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Classification of Prostanoid Receptors: Properties, Distribution, and Structure of the Receptors and Their Subtypes

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I. Introduction

A. Historical Background

The activity associated with the PGs* was first observed in 1930 by Kurzrok and Lieb in human seminal fluid. This observation was supported and extended by both Goldblatt (1933) and von Euler (1934). However, it was not for another 20 years that Bergström and Sjövall (1957) successfully purified the first PGs, PGE₁ and PGF₁₅. During the next decade or so, it became clear that the biological activities of the PGs were extremely diverse and that the family included members other than the original two, these being named alphabetically from PGA₂ to PGH₂. Of these, PGA₂, PGB₂, and PGC₂ are prone to extraction artifacts (Schneider et al., 1966; Horton, 1979). PGG₂ and PGH₂ are unstable intermediates in the biosynthesis of this family of hormones (Hamberg and Samuelsson, 1973). PGs can be biosynthesized from three related fatty acid precursors, 8,11,14-eicosa-trienoic acid (dihomo-γ-linolenic acid), 5,8,11,14-eicosatetraenoic acid (arachidonic acid), and 5,8,11,14,17-eicosapentaenoic acid (timodonic acid), giving rise to 1-, 2- and 3-series PGs, respectively (van Dorp et al., 1964); the numerals refer to the number of carbon-carbon double bonds present. In most animals, arachidonic acid is the most important precursor; therefore, the 2-series PGs are by far the most abundant.

By the middle 1970s it was clear that PGs were capable of causing a diverse range of actions, but few efforts were made to investigate the receptors at which PGs acted. Indeed, some doubted that they acted at receptors in the "classical" sense at all, believing, rather, that by virtue of their lipid nature they dissolved in cell membranes and caused their biological actions by altering the physical state of those membranes. However, despite this, interest was increasing in this new class of hormones, and there was optimism about their potential as new drugs. This interest peaked with the discovery of the two unstable PG-like compounds, TXA₂ (Hamberg et al., 1975) and PGI₂ (Moncada et al., 1976). The collective term for this family of hormones is "the prostanoids." At that time, the main problem with prostanoids as drugs was perceived to be one of stability, both chemical and metabolic, and there was an enormous amount of chemical effort directed toward developing more stable prostanoids. Despite successes in this regard, another problem soon became apparent, and that was one of side effect liability. Indeed, the very range of the actions of this class of compounds, which on the one hand offered such opportunities for drug development, began conversely to appear to be their limitation, because it appeared not to be possible to produce prostanoids as drugs without use-limiting side effects. It was this challenge that prompted a small number of groups of scientists to attempt to rationalise the "bewildering array" of actions of prostanoids by means of the identification and classification of prostanoid receptors. Initially, in the 1970s, most of the work directed toward the study of prostanoid receptors was designed to characterise specific binding sites for the radiolabeled natural ligands (Kuehl and Humes, 1972; Rao, 1973; Powell et al., 1974). Although this served to support the existence of specific membrane-binding sites, these sites may or may not have represented functional receptors.

B. Studies of Receptor Identification and Classification

1. Functional studies. The use of functional data to classify hormone receptors was pioneered by Ahlquist in 1948, in an attempt to classify the receptors responsible for the biological actions of the catecholamines, adrenaline and noradrenaline. Despite the limited tools at his disposal, the outcome of these studies was the classification of adrenoceptors into α and β subtypes, a classification scheme that has stood to the present day. This work was subsequently extended by Lands and colleagues.
in 1967 who, using the same approach, demonstrated that, although the classification proposed by Ahlquist was essentially correct, it was an oversimplification and one of Ahlquist's receptors, the β-adrenoceptor, could be further divided into two subtypes, termed β₁ and β₂. This approach to receptor classification, although now largely taken for granted, was revolutionary.

The relatively large number of naturally occurring prostanoids, their high potencies, and the variety of the responses elicited by them in different cells throughout the mammalian body made this an ideal area in which to study receptor subtypes. This was first recognised by Pickles in 1967, when he demonstrated that a range of different prostanoids, both natural and synthetic, showed different patterns of activity on a variety of isolated smooth muscle preparations. Yet, Pickles did not extend this work, and during the next 15 years little further work was reported extending his original observations. The few studies that were published (Andersen and Ramwell, 1974; Andersen et al., 1980; Gardiner and Collier, 1980) demonstrated that not only were different rank orders of agonist activity observed with a relatively small range of both natural and synthetic prostanoids, over a wide range of isolated preparations, but certain consistent patterns emerged. However, this work was not developed to describe a comprehensive receptor classification. In 1982, Kennedy and his coworkers described a comprehensive, working classification of prostanoid receptors based on functional data with the natural agonists, some synthetic agonists, and a small number of antagonists (Kennedy et al., 1982; Coleman et al., 1984). Their classification of receptors into DP, EP, FP, IP, and TP recognised the fact that receptors exist that are specific for each of the five naturally occurring prostanoids, PGs D₂, E₂, F₃₅, I₂, and TXA₂, respectively. It was clear that at each of these receptors one of the natural prostanoids was at least one order of magnitude more potent than any of the other four. Although in hindsight this observation may not seem remarkable, there are two other examples of a family of hormones that demonstrate such receptor selectivity; it is certainly not true of catecholamines, tachykinins, or leukotrienes. Although this broad classification into five classes of prostanoid receptors remains intact, evidence arose for a subdivision within the EP receptor family. There is now evidence for the existence of four subtypes of EP receptors, termed arbitrarily EP₁, EP₂, EP₃, and EP₄. The recent cloning and expression of receptors for the prostanoids has not only confirmed the existence of at least four of the five classes of prostanoid receptor, EP, FP, IP, and TP, but has also supported the subdivision of EP receptors into at least three subtypes, corresponding to EP₁, EP₂ (or EP₄), and EP₃. The current classification and nomenclature of prostanoid receptors is summarised in table 1.

2. Radioligand-binding studies. During the 1970s, there were a large number of ligand-binding studies performed in a wide range of tissues using radiolabeled PGs (Robertson, 1986). These studies made it clear that there are specific prostanoid-binding sites in the plasma membranes of such diverse tissues as liver, smooth muscle, fat cells, corpus luteum, leukocytes, platelets, and brain. In many of these, the ligand affinity (K_d) is of the order of 1 to 10 nM, and the receptor density is in the range of 1 pmol/mg protein. Furthermore, in many of the tissues exhibiting high affinity, and high density prostanoid-binding sites, it was known that prostanoids had biological activity, thus providing circumstantial support for these binding sites being functional receptors. Nonetheless, these studies did not further our understanding of prostanoid receptor classification, because in most cases, radioligands were confined to [³H]PGs E₁, E₂, or F₂, and either no competition studies were performed or competition studies were undertaken with prostanoids that do not discriminate among receptor subtypes (Coleman et al., 1990). It was not until the 1980s that studies were performed using [³H]PGs D₂ and I₂, and the evidence for a wider range of different types of prostanoid ligand-binding site emerged.

