysis is the failure of the corpus luteum to secrete progesterone. In domestic animals such as the sheep and cow, luteolysis is evoked by a substance in blood flow from the uterus that contains $PGF_{2\alpha}$, inhibited by treatment with aspirin-like drugs, and this inhibition is reversed by the addition of exogenous $PGF_{2\alpha}$ (86, 135). However, species differences in the actions of $PGF_{2\alpha}$ and in the effects of hysterectomy on the estrous cycle exist, raising the question regarding the universality of $PGF_{2\alpha}$ action on luteolysis. It has therefore remained controversial as to what role the luteolytic action of $PGF_{2\alpha}$ plays in the reproductive functions in each species, including the human. The most familiar actions of PG in reproduction are those on reproductive tract motility. Changes in the motility of the reproductive tract affect processes leading to the evacuation of the uterine contents, such as abortion and parturition. Aspirin-like drugs are known to delay parturition in many species including humans (33, 121). Because $PGF_{2\alpha}$ is the dominant PG found in intrauterine tissues during late pregnancy, and is a strong uterotonic substance, this PG has been thought to play important roles in parturition with its contractile actions (89, 207). However, its exact role in this process has not yet been defined. Thus a number of studies have been carried out in this field, but there is no clear evidence indicating that endogenous PG have physiological roles in these processes.

Recent studies on COX-2-deficient mice showed multiple reproductive failures in early pregnancy, such as in ovulation, fertilization, implantation, and decidualization, suggesting that PG play essential roles in these processes (51, 123). Because IP-, EP_1-, EP_3-, EP_4-, and TP-deficient females are fertile, these receptors may be dispensable in female reproduction. Likewise, ovulation, fertilization, and implantation are normal in mice lacking the FP receptor gene (221), suggesting that $PGF_{2\alpha}$ is not crucial for these processes. Quite recently, two groups reported failure in early pregnancy in $EP_2-/-$ female mice (83, 106). Kennedy et al. (106) found that $EP_2-/-$ female mice consistently deliver fewer pups than their wild-type counterparts irrespectively of the genotypes of mating males. They detected slightly impaired ovulation and dramatic reduction in fertilization in $EP_2-/-$ mice and concluded that failure in early pregnancy in COX-2$-/-$ mice is due to dysfunction of the EP_2 receptor. Hizaki et al. (83) observed the identical phenotype in the $EP_2-/-$ mice they independently generated and further found that this phenotype is due to impaired expansion of cumulus of oophorus. Because the EP_2 receptor and COX-2 are induced in the cumulus in response to gonadotropins and PGE_2 can induce cumulus expansion by elevating cAMP, these authors suggest that the PGE_2 and EP_2 receptor system work as a positive-feedback loop to induce oopho-

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rus maturation required for fertilization during and after ovulation.

As described above, the FP−/− mice did not show any abnormality in early pregnancy and any change in the estrous cycle. The latter finding appears enigmatic at the first glance, since the FP receptor is abundantly expressed in the corpora lutea in the ovary of mice with normal estrous cycles (219), and the expression of FP mRNA is closely associated with luteal cell apoptosis in pseudopregnancy (76). This may be probably due to the fact that luteolysis is not required for entrance into a new estrous cycle in mice and their ovaries contain corpora lutea from a few previous estrous cycles. Thus $PGF_{2\alpha}$ synthesis and action are not synchronized with the estrous cycle of mice, although the FP receptor is still present in the corpora lutea in these animals (119, 189). Sugimoto et al. (221) found that, despite no alteration in the above processes, FP-deficient mothers do not perform parturition, apparently due to the lack of labor. They further found that FP-deficient mice do not undergo parturition even when given exogenous oxytocin and show no prepartum decline in progesterone. A reduction in progesterone levels by ovariectomy 24 h before term caused an upregulation of uterine receptors for oxytocin and normal parturition in FP-deficient mice. These experiments indicate that luteolytic action of $PGF_{2\alpha}$ is required in mice to diminish progesterone levels and thus permit the initiation of labor. They also indicate that the uterotonic action of $PGF_{2\alpha}$ in myometrium is not essential for parturition. Although uterine oxytocin receptors were upregulated in conjunction with labor in mice, it is not clear whether oxytocin itself is essential for labor in mice, since parturition is normal in oxytocin-deficient mice, although they fail to lactate (164, 253). It has been shown in many species that a large amount of PG are produced in intrauterine tissues during labor, but their exact roles remain unknown at present. The luteolytic role for $PGF_{2\alpha}$ in the induction of labor in mice is supported by the finding that mice lacking the gene encoding cytosolic PLA_2 also had abnormal parturition (23, 236). The observed reduction in PG production in these mice is in keeping with the key role of this PLA_2 species in cleaving the PG precursor arachidonic acid from phospholipids. Moreover, the administration of a progesterone receptor antagonist (RU-486) at term to substitute for the luteolytic decline in progesterone corrected the defect in labor seen in the PLA_2-deficient mice (236).

