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PERSPECTIVE

Prostaglandin EP_1 Receptor Subtype Selectivity Takes Shape

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The studies of Ungrin et al. (2001) describe the ligand binding and activation of the human prostaglandin (PG) receptor assayed with an extensive panel of ligands. PGs are a diverse family of autocoids whose synthesis is initiated by cyclooxygenase-mediated metabolism of the unsaturated 20-carbon fatty acid arachidonic acid to PGG/H_2, generating five primary bioactive prostanoids: PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 (Bonvalet et al., 1987; Smith, 1992). These arachidonic acid metabolites, including PGE_2, are potent modulators of a wide variety of physiological responses including inflammation, pain, fever, and modulation of smooth muscle tone (Needleman et al., 1986; Coleman et al., 1990; Gardiner, 1990). The importance of this pathway in the treatment or promotion of a broad array of pathophysiologic conditions including of cancer, arthritis and hypertension is underscored by the classic and novel uses of cyclooxygenase-inhibiting nonsteroidal anti-inflammatory drugs, which nonselectively inhibit the synthesis of all of these compounds. PGs act within the tissue where they are synthesized, in part via specific G protein-coupled receptors, designated EP (for E-prostanoid), DP, FP, IP, and TP receptors, for the other prostanoids (Coleman et al., 1994; Narumiya et al., 1999). PGE_2, a major cyclooxygenase product, may have multiple and at times apparently opposing functional effects on a given target tissue such as vascular smooth muscle (Bonvalet et al., 1987; Smith, 1992). The differential effects of PGE_2 analogs are important functional evidence for the existence of multiple PGE_2 receptors (EP receptors) and molecular cloning has now confirmed the existence of multiple PGE_2 receptor subtypes, each encoded by distinct genes. These receptors are designated EP_1, EP_2, EP_3, and EP_4 (Boie et al., 1997; Kiriyama et al., 1997) and probably account for the diverse effects of PGE_2 (Fig. 1).

Although some EP receptor subtype selective ligands exist, many of these compounds act at multiple PG receptor subtypes. The development of subtype selective EP receptor ligands has been attempted for more than 20 years with only partial success, partly because of the relatively recent discovery of the existence of multiple EP receptor subtypes and partly because of the difficulties associated with the synthesis of prostanoid analogs. Thus, the structural requirements for EP subtype selectivity are largely uncharacterized. Selective agonists that bind to the EP_1 receptor exist; however, these also have significant affinity for other receptor subtypes: the $EP_{1/3}$ selective agent sulprostone and the EP_1/IP selective agonist iloprost. Structure-activity relationship (SAR) studies may identify key structural requirements that would allow the synthesis of novel EP selective agonists and/or antagonists as well as provide insights as to the mechanism of receptor ligand selectivity. In the studies by Ungrin et al. (2001), an extensive panel of PG analogs was used in a high-throughput screening assay to perform SAR analysis for the cloned human EP_1 receptor. The EP_1 receptor was originally described as a smooth muscle constrictor (Kennedy et al., 1982) and, consistent with this function, activation of the recombinant human EP_1 receptor leads to signals via increased intercellular Ca^{2+} (Funk et al., 1993). Ungrin et al. (2001) used a calcium-responsive, aequorin-based reporter assay to analyze the activity of 55 prostanoid compounds (Ungrin et al., 2001).

Their SAR studies at the EP_1 receptor uncovered several notable findings. One of the most sensitive positions for agonist-activity at the EP_1 receptor is the hydroxyl group at the carbon 15 position. This is especially notable because conversion of PGE_2 to the 15-keto derivative is one of the primary pathways of metabolic inactivation in vivo (Anggard and Larsson, 1971). Moreover, the EP_1 receptor is more sensitive to 15 OH oxidation than the other EP receptors by 3- to 10-fold. This enhanced sensitivity of the EP_1 receptor to the metabolism of PGE_2 suggests that there may be differential inactivation of the signaling response at the EP receptors as a result.

Modifications of the C-1 carboxylate have been well characterized to cause a decrease in agonist affinity for the EP_2, EP_3, and EP_4 receptors. A similar sensitivity to modification of the C-1 carboxylate by esterification was seen for the EP_1 receptor. One notable exception to the low affinity of methyl ester analogs is the agonist enprostil, which has a relatively

ABBREVIATIONS: SAR, structure-activity relationship; PG, prostaglandin.