That some of these binding sites truly represented functional receptors was supported by the demonstration that they were capable of autoregulation, whereby binding site numbers are modulated by exposure to ligand. Thus, exposure of the animal or tissue to high levels of unlabelled ligand resulted in a "down-regulation" or loss of binding sites (Robertson et al., 1980; Robertson and Little, 1983), and conversely, treatment with inhibitors of endogenous prostanoid synthesis led to a corresponding "up-regulation" of binding sites (Rice et al., 1981). In some of these studies, attempts were made to associate modulation of binding sites with alterations in function; for example, Richelsen and Beck-Nielsen (1984) demonstrated that down-regulation of PGE₂-binding sites was accompanied by a reduction in PGE₂-induced inhibition of lipolysis. However, it was not until more selective, synthetic prostanoid agonists and antagonists became available, and distinct rank orders of agonist activity in functional studies became apparent, that the association between binding sites and functional receptors became possible (see section I.B.3).

3. Second-messenger studies. Almost all of the studies of prostanoids and second messengers until the late 1980s were concerned with cyclic nucleotides, particularly cAMP. Butcher and colleagues were the first to demonstrate an association between PGs and cAMP (Butcher et al., 1967; Butcher and Baird, 1968), and although their observation made little initial impact, it became increasingly accepted that E-series PGs at least were capable of stimulating adenylyl cyclase to cause increases in intracellular cAMP (Kuehl et al., 1972, 1973). However, it became clear that prostanoid effects on adenylyl cyclase were not solely excitatory, and in 1972, a more complex
relationship between PGEs and adenylyl cyclase was reported in platelets (Shio and Ramwell, 1972). PGE₁ caused an elevation of platelet cAMP, but PGE₂ caused a reduction. Interestingly, this distinction was reflected in the effects of PGE₁ and PGE₂ on platelet aggregation. PGE₁ inhibited aggregation, whereas PGE₂ potentiated the effect of aggregatory agents such as adenosine diphosphate (Shio and Ramwell, 1971). This parallel between cAMP and function not only provided evidence that the effect on cyclic nucleotide levels had functional relevance but also suggested that these might be receptor subtypes. A further distinction was observed at this time between the effects of E- and F-series PGs. Whereas PGE₁ and PGE₂ were seen to exert marked effects on levels of cAMP, both stimulatory and inhibitory, PGF₂α, despite its marked functional activity in many different cell types, was virtually devoid of effect on cAMP (Kuehl and Humes, 1972; Smith et al., 1992). In fact, it became accepted that the actions of PGF₂α were mediated through elevation of cyclic guanosine 3′,5′-monophosphate (Dunham et al., 1974; Kadowitz et al., 1975), although this idea has now lost support.

As with studies of radioligand binding, studies of second-messenger systems in the 1970s were limited, there being few studies in which ranges of receptor-selective agonists were compared for both function and modulation of cyclic nucleotide levels. Where comparisons were reported, as with the binding studies, they involved comparisons of the then available PGs, E, F, A, and B (Kuehl and Humes, 1972), and these give little insight into the receptor subtypes involved (Coleman et al., 1990). It was not until the 1980s, when more selective agonists became available, that studies of intracellular levels of cAMP provided real evidence for the existence of prostanoid receptor subtypes (see section I.B.4).

### 4. Molecular biology

Development of highly potent TP receptor antagonists and introduction of their high-affinity radiolabeled derivatives in binding experiments in the 1980s enabled solubilization and purification of the TP receptor. Using one of these compounds, S-145 (table 2), and its ³H-labeled derivative, Ushikubi et al. (1989) purified the human TP receptor from human platelets to apparent homogeneity, and based on the partial amino acid sequence of the purified protein, its cDNA was isolated in 1991 (Hirata et al., 1991). Subsequently, the cDNAs for numerous types and subtypes of prostanoid receptors have been cloned by homology screening, and the structures of the receptors that they encode have been elucidated. These receptors include the mouse TP receptor, the human EP₁ receptor, the mouse EP₁, EP₂, EP₃, EP₄, and FP receptors.
and EP₃ receptors, the rat EP₂ receptor, the mouse and bovine FP receptors, and the mouse IP receptor. The deduced amino acid sequences of the recombinant mouse receptors are shown in figure 1. Hydrophobicity and homology analysis of these sequences has revealed that all of them have seven hydrophobic segments characteristic of transmembrane domains, indicating that they are G-protein-coupled, rhodopsin-type receptors. The overall homology among the receptors is not high, and the amino acid identity is scattered over the entire sequences, showing that they are derived from different genes. Indeed, Taketo et al. (1994) have identified the genetic loci of mouse EP₂, EP₃, and TP receptors on chromosomes 15, 3, and 10, respectively. On the other hand, as shown

