Prostaglandin E Receptors*

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Prostaglandin (PG) E2 exerts its actions by acting on a group of G-protein-coupled receptors (GPCRs). There are four GPCRs responding to PGE2 designated subtypes EP1, EP2, EP3, and EP4 and multiple splicing isoforms of the subtype EP3. The EP subtypes exhibit differences in signal transduction, tissue localization, and regulation of expression. This molecular and biochemical heterogeneity of PGE receptors leads to PGE2 being the most versatile prostanooid. Studies on knock-out mice deficient in each EP subtype have defined PGE2 actions mediated by each subtype and identified the role each EP subtype plays in various physiological and pathophysiological responses. Here we review recent advances in PGE receptor research.

Prostanoids including various prostaglandins (PGs)2 and thromboxanes (TXs) are cyclooxygenase (COX) metabolites of C20-unsaturated fatty acids such as arachidonic acid. These substances are synthesized in response to various stimuli in a variety of cells, released immediately after synthesis, and act in the vicinity of their synthesis to maintain local homeostasis (1). Among prostanoids, the E type PGs, particularly PGE2 derived from arachidonic acid, is most widely produced in the body, most widely found in animal species, and exhibits the most versatile actions. Receptors mediating prostanoid actions were characterized first by pharmacological analysis, which indicated the presence of one receptor each, named DP, FP, IP, and TP, for PGs of the D, F, and I types and TXA, respectively, and four different receptors designated EP1, EP2, EP3, and EP4 for the E type PGs (reviewed in Refs. 2 and 3). Molecular identification of these receptors was achieved by their cDNA cloning, which revealed that the prostaglandin receptors are G-protein-coupled receptors (GPCRs) and that there is indeed a family of eight GPCRs that correspond to the pharmacologically defined receptors. In addition, a recent study revealed the presence of the ninth prostaglandin receptor that belongs not to the prostanooid receptor family described above but to the chemoattractant receptor family (4). This receptor called CRTH2 or DP2 is expressed in Th2 cells and eosinophils and mediates some of the PGD2 actions on these cells such as chemotaxis. cDNA cloning also revealed the presence of several splicing variants for EP3. Thus, there are four GPCRs designated subtypes EP1, EP2, EP3, and EP4 and EP3 variants mediating PGE2 actions. Subsequent analysis has revealed distinct biochemical properties and tissue and cellular localization of each EP subtype. The cloned EP subtypes have also been used in the development of compounds specific to each subtype.

Biochemical Properties of PGE Receptor Subtypes and Isoforms

Molecular Structures—Fig. 1 shows an alignment of the primary amino acid sequences of the mouse EP1, EP2, and EP4 and three isoforms of mouse EP3 receptors. The mouse EP1, EP2, and EP3 (EP3α), and EP4 receptors consist of 405, 362, 366, and 513 amino acids, respectively. EP4 has the longest intracellular C terminus and a relatively long intracellular third loop. The EP1 receptor also has a long third loop, whereas the EP2 and EP3 receptors have a more compact structure. A remarkable feature distinguishing the EP3 receptor from the other EP receptors is the existence of multiple variants generated by alternative splicing of the C-terminal tail. In mouse, alternative splicing creates three EP3 splice isoforms, α, β, and γ, containing C-terminal tails of 30, 26, and 29 amino acids that do not share any structural motifs or hydrophobic features (5, 6). These isoforms show similar ligand binding properties but have different signal transduction properties as described below. Multiple splice isoforms for EP3 also exist in other species including rat, rabbit, bovine, and human (3). Although all of the four EP subtypes respond to PGE2, the amino acid identity among the EPs is limited; the identity of EP1 to EP2, EP3, and EP4 is 30, 33, and 28%, respectively. The amino acid identity is only 31% even between the two EPs (EP2 and EP4) that couple to the activation of adenylate cyclase. The EP2 receptor is more homologous to IP (40%) and DP (44%), the other two adenylate cyclase-stimulatory prostanooid receptors, than any other EPs, and the EP1 receptor is more homologous to FP (35%) and TP (34%) than other EPs. This limited homology among EPs probably reflects the phylogenetic relationship among the prostanooid receptors (7).

Ligand Binding Properties—The EP subtypes bind most potently to PGE2 with Kd values in the range of 1–40 nM. Iloprost, an IP agonist, also binds to EP1 and EP3 with Kd values of about 20 nM. The PGE analogs that have been used in conventional studies are not specific for any given EP subtype except butaprost, which is specific for EP2. Several compounds highly selective for each EP subtype have been developed using cultured cell lines stably expressing each subtype. Examples are shown in Fig. 2 (8–11).