E. Bone

Bones undergo continuous destruction and renewal, a process termed bone remodeling. Bone resorption in the former process is carried out by osteoclasts, and bone formation in the latter process by osteoblasts. These processes are controlled by systemic humoral factors such as parathyroid hormone, estradiol, and vitamin D as well as by local cytokines such as IL-1β, IL-6, and insulin-like growth factor I. Prostaglandin, particularly type E, can also affect bone remodeling, in both bone formation and resorption. The bone resorptive activity of PGE was first noted in vitro in bone organ cultures (111) and then in vivo in animals receiving systemic as well as local injections of PG (see, for example, Ref. 194). This is associated with the occurrence of an increase in the number of osteoclasts. Osteoclasts develop from precursor cells of macrophage lineage in the microenvironment of the bone. A recent study by Yasuda et al. (251) revealed that humoral bone-resorbing factors such as parathyroid hormone, vitamin D, IL-1, and IL-6 act first on osteoblasts to induce formation of the osteoclast differentiation factor, a member of the membrane-bound TNF ligand family, which then stimulates the formation of mature osteoclasts from hematopoietic precursors by cell-cell interaction. Interestingly, IL-1 induces COX-2 in osteoblasts to release PGE_2, and osteoclast induction by IL-1 is inhibited by aspirin-like drugs, indicating the obligatory role of PGE_2 in this process (222). Sakuma et al. (192) used several PGE analogs and cocultures of primary osteoblasts and bone marrow cells that contain osteoclast precursors and found that osteoclast formation is most potently induced by analogs with EP_4 agonistic activity. Based on this finding, they suggested the EP_4 receptor as the PGE receptor subtype for osteoclast induction. They then used EP_4 receptor-deficient mice and confirmed their proposal. Although EP_4-deficient mice did not show gross skeletal abnormalities, PGE_2-induced osteoclast formation was impaired in the culture of osteoblasts from the EP_4-deficient mice and osteoclast precursors from the spleen of wild-type mice. Interestingly, IL-1α, TNF-α, and basic fibroblast growth factor failed to induce osteoclast formation in these cultures. Suzawa et al. (221a) added PGE_2 to culture of parietal bone from mice deficient in each of the subtypes of PGE receptors as well as wild-type mice and examined the bone resorptive activity of this PG by measuring Ca^{2+} released into the medium. They found that bone resorption by PGE_2 was much decreased in the bone from EP_4-deficient mice, which, on the other hand, showed an equal extent of response to dibutyryl cAMP added to the culture as the bones from control mice. These studies unequivocally established the role of the EP_4 subtype of PGE receptors in PGE_2-mediated bone resorption. However, the contribution of this receptor-mediated process to bone resorption under various physiological and pathophysiological conditions in intact mice has not been fully examined, because an adequate number of adult EP_4−/− mice are not available because of their premature death from patent ductus arteriosus (see sect. v*C3*).

In addition to bone resorption, PGE_2 added exog-

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enously also induces bone formation. This was first noticed as a reversible increase in the bone cortex of infants with congenital heart diseases, who were receiving PGE_1 as a preservative measure (210). This bone-forming activity of the E type of PG was then confirmed in young rats receiving daily subcutaneous injections of PGE_2 (235). Histological examinations indicated that PGE_2 reduced bone resorption and increased the number of osteoblasts in these animals (93). Hakeda et al. (72) used MC3T3-E1 clonal osteoblast cells and found that PGE_2 at concentrations lower than 10^{-6} M arrests growth and induces differentiation of these cells through the elevation of cAMP levels, whereas at higher concentrations stimulates their growth. Suga et al. (215) found that the EP_1 and EP_4 subtypes of the PGE receptor are expressed in MC3T3-E1 cells and, using various PGE agonists, showed that the former receptor works in growth stimulation and the latter in differentiation. The physiological importance of these findings and the bone-forming activity of PGE will be clarified by studies of mice deficient in each receptor.

VI. CONCLUSIONS

Molecular cloning of the prostanoid receptors and elucidation of their properties and distribution have been a pivotal step in our understanding of prostaglandin physiology. Before the cloning of the prostanoid receptors, the physiological roles of prostanoids were tested by comparing the effects of aspirin-like drugs and the effects of exogenously applied prostanoids in a particular system. Although this approach proved to be generally useful, the exact picture of prostanoid actions was in many cases elusive, since exogenous molecules sometimes act on more than one receptor in tissues, and we were not certain as to how well they mimic the actions of endogenous prostanoids. The physiological and pathophysiological importance of the identified prostanoid actions had also been unclear. We can now properly evaluate our experiments using various prostanoids, based on our knowledge of receptor distribution and on the properties of each receptor. The importance of each prostanoid action can also be assessed by the use of knockout mice deficient in each receptor in various physiological and pathophysiological settings. Moreover, the cloned receptors have been exploited in mass compound screening, and we expect that agonists and antagonists highly selective for each receptor should be available in a few years. The availability of such compounds will further widen our understanding of prostanoid physiology and may lead to the development of novel therapeutics that selectively manipulate prostanoid-mediated disease processes.

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