<table>
<thead>
<tr>
<th>Code</th>
<th>Chemical name</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH6809</td>
<td>6-isopropoxy-9-oxoanethene-2-caboxic acid</td>
</tr>
<tr>
<td>AH13205</td>
<td>trans-2-[4-(1-hydroxyhexyl)phenyl]-5-oxocyclopentaneeheptanoic acid</td>
</tr>
<tr>
<td>AH19437</td>
<td>1α(Z),2ß,5α(±)-methyl,7-2-(4-morpholinyl)-3-oxo-5(phenylmethoxy)cyclopentyl-5-heptenoate</td>
</tr>
<tr>
<td>AH22921</td>
<td>[1α(Z),2ß,5α]-7-[6-[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-5-oxocyclopentyl-5-heptenoic acid</td>
</tr>
<tr>
<td>AH23848</td>
<td>[1α(Z),2ß,5α]-7-[6-[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-5-oxocyclopentyl-5-heptenoic acid</td>
</tr>
<tr>
<td>AY23626</td>
<td>11-deoxy-prostaglandin F₆</td>
</tr>
<tr>
<td>BAY u3405</td>
<td>3(R)-3-(4-fluorophenylsulphonamido)-1,3,4,4-tetrahydro-9-carbazolepropanoic acid</td>
</tr>
<tr>
<td>BW245C</td>
<td>5-(6-carboxylhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin</td>
</tr>
<tr>
<td>BW A868C</td>
<td>3-benzyl-5-(6-carboxylcyclopentyl-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin</td>
</tr>
<tr>
<td>EP11</td>
<td>17,18,19,20-tetranor-16-p-fluorophenoxy-9,11-etheno PGF₂</td>
</tr>
<tr>
<td>EP105</td>
<td>(±)-5-endo-(6'-carboxyb-2'-Z-ened)-6-endo[N-(phenylcarbamoyl)hydrazono-methyl]-bicyclo[2.2.1]heptane</td>
</tr>
<tr>
<td>EP171</td>
<td>10α-homo-15ß-hydroxy-9α,11α-epoxy-16g-fluorophenoxy-ω-tetranor-5Z,13E-dienoic acid</td>
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<td>FCE22176</td>
<td>(+)-13,14-didehydro-20-methyl-carboptroctacyclin</td>
</tr>
<tr>
<td>GR32191</td>
<td>[1ß-[1α(Z),2ß,3α,5α]-]+-7-[6-[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidiny)cyclopentyl-5-heptenoic acid</td>
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<td>GR63799</td>
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</tr>
<tr>
<td>I-BOP</td>
<td>[1ß-(1αZ),(3αE)-3ß[(1-E,3S*,4-a)-[7-3-(3-carboxyb-4(4'-iodophenoxy)-1-butenyl]-7-oxobicyclo[2.2.1]heptan-2-yl]-5-heptenoic acid</td>
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<td>ICI192605</td>
<td>4(Z)-6-(2-o-chlorophenyl-1,3-dioxan-5-yl) hexenoic acid</td>
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<td>K10158</td>
<td>13,14-didehydro-20-methyl PGF₅</td>
</tr>
<tr>
<td>L-655240</td>
<td>3-[(4-chlorobenzyl)-5-fluoro-3-methyl-indol-2-yl]2,2-dimethylpropanoic acid</td>
</tr>
<tr>
<td>M&amp;B 28767</td>
<td>15ß-hydroxy-9-oxo-16-phenoxy-ω-tetranorprior-13E-enolic acid</td>
</tr>
<tr>
<td>N-0164</td>
<td>Sodium p-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl] phenyl phosphate</td>
</tr>
<tr>
<td>RS9520</td>
<td>Z-4-[(C'3,S,1ß,2Z,3ß,6ß)-2ß-3'-cyclohexyl-3'-hydroxyprop-1-ynyl]-3-hydroxybicyclo[4.2.0]oct-7-ylidene butyric acid</td>
</tr>
<tr>
<td>S-145</td>
<td>(1ß,2ß,3ß,4ß)-[5Z]-7-[3(p-sulphonyl-aminobicyclo[2.2.1]heptan-2-yl]-5-heptenoic acid</td>
</tr>
<tr>
<td>SC-19220</td>
<td>1-acetyl-2-(8-chloro-10,11-dihyodibenzo[b,f][1,4]oxazepine-10-carboxyl) hydrazine</td>
</tr>
<tr>
<td>SQ26555</td>
<td>(1ß-(6α,2ßS)-[5Z],[3α(1E,3a<em>S</em>,4a*)]-7-[3-(3-hydroxy-1-octenyl)-7-oxobicyclo[2.2.1]heptan-2-yl]-5-heptenoic acid</td>
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<tr>
<td>SQ29548</td>
<td>[15ß-[1α,2ß(5Z),3ß,4ß]-7-[(2-[phenylamino)-carbonyljhydrazino]methyl]-7-oxobicyclo[2.2.1]heptan-2-yl]-5-heptenoic acid</td>
</tr>
<tr>
<td>STA₃</td>
<td>9α,11α-thia-11α-carba-prosta-5Z,13E-dienoic acid</td>
</tr>
<tr>
<td>U44069</td>
<td>9α,11α-epoxymethano-15ß-hydroxy-prosta-5Z,13E-dienoic acid</td>
</tr>
<tr>
<td>U46819</td>
<td>11α,5α-epoxymethano-15ß-hydroxy-prosta-5Z,13E-dienoic acid</td>
</tr>
<tr>
<td>ZK110841</td>
<td>9-deoxy-9ß-chloro-16,17,18,19,20-pentanor-15-oxocyclopentyl-PGF₅</td>
</tr>
</tbody>
</table>
in the figure, these receptors show strong conservation of sequence in several regions, indicating that they probably evolved from a common ancestor. The most conserved region is the seventh transmembrane domain, which is located at a position analogous to lysine in the rodopsin-type receptors. Because sequences are shared by the prostaglandin receptors from various species, but not by other rhodopsin-type receptors, Narumiya et al. (1993) suggested that they are related to structural requirements of prostaglandin recognition. Particular attention has been paid to the conserved arginine in the seventh transmembrane domain, which is located at a position analogous to lysine in the rodopsin-type receptors. According to this assumption, the arginine serves as the binding site for the α-carboxyl group of prostaglandin molecules. The second extracellular loop between the fourth and fifth transmembrane regions is also conserved. Another region of significant homology is the outer half of the seven transmembrane segments (Saurese and Fraser, 1992). Because the carboxyl group is essential for biological activity of most prostaglandins, it was proposed that the arginine serves as the binding site for the α-carboxyl group of prostaglandin molecules. The other transmembrane domains of the prostaglandin receptors are more hydrophobic than those of the monoamine receptors, which may facilitate binding to the cypelopentane ring and aliphatic side chains of prostaglandin molecules. All of the recombinant receptors have been expressed in cultured cells and their ligand-binding activities and signal transduction pathways studied. The results obtained with each receptor type are described in detail in subsequent sections. These studies may give us more accurate information than those obtained by pharmacological and biochemical studies in native tissues, because the expressed receptor system permits the study of homogeneous populations of receptors without the complications of the presence of other receptor types. Of course, there are also limitations to this approach. The fact that...
only the receptor cDNAs of a limited number of species have been cloned means that we do not know whether discrepancies between the properties of the recombinant receptors and the pharmacological analyses are attributable to species differences or to receptor subtypes (see discussion by Hall et al., 1993). Another limitation is that the recombinant receptors have been expressed and analysed only in Chinese hamster ovary cells or simian kidney COS cells, and this results in coupling of a receptor from one species with a G-protein from a different species; moreover, the pool of G proteins in CHO cells and COS cells may differ from that of the tissue in which a receptor is normally expressed. With prostanoid receptors, effects on the ligand-binding properties may be minimal, because, unlike the monoamine receptors, guanosine triphosphate analogues appear to exert little effect on prostanoid binding. Nonetheless, the ligand-binding profiles of the recombinant receptors (detailed in section II.A.2) are generally in good agreement with those characterized earlier in pharmacological and biochemical experiments.