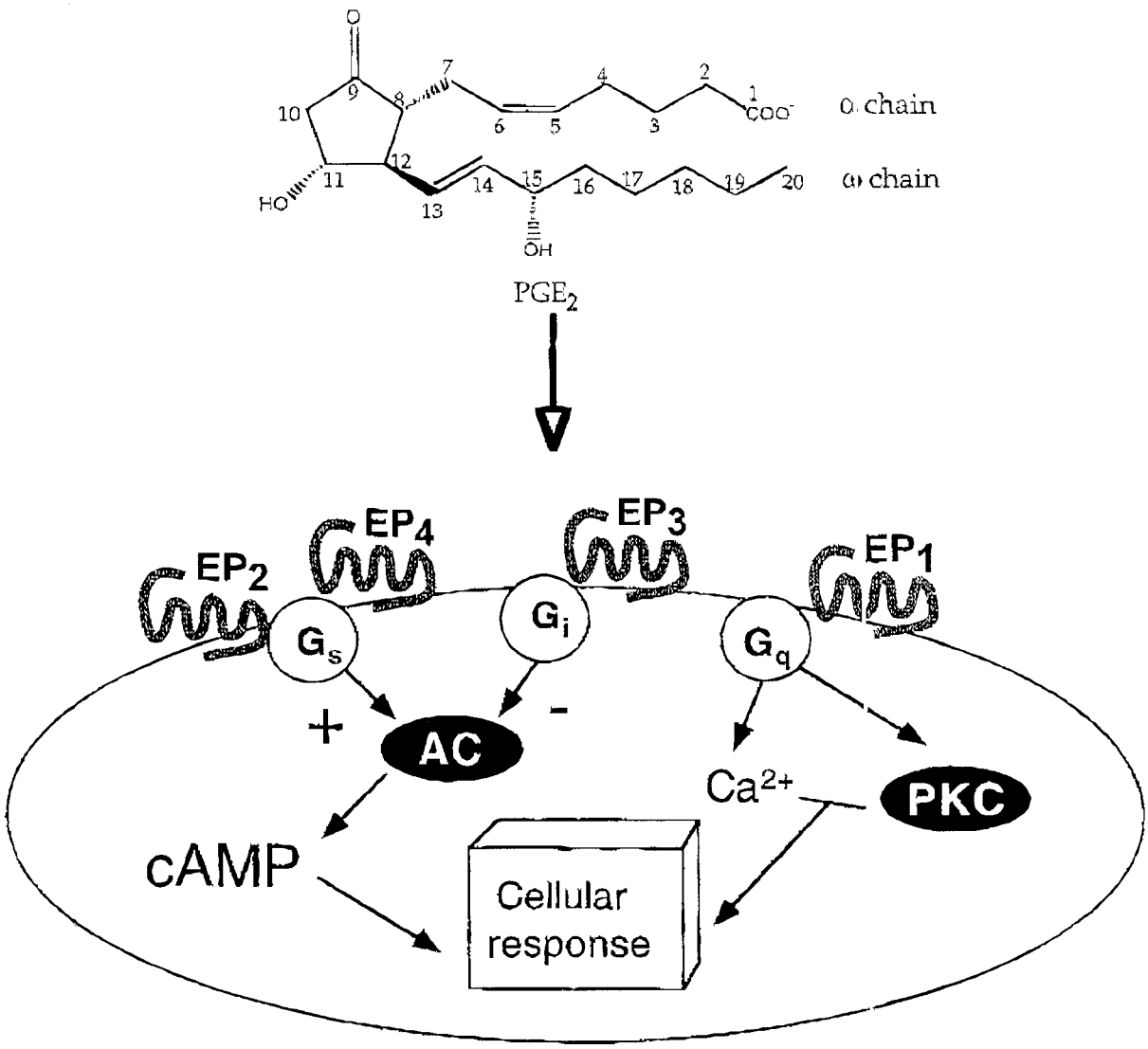

Fig. 1. Structure of PGE2, and primary signal transduction pathway activated by each of the EP receptor subtypes.

high potency despite the methyl ester found at the C-1 position. This demonstrates that there is not an absolute requirement for a negative charge at the C-1 ligand position and it calls into question a proposed ionic interaction between the C-1 carboxylate and a conserved arginine found in the seventh transmembrane region of the EP receptors. However, an equally plausible explanation put forth by Ungrin et al. is that the modifications of the ω-side chain of enprostil may increase the affinity of the ligand and overcome the energetic loss of the esterification of the C-1 carboxylate.

There are limited antagonists available for the EP receptors. Selective EP_1 antagonists have been described, including such nonprostanoid compounds as SC51089, or SC53122, that can aid in characterizing effects mediated by this receptor subtype (Hallinan et al., 1993; Hallinan et al., 1994; Lanthorn et al., 1995). These antagonists seem to have analgesic activity, prompting the search for clinically active drugs that would reduce pain without causing the gastric and renal side effects of nonsteroidal anti-inflammatory drugs (Hallinan et al., 1993, 1994). The role of the EP_1 receptor in nociception has recently been confirmed by genetic studies using a mouse targeted gene disruption model (Stock et al., 2001). It is of great interest to note that substitution of a Cl atom at the C-9 position led to an increase in affinity with no increase in potency, suggesting a route for the generation of novel competitive antagonists.

This new study by Ungrin et al. (2001) has assessed a large array of compounds on the EP_1 receptor. Comparison of these results with future studies for each of the other EP receptors will provide insights into the design of novel subtype selective agonist and antagonists. These studies may also provide clues about the structural basis for EP receptor-ligand interaction. Ultimately, the identification of EP selective ligands may allow the development of novel therapeutic agents.

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