Signal Transduction Properties—Signal transduction pathways of EP subtypes have been studied by examining agonist-induced changes in second messengers such as cAMP, Ca2+2, and inositol phosphates and agonist-induced changes in activities of downstream kinases. The EP1 receptor mediates a PGE2-induced elevation of the free Ca2+ concentration in Chinese hamster ovary cells. This increase is dependent on extracellular Ca2+ and occurs without a detectable phos-
phatidylinositol response (12), suggesting that EP1 regulates Ca\textsuperscript{2+}/H\textsubscript{11001} channel gating via an unidentified G protein. It was reported that EP1 expressed in Xenopus oocytes can couple to TRP5, a candidate for the receptor-activated Ca\textsuperscript{2+}/H\textsubscript{11001} channel, and this coupling is inhibited by an antisense oligonucleotide for G\textsubscript{q}/G\textsubscript{11} but not by one for G\textsubscript{i1} (13). The EP2 and EP4 receptors couple to Gs and mediate increases in cAMP concentrations. The major signaling pathway of the EP3 receptor is inhibition of adenylate cyclase via G\textsubscript{i1}. It should be noted, however, that the EP receptors do not couple exclusively to the pathways described but often to more than one G protein and signal transduction pathway (Table 1). Of interest in this respect is the presence of two EPs, EP2 and EP4, that are coupled to increases in cAMP. They apparently function redundantly in some processes. For example, both EP2 and EP4 mediate induction of RANKL through cAMP by PGE\textsubscript{2} in osteoclastogenesis, although the extent of the contribution by each receptor may be different (14, 15). On the other hand, there are processes in which EP2 and EP4 play distinct roles. Some of these may be because of selective expression of either of them in relevant cells such as the action of EP2 during cumulus expansion in ovulation and fertilization (16) and that of EP4 in closure of the ductus arteriosus (17). However, only EP4 regulates migration of dendritic cells in the mouse although both EP2 and EP4 are expressed in these cells (18). This EP4-selective action may be related to the fact that EP4 but not EP2 couples to phosphatidylinositol 3-kinase, probably via G\textsubscript{i1}, in addition to activation of adenylate cyclase (19, 20). It is interesting in this respect that EP4 is also implicated in cell migration during tumor invasion (21) for ductus arteriosus closure (22) and for zebrafish gastrulation (23). As described, the EP3 receptor consists of multiple isoforms generated by alternative splicing of the C-terminal tail. Functional differences among these splice variants have been reported, including coupling to different signal transduction pathways (Table 1) (24), different sensitivities to agonist-induced desensitization (25), different extents of constitutive activity (26), different intracellular trafficking patterns (27), and different agonist-induced internalization patterns (28).

**Tissue Distribution and Cellular Localization**—Northern blot analysis and in situ hybridization have provided detailed information about EP receptor distribution and have shown that each receptor is specifically distributed in the body and that the expression levels are variable among tissues. The tissue distribution of the mouse EP subtypes assessed by Northern blot analyses is presented in Fig. 3A (29–32). Among the four EPs, EP3 and EP4 receptors are the most widely distributed with their mRNAs being expressed in almost all mouse tissues examined. In contrast, the distribution of EP1 mRNA is restricted to several organs, such as...
Physiological Functions of EP Subtypes

Mice deficient in each EP subtype individually have been generated, and studies using these knock-out mice and subtype-specific EP agonists/antagonists have identified EP subtypes mediating various PGE₂ actions (Table 2). EP subtypes mediate many processes known to be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs). For example, the EP3 receptor mediates generation of pyrogenic fever (54), and EP1 mediates anti-inflammatory effects of glucocorticoids (55). EP1 and EP3 are also involved in the regulation of steroidogenesis. The EP1 receptor mediates the effects of PGE₂ on steroidogenesis in the adrenal glands (56), while EP3 is involved in the regulation of progesterone production in the ovary (57). The EP2 receptor plays a role in the regulation of gonadotropin-stimulated luteinization in the ovary (58). Furthermore, EP4 is involved in the regulation of COX-2 expression in the ovary and uterus (59). These findings suggest that EP receptors play important roles in the regulation of various physiological processes in the female reproductive system.
and EP3 signals converge at the paraventricular nucleus of the hypothalamus and mediate neuroendocrine stress response by facilitating release of corticotropin-releasing hormone (43). EP2 facilitates ovulation and fertilization by inducing expansion of the cumulus, thus clarifying the mechanism for the inhibitory effect of NSAIDs on ovulation (16). Other studies have revealed that different EP subtypes as well as the IP receptor function in hyperalgesia both at the periphery and in the CNS. For example, the acetic acid writhing test revealed the involvement of both IP and EP3 in hyperalgesia (57, 61). Pain sensation that is induced by pH and heat and mediated by the capsaicin receptor TRPV1 is augmented by PGE2 and PGI2 acting on EP1 and IP, respectively (46). Furthermore, in the spinal cord, PGE2 acting on EP2 in glycinergic neurons abolishes the glycine-induced tonic inhibition of pain neuron in the dorsal horn (40). Recent studies on mice deficient in each EP receptor subtype receptors. Furthermore, development of highly selective agonists and antagonists to each EP subtype and information obtained by studies on mice deficient in each EP receptor now provide opportunities to apply our knowledge to manipulate various PGE2-mediated pathological processes.

### Concluding Remarks

The mechanisms whereby PGE2 exerts its pleiotropic effects, once a mystery in physiology, have been clarified through the biochemical identification and cDNA cloning of the four EP subtype receptors. Furthermore, development of highly selective agonists and antagonists to each EP subtype and information obtained by studies on mice deficient in each EP receptor now provide opportunities to apply our knowledge to manipulate various PGE2-mediated pathological processes.

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