II. Types, Subtypes, and Isoforms of Prostanoid Receptors

A. DP Receptors

1. Functional studies. a. SELECTIVE AGONISTS AND ANTAGONISTS. Although PGD_2 and various close analogues do behave as DP agonists, none is particularly selective (Giles and Leff, 1988). Indeed, PGD_2 itself possesses relatively potent FP and even TP receptor agonist activity. However, there are a number of potent and highly selective DP receptor agonists. One of these is 9-deoxy-Δ^9-PGD_2 (PGJ_2, Bundy et al., 1983), but the first to be identified, and the most widely used was BW 245C, a hydantoin prostanoid analogue (Caldwell et al., 1979). This compound is interesting in that it is at least one order of magnitude more potent than the natural ligand, PGD_2, as a DP receptor agonist but, on the other hand, appears to be several orders of magnitude less potent at other prostanoid receptors and lacks the relatively high FP and TP receptor agonist activity associated with PGD_2 itself. Since the discovery of BW 245C, another selective DP receptor agonist, ZK110841, was reported by Thierauch et al. (1988), although there is much less information concerning this compound. ZK110841 is interesting in that it is not an analogue of PGD_2 but of PGE_2. One additional compound of structural significance is RS 93520, which is superficially a PGF_2 analogue, and has some weak IP agonist activity but is much more potent as a DP receptor agonist (Alvarez et al., 1991). Quantitative data for some selective DP receptor agonists and antagonists are summarised in table 3.

The study of DP receptors has been facilitated by the availability of antagonists. The first compound shown to possess DP receptor-blocking activity was the phloretin derivative, N-0164, which weakly, but selectively, antagonised inhibitory activity of PGD_2 on human platelets (Maclntyre and Gordon, 1977). Subsequently, an EP1 receptor-blocking drug, AH6809, was shown to exhibit DP receptor-blocking activity but was, again, rather weak, with a pA_2 of about 6.0 (Keery and Lumley, 1988). The most significant development was that of BW A868C (Giles et al., 1989), an analogue of the agonist, BW 245C. In fact, the Wellcome group synthesised a wide range of analogues of a related series of high-efficacy agonists to antagonists.

b. DISTRIBUTION AND BIOLOGICAL FUNCTION. DP receptors are perhaps the least ubiquitous of the prostanoid receptors. Only in American Type Culture Collection CCL 44 cells, a cell line derived from bovine embryonic trachea (Ito et al., 1990), have DP receptors been shown to exist as a homogeneous receptor population; in all other tissues in which they have been identified, they exist only in association with one or more other prostanoid receptor types. Therefore, it is difficult to study them in isolation. Fortunately, the available potent and selective DP receptor agonists and antagonists have proved valuable in the study of this receptor type.

DP receptors are distributed largely in blood platelets, vascular smooth muscle, and nervous tissue, including the central nervous system. There are also examples of DP receptors in gastrointestinal, uterine, and airway smooth muscle in some animal species (Coleman et al., 1990). Responses mediated by DP receptors are predominantly inhibitory in nature, e.g., inhibition of platelet aggregation and relaxation of smooth muscle and possibly inhibition of autonomic neurotransmitter release. However, DP receptors are associated with excitatory events in some afferent sensory nerves, where they can induce pain or, probably more correctly, hyperalgesia (Ferreira, 1983; Horiguchi et al., 1986). The distribution of DP receptors is highly species specific, e.g., human platelets appear to have a particularly rich population of inhibitory DP receptors (MacIntyre and Armstrong, 1987), whereas the platelets of most laboratory species appear to contain few, if any, DP receptors, and as far as uterine smooth muscle is concerned, DP receptors appear to be confined to the human (Sanger et al., 1982).

2. Ligand-binding studies. Few ligand-binding studies have been reported for DP receptors. PGD_2-specific binding sites have been identified in human platelet membranes, at which PGs of the E, F, and I series have substantially lower binding affinities but at which the DP receptor agonist, BW 245C, has high affinity (Cooper and Ahern, 1979; Town et al., 1983). Binding sites for [^3H]PGD_2 have also been identified in rat brain synaptic membranes (Shimizu et al., 1982).

3. Second-messenger studies. The evidence relating to DP receptors and second-messenger coupling is largely indirect. Simon et al. (1980) demonstrated that PGs D_2, E_2, and I_2 are approximately equipotent in stimulating
adenylate cyclase activity in human colonic mucosa. Because PGD2 is only a very weak agonist at EP and IP receptors, this argues that, among others, DP receptors must be present in this preparation, coupling positively to adenylyl cyclase, presumably via Gα. However, the demonstration by Ito et al. (1990) that activation of DP receptors in American Type Culture Collection CCL 44 cells (see above) results in an increase in levels of intracellular cAMP supports this association. Furthermore, several selective agonists exist for the DP receptor (Giles et al., 1989), and both of these and PGD2 itself have been shown to bind to a specific DP receptor in platelets to cause an increase in cAMP formation (Gorman et al., 1977b; Schafer et al., 1979; Siegl et al., 1979a; Whittle et al., 1978), again suggesting that DP receptors can couple to Gα to stimulate adenylyl cyclase (Halushka et al., 1989).

**B. EP Receptors**

1. **Functional studies.**
   a. **SELECTIVE AGONISTS AND ANTAGONISTS.**
      i. **EP1 receptors.** Although sulprostone was first identified as a potent EP1 receptor agonist, it is more potent at EP3 receptors (Bunce et al., 1990). In fact, to date, there is no reported example of a highly selective EP1 receptor agonist. Two compounds that have proven useful are 17-phenyl-ω-trinor PGE2 (Lawrence et al., 1992) and iloprost (Sheldrick et al., 1988). The selectivity of 17-phenyl-ω-trinor PGE2 for EP1 receptors other than for EP2 and EP3 receptors is only about 10-fold, and thus, it is of limited value in the characterisation of EP receptors. In contrast, iloprost is more selective for EP1 receptors over EP2 and EP3 receptors but is a partial agonist and, also, being a PGI2 analogue, is a highly potent IP receptor agonist, being at least as potent as PGI2 itself. As far as antagonists are concerned, there are a number of compounds with specific EP1 antagonist activity, and at least some of these appear to be competitive receptor-blocking drugs, particularly SC-19220 and AH6809 (Sanner, 1969; Coleman et al., 1985). However, both of these compounds are weak. SC-19220 is also highly insoluble and possesses some local anaesthetic activity (Poll et al., 1989), and AH6809 has DP receptor-blocking activity and binds albumin avidly (Coleman et al., 1985). Quantitative data for some selective EP1 receptor agonists and antagonists are summarised in table 4.
      
      ii. **EP2 receptors.** There are some EP receptor agonists that have proven useful in characterizing EP2 receptors. The first identified EP2 receptor agonist was the PGE2 analogue, AY23626, but this compound was later found to possess relatively potent EP3 receptor agonist activity (Coleman et al., 1987b). Another compound that has clearly demonstrable EP2 receptor agonist activity is misoprostol, but like AY23626, it is also a potent EP3 receptor agonist (Coleman and Humphrey, 1993). Both of these compounds are weak EP1 receptor agonists and, therefore, can provide valuable information concerning EP2 receptors in preparations devoid of EP3 receptors. The only two compounds that have been reported to be selective for EP2 receptors as opposed to the other three subtypes are butaprost (Gardiner, 1986) and AH13205 (Nials et al., 1993). However, both of these compounds suffer from low potency; thus, although they are selective for EP2 receptors, both are between about 10- and 100-fold less potent than PGE2 and their usefulness is limited. There are to date no reports of any compounds with EP2 receptor-blocking activity. Quantitative data for some selective EP2 receptor agonists are summarised in table 5.

      iii. **EP3 receptors.** There are many potent EP3 receptor agonists, because they have been developed by various drug companies as potential antiulcer drugs (Collins, 1986). Misoprostol is an example of such a drug. However, many of these compounds also possess agonist activity at other EP receptors and even at other prostanoid receptors. Examples of these are rioprostil, M&B 28767, and nocloprost (Shriver et al., 1985; Lawrence and Jones, 1992; Täuber et al., 1988). Two compounds have been developed that are more selective for EP3 receptors than the others, and these are enprostil and GR63799, both of which show a high degree of EP3 receptor selectivity (Reeves et al., 1988; Bunce et al., 1990). These two compounds are not only highly selective but are also highly potent, both being at least 10-fold more potent than PGE2 at EP3 receptors. Despite the

### Table 3: Potencies of some DP receptor agonists and antagonists

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Equieffective concentration (PGD2 = 1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW 245C</td>
<td>0.03-0.7</td>
<td>Town et al., 1983; Narumiya and Toda, 1985</td>
</tr>
<tr>
<td>ZK110841</td>
<td>0.2-1.0</td>
<td>Thiersch et al., 1988; Ito et al., 1990</td>
</tr>
<tr>
<td>RS93520</td>
<td>1.0</td>
<td>Alvarez et al., 1991</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>pA2</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW A8685C</td>
<td>9.3</td>
<td>Giles et al., 1989</td>
</tr>
<tr>
<td>AH6809</td>
<td>6.0-6.6</td>
<td>Keery and Lumley, 1988; Ito et al., 1990</td>
</tr>
</tbody>
</table>

* Data obtained on human platelets, rabbit transverse stomach strip, rat peritoneal mast cells, and bovine embryonic trachea cells.
large amount of activity in this area of prostanooid chemistry, there are no reports of the discovery of any compound with EP₃ receptor-blocking activity. Quantitative data for some selective EP₃ receptor agonists are summarised in table 6.

iv. EP₄ receptors. The most recently identified of the subtypes of EP receptor is the EP₄ receptor (Coleman et al., 1994). There are currently no selective agonists for EP₄ receptors, but they were first identified in piglet saphenous vein, where prostanooid-induced smooth muscle relaxation was clearly mediated by an EP receptor but one at which selective agonists for EP₁, EP₂ and EP₃ receptors were weak or inactive. One further characteristic that distinguishes EP₄ receptors from the other three subtypes is their blockade by the TP receptor-blocking drugs, AH22921 and AH23848. Although the EP₁ receptors in piglet saphenous vein are blocked by these two TP antagonists, their potencies are low (pA₂ = 5.3 and 5.4, respectively), being at least 100-fold weaker than they are at TP receptors. AH23848, at least, has no antagonist activity at other EP receptors (Coleman et al., 1994).

b. DISTRIBUTION AND BIOLOGICAL FUNCTIONS. EP receptors mediate an impressive range of biological activities, including contraction and relaxation of smooth muscle, inhibition and enhancement of neurotransmitter release, inhibition of lipolysis, inhibition of gastric acid secretion, inhibition and enhancement of nonacid (water) secretion, inhibition of inflammatory mediator release, inhibition of immunoglobulin expression, immunoregulation, etc. (Coleman et al., 1990). It is partly because of the multitude of their biological actions that it first became obvious that there must be more than a single subtype of EP receptor. For example, looking at the effects of PGE₂ on smooth muscle, there are preparations, such as guinea pig trachea, where PGE₂ can cause contraction, relaxation, or both, depending on the conditions of the study; both responses appear to be mediated by EP receptors. This situation was clarified first by the use of the antagonists, SC-19220 and AH6809, that specifically blocked the contractions caused by PGE₂ but had no effect on the relaxations (Coleman et al., 1987a). This subdivision of EP receptors into two subtypes explained many otherwise puzzling observations; however, it soon became clear that more than two EP receptor subtypes were needed to account for all of the findings with PGE₂ and other, more selective EP receptor agonists. The first obvious inconsistency to the two-subtype system was contraction of the chick ileum, where the selective EP₁ receptor agonist, sulprostone, and the selective EP₃ receptor agonist, AY23626, both potently contracted the preparation, but neither

| TABLE 4 |
| Potencies of some EP₁ receptor agonists and antagonists* |

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Equieffective concentration (PGE₂ = 1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulprostone</td>
<td>4–6</td>
<td>Coleman et al., 1987a</td>
</tr>
<tr>
<td>Iloprost</td>
<td>~1†</td>
<td>Dong et al., 1986; Sheldrick et al., 1988</td>
</tr>
<tr>
<td>17-Ph PGE₂</td>
<td>1</td>
<td>Lawrence et al., 1992</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>pA₂</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-19220</td>
<td>5.2–5.6</td>
<td>Coleman et al., 1985, 1987a</td>
</tr>
<tr>
<td>AH6809</td>
<td>6.4–7.0</td>
<td>Coleman et al., 1985, 1987a</td>
</tr>
</tbody>
</table>

* Data obtained on guinea pig isolated ileum and gastric fundus.
† Partial agonist.

| TABLE 5 |
| Potencies of some EP₂ receptor agonists* |

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Equieffective concentration (PGE₂ = 1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butaprost</td>
<td>6–30</td>
<td>Gardiner, 1986; Humbles et al., 1991</td>
</tr>
<tr>
<td>AH13205</td>
<td>30–100</td>
<td>Nials et al., 1993; Humbles et al., 1991</td>
</tr>
<tr>
<td>AY23626†</td>
<td>2–14</td>
<td>Coleman et al., 1987a</td>
</tr>
<tr>
<td>Misoprostol†</td>
<td>1–4</td>
<td>Coleman et al., 1988; Humbles et al., 1991</td>
</tr>
<tr>
<td>Rioprostil†</td>
<td>4</td>
<td>Coleman et al., 1988</td>
</tr>
</tbody>
</table>

* Data obtained on cat trachea and rabbit ear artery.
† Also potent EP₁ receptor agonist activity.

| TABLE 6 |
| Potencies of some EP₃ receptor agonists |

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Equieffective concentration (PGE₂ = 1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enprostil</td>
<td>0.02–0.1</td>
<td>Reeves et al., 1988; Bunce et al., 1990; Strong et al., 1992</td>
</tr>
<tr>
<td>GR63799</td>
<td>0.1</td>
<td>Bunce et al., 1990</td>
</tr>
<tr>
<td>Sulprostone†</td>
<td>0.05–0.3</td>
<td>Coleman et al., 1988; Bunce et al., 1990; Strong et al., 1992; Lawrence et al., 1992</td>
</tr>
<tr>
<td>Misoprostol‡</td>
<td>0.2–1.0</td>
<td>Reeves et al., 1988; Coleman et al., 1988; Bunce et al., 1990; Lawrence et al., 1992</td>
</tr>
<tr>
<td>Rioprostil‡</td>
<td>0.9–1.1</td>
<td>Reeves et al., 1988</td>
</tr>
<tr>
<td>M&amp;B 28767</td>
<td>0.6</td>
<td>Lawrence et al., 1992</td>
</tr>
<tr>
<td>AY23626‡</td>
<td>3.0–7.7</td>
<td>Coleman et al., 1987a; Reeves et al., 1988</td>
</tr>
</tbody>
</table>

* Data obtained on guinea pig vas deferens, chick ileum, rat gastric mucosa, and rat adipocytes.
† Also has potent EP₁ receptor agonist activity.
‡ Also has potent EP₁ receptor agonist activity.
by Northern blot analysis in mouse tissue, EP2 receptors were highly potent in contracting chick ileum. The discovery of such compounds revealed that there were many preparations exhibiting "atypical" EP receptors; for example, guinea pig vas deferens and rat gastric mucosa, where PGE2 caused inhibition of autonomic neurotransmitter release and inhibition of acid secretion, respectively (Coleman et al., 1987a,b,c). It became apparent that these receptors represented a novel subtype of EP receptor, which were termed EP3 (Coleman et al., 1987c). Additional functional studies by many different groups have now been published in support of the existence of these three subtypes of EP receptors, and their existence has gained widespread acceptance. It is now clear, however, that there also exists a fourth subtype of EP receptor, the EP4 receptor (Coleman et al., 1994).

EP receptors, although the first characterised of the EP receptor subtypes, do not appear to have a wide distribution, being most evident in smooth muscle from the guinea pig, where they exist in the trachea, the gastrointestinal tract, the uterus, and the bladder, where they mediate smooth muscle contraction. EP1 receptors have also been demonstrated in at least some of these same tissues from rat and hamster. Although EP1 receptors are more sparse in non-rat species, they do exist, for example in dog gastric fundus (Coleman, 1987), bovine iris sphincter (Dong et al., 1986), and human myometrium (Senior et al., 1991). The tissue distribution of EP1 receptors in mouse has been examined by Northern blot analysis (Watabe et al., 1993). Expression of EP1 is generally low compared with other subtypes of EP receptor, although moderate expression was found in kidney and some was noted in lung. Sugimoto et al. (1994a,b,c) detected mRNA for the EP1 receptor in the papillary collecting duct of mouse kidney.

EP2 receptors are more widespread and the responses mediated much more varied. They are widely distributed in smooth muscle, where they invariably mediate relaxation, on epithelial cells, where they mediate nonacid secretion, on inflammatory cells, such as mast cells and basophils, where they mediate inhibition of mediator release, and on sensory afferent nerves, where they mediate activation (Coleman et al., 1990). When examined by Northern blot analysis in mouse tissue, EP2 receptors were found to be expressed most highly in ileum, followed by thymus, lung, spleen, heart, and uterus, in this order (Honda et al., 1993). EP2 receptor mRNA in mouse kidney was specifically localised to the glomeruli (Sugimoto et al., 1994a,b,c).

Of the four subtypes, EP3 receptors appear to be the most ubiquitous. They are present in smooth muscle of gastrointestinal, uterine, and vascular origin, where they mediate contraction, in autonomic nerves, where they mediate inhibition of neurotransmitter release, in adipocytes, where they mediate inhibition of lipolysis (Strong et al., 1992), in gastric mucosal cells, where they mediate inhibition of acid secretion (Reeves et al., 1988), and in renal medulla, where they mediate inhibition of water reabsorption (Sonnenburg and Smith, 1988). Their presence in the gastric mucosa has been exploited in the development of EP3 receptor agonists as gastric antisecretory agents for the treatment of gastric ulcer and for the prevention of the side effects associated with long-term therapy with nonsteroidal anti-inflammatory drugs. They also appear to be present in human platelets, where they mediate a proaggregatory effect (Jones and Wilson, 1990), i.e., they do not induce aggregation in their own right but potentiate that to other aggregatory agents. EP3 receptor mRNA is expressed very highly in kidney and uterus, and expression is also seen in stomach, thymus, spleen, lung, and brain (Sugimoto et al., 1992). Takeuchi et al. (1993) cloned the rat EP3 receptor and examined its mRNA distribution in kidney by reverse transcription and polymerase chain reaction on microdissected nephron segments. Their results indicate that EP3 mRNA is expressed in the thick ascending limbs of Henle's loop and in the collecting ducts in the cortex and medulla. The cellular distribution of the three EP3 receptors in the kidney was examined by in situ hybridization by Sugimoto et al. (1994b); this work identified mRNA for EP3 receptors in the tubules in the medulla, the distal tubules, and macula densa. The same authors also reported the distribution of the EP3 receptor in nervous tissue. This receptor mRNA is expressed only in neurons and highly expressed in dorsal root ganglion and brain regions such as hippocampus, preoptic area, hypothalamus, maxillary body, locus coeruleus, and raphe nuclei (Sugimoto et al., 1994c).

There is currently little information concerning the distribution of EP4 receptors, but after the first report of the existence of this receptor in piglet saphenous vein (Louttit et al., 1992a,b), evidence has been presented for the existence of what may be EP4 receptors in rabbit jugular vein and saphenous vein, rat trachea, and hamster uterus (Lawrence and Jones, 1992; Yeardley et al., 1992; Lydford and McKechnie, 1993, 1994).

2. Ligand-binding studies. a. EP RECEPTORS. Although in the 1970s many studies were performed on specific [3H]PGE2 binding to membranes from a range of different cell types, and the evidence from competition studies strongly pointed to these binding sites being EP receptors, there is very little information with which to identify the EP receptor subtype involved and none that any of them were EP1 receptors (Coleman et al., 1990). Indeed, to date, there are still no reports of ligand-binding studies of native EP1 receptors. However, such studies have been performed on recombinant EP1 receptors from mouse (Watabe et al., 1993) and human (Funk et al.,...
1993). With the mouse receptors, expressed in Chinese hamster ovary cells, [3H]PGE2 had an affinity of 21 nM, this being displaced not only by unlabeled PGE2 but also in approximately equimotent fashion by sulprostone, iloprost, and 17-phenyl-trinor PGE2, all of which are essentially functionally equipotent with PGE2 at EP1 receptors. Furthermore, PGE2, which is approximately 10-fold less potent than PGE2 as an EP1 receptor agonist, was one order of magnitude less potent than PGE2 in displacing [3H]PGE2 from the recombinant EP1 receptor; the selective EP2 receptor agonist, butaprost, was virtually ineffective. Interestingly, the EP1 receptor-blocking drug, AH6809, was virtually ineffective in displacing bound [3H]PGE2. This finding is difficult to explain in view of the established effectiveness of AH6809 in functional studies, although these have not been conducted in mouse tissues, and this may reflect species differences in ligand specificities of EP1 receptors. With the human EP1 receptors expressed in COS cells, [3H]PGE2 had an affinity of 1 nM and displacement characteristics similar to those observed with the recombinant mouse receptors in that the rank order of displacement was PGE2 > PGE1 > PGF2α > PGD2, with butaprost being virtually ineffective. However, with the cloned human receptor, both AH6809 and SC-19220 were effective in displacing bound ligand, with potencies consistent with their affinity values for EP1 receptors determined in functional studies.

b. EP2 RECEPTORS. As with EP1 receptors, it is by no means certain that any of the [3H]PGE2-binding sites described in early studies mentioned above correspond to EP2 receptors. It is possible that the high-affinity sites (Ka ≈ 1 nM) identified by Kimball and Lauderdale (1975) and Rao (1976) in bovine corpus luteum were EP2 receptors and were positively correlated with enhancement of adenylate cyclase activity. Beyond this, there have been no ligand-binding studies reported on native EP2 receptors. There are reports of binding to recombinant EP2 receptors from mouse (Honda et al., 1993) and human (An et al., 1993), both of which have been expressed in COS cells. Both of these receptors demonstrated high specific binding for [3H]PGE2 (Ka ≈ 2 and 11 nM), with much lower affinities for the other naturally occurring prostanoids, and for synthetic prostanoids selective for EP1 and EP3 receptors.

c. EP3 RECEPTORS. In ligand-binding studies performed on membranes prepared from rat adipocytes and guinea pig and human myometrium and renal collecting tubule cells (Kuehl, 1974; Oien et al., 1975; Losert et al., 1979; Schillinger et al., 1979; Sonnenburg et al., 1990), there is evidence that the high-affinity (Ka < 10 nM) [3H]PGE2-binding sites identified are of the EP3 subtype. First, on the basis of functional evidence, all of these tissues are known to contain EP3 receptors, and second, two compounds with established EP3 receptor agonist activity, sulprostone and AY23626, were shown to potently inhibit PGE2 binding. It is interesting that the relative binding affinities of these two selective agonists relative to PGE2 reflect their relative agonist potencies at EP3 receptors (Coleman et al., 1990). However, Sewing and Beinborn (1990), in a study comparing binding affinities of a diverse range of prostanoids for the high-affinity PGE-binding sites in pig gastric mucosa with their abilities to inhibit pepsinogen secretion in the same tissue, found an EP3 receptor-mediated response and an impressive correlation was obtained. A diagnostic feature of EP3 receptors coupled to G1 is that guanine di- and trinucleotides actually decrease the Kd for ligand binding (Grandt et al., 1982; Watanabe et al., 1986, Sonnenburg et al., 1990).

Recombinant EP3 receptors from mouse (Sugimoto et al., 1992), rat (Takeuchi et al., 1993), rabbit (Breyer et al., 1993), and human (Adam et al., 1994) have been expressed in COS cells, where they exhibit high affinities for ligand binding (Ka = 0.3 to 6.6 nM) for [3H]PGE2. In each case, in competition studies, the recombinant receptor exhibited a marked selectivity for PGE2 over the other naturally occurring prostanoids or their synthetic mimetics. In each case, PGE1 was as potent as PGE2 in displacing radiolabeled ligand from the receptors, and in mouse, rabbit, and human homologues, potent, selective EP3 receptor agonists, including M&B 28,767, sulprostone, and GR63799, were all potent competitors of [3H]PGE2 binding.

3. Second-messenger studies. a. EP1 RECEPTORS. Creese and Denborough (1981) demonstrated that the PGE2-induced contraction of guinea pig trachea is absolutely dependent on extracellular Ca2+. Because PGE2 is known to contract this preparation via EP1 receptors (Coleman and Kennedy, 1985), this implicates a key role for extracellular Ca2+ in EP1 receptor activation. However, in RCCCT cells, PGE2 and sulprostone both stimulate mobilization of Ca2+ from intracellular stores, and AH6809 causes about 50% inhibition of PGE2-induced Ca2+ mobilization. Thus, Ca2+ mobilization in this system appears to be mediated, at least in part, via EP1 receptors; PGE2-induced Ca2+ mobilization was not blocked by pertussis toxin and does not require extracellular Ca2+. Somewhat surprisingly, no changes in IP3 formation were observed in response to treating RCCCT cells with PGE2. Thus, in the RCCCT cell system, occupancy of EP1 receptors appears to cause Ca2+ mobilization from intracellular stores via an IP3-independent mechanism.

Studies with recombinant mouse and human EP1 receptors (Watabe et al., 1993; Funk et al., 1993) also indicate that this receptor is involved in Ca2+ mobilization. Watabe et al. (1993) found that PGE2 caused an increase in intracellular Ca2+ concentration in cells expressing the recombinant murine EP1 receptor. This response consisted of two phases: a peak of about 30 s duration, followed by a slower but sustained increase of more than 3 min. The PI response evoked by this receptor was weak (about only 20% above control level) and
occurred slowly, making it difficult to assess the contribution of the PI response to the rapid transient increase in free Ca$^{2+}$ concentration in the cells. Changes in the intracellular cAMP level were negligible in the EP$_1$-expressing cells.

b. EP$_2$ RECEPTORS. The results of Simon et al. (1980), referred to above (see section II.A.3), provide indirect evidence for positive coupling of an EP receptor to adenylate cyclase, but more direct evidence has been provided by Hardcastle et al. (1982), in their demonstration of an association between EP$_2$ receptors and cAMP generation in enterocytes. The latter findings are supported and extended by Reimer et al. (1992), who examined a wide range of EP receptor subtype-selective agonists and the EP$_1$ antagonist, SC-19220. Similarly, Jumblatt and Peterson (1991) found an association between EP$_2$ receptor stimulation and cAMP generation in corneal endothelial cells. In both freshly isolated RCCT cells and in RCCT cells cultured for 4 to 5 days, PGE$_2$ acting at relatively high concentrations (ED$_{50}$ = 500 nM) causes stimulation of cAMP formation (Sonnenburg et al., 1988). PGs of the E-series are the most effective in stimulating RCCT cell cAMP synthesis, indicating that this process is mediated via EP receptors. Despite this, the PGE$_2$ analogue sulprostone, which as indicated (section II.B.2.b) is not an effective agonist at EP$_2$ receptors, fails to stimulate cAMP formation in RCCT cells. These results imply that EP$_2$ receptors are coupled to adenylate cyclase presumably through G$_s$, at least in RCCT cells. Evidence that EP$_2$ receptors interact directly with a G$_s$ is as follows. Depending on its concentration, PGE$_2$ can exert either stimulatory or inhibitory effects on cAMP formation by freshly isolated RCCT cells; furthermore, there are high- and low-affinity PGE$_2$-binding sites in these cells (Sonnenburg et al., 1990). In contrast to what is observed with freshly isolated RCCT cells, only the cAMP stimulatory effect of PGE$_2$ is observed with cultured RCCT cells; moreover, these cultured RCCT cells contain only one class of binding sites. Binding to this single class of sites is inhibited by guanine nucleotide derivatives. This latter finding argues that the EP$_2$ receptor of RCCT cells interacts with G$_s$.

In cells expressing the recombinant murine EP$_2$ receptor, PGE$_2$ increased the intracellular cAMP level without any change in inositol phosphate content (Honda et al., 1993). A threshold response was observed at 1 nM PGE$_2$, and a plateau was reached at 1 nM.

c. EP$_3$ RECEPTORS. The EP$_3$ receptor is an example of a promiscuous receptor that may couple to different second-messenger systems. Functionally, EP$_3$ receptors appear to be coupled to at least two different intracellular processes, one of which results in inhibition of autonomic neurotransmitter release, gastric acid secretion, and lipolysis, and another of which is involved in smooth muscle contraction (see section II.B.1.a.iii). The former are all responses classically mediated by inhibition of adenylate cyclase and the latter by increases in intracellular free Ca$^{2+}$, whether of intracellular or extracellular origin. In renal collecting tubule epithelia, occupancy of receptors having pharmacological properties of the EP$_3$ receptor causes inhibition of cAMP generation induced by treatment of the cells with arginine vasopressin (Sonnenburg and Smith, 1988). This receptor is apparently involved in vivo in inhibition of arginine vasopressin-induced water reabsorption (Smith, 1989). The activity of the renal EP$_3$ receptor is blocked by pertussis toxin, indicating that the receptor is coupled to a G$_i$ (Sonnenburg et al., 1990); furthermore, this same receptor has been partially purified in association with a pertussis toxin-sensitive G-protein (Watanabe et al., 1986, 1991a).

Thus, renal EP$_3$ receptors appear to be coupled to G$_i$ and are involved in the inhibition of cAMP formation. EP$_3$ receptors associated with inhibition of hormone-induced cAMP synthesis are also present in stomach, where they are involved in the inhibition of histamine-induced acid secretion (Reeves et al., 1988), and in adipose tissue, where they inhibit epinephrine-induced lipolysis (Butcher and Baird, 1968; Strong et al., 1992). Both of these latter EP$_3$-mediated events are inhibited by pertussis toxin.

PGE$_2$ and an EP$_3$ agonist, M&B 28767, decreased forskolin-stimulated cAMP level in cells expressing the mouse EP$_3$ receptor. Interestingly, although PGE$_2$ and M&B 28767 have similar affinities for the recombinant EP$_3$ receptor, the potency of the M&B compound on native EP$_3$ receptor-containing preparations is more than two orders of magnitude higher than that of PGE$_2$. This difference appears to reflect the greater efficacy of the M&B compound, resulting in a more efficient catalysis by the M&B-EP$_3$ receptor complex in stimulation of the coupling G-protein than the PGE$_2$-EP$_3$ receptor complex, as examined by Sugimoto et al. (1993).

It should also be noted that an EP$_3$ receptor is coupled via a pertussis toxin-insensitive G$_s$ to phospholipase C in bovine adrenal glands to cause catecholamine release (Negishi et al., 1989, 1990; Yokohama et al., 1988). The identity of this receptor has not been established. It could be either an EP$_1$ receptor or the EP$_{3d}$ receptor isoform recently cloned by Namba et al. (1993).

4. Molecular biology. a. EP RECEPTOR SUBTYPES. As discussed in more detail below, four EP$_3$ receptor isoforms were expressed from a bovine adrenal gland cDNA library by Namba et al. (1993) and designated EP$_{3A}$, EP$_{3B}$, EP$_{3C}$, and EP$_{3D}$ receptors when expressed in Chinese hamster ovary cells. EP$_{3A}$ and EP$_{3C}$ receptors couple to G$_i$ to induce inhibition of adenylate cyclase, EP$_{3B}$ and EP$_{3D}$ couple to G$_s$ to increase cAMP, and EP$_{3D}$ couples to G$_q$, in addition to G$_i$ and G$_s$, to induce pertussis toxin-insensitive PI turnover and calcium mobilization.

As described in section II.B.1.a.iii, EP$_3$ receptors mediate a variety of actions. Although some of these actions such as the inhibition of vasopressin-induced water reab-
sorption in kidney or histamine-induced gastric acid secretion and autonomic neurotransmitter release can be explained by EP3-mediated inhibition of adenylate cyclase (Garcia-Perez and Smith, 1984; Chen et al., 1988; Sonnenburg and Smith, 1990), this mechanism cannot explain other EP actions such as contraction of uterine and gastrointestinal smooth muscles, and it has been suggested that activation of EP receptors causes calcium mobilization in these tissues (Coleman et al., 1987a,b,c; Goureau et al., 1992). Although the tissue distribution of each EP isoform is not yet known, selected distribution could explain the various actions mediated by EP receptors. Again, it is interesting that to date no responses to EP receptor stimulation consistent with coupling to G, and elevation of intracellular CAMP have been reported, although little work has been performed in the cow.

Sugimoto et al. (1992) and Watabe et al. (1993) used the mouse TP receptor cDNA as a probe and isolated two independent cDNA clones by cross-hybridization from a mouse lung cDNA library. The receptors encoded by both clones were identical, but displayed specific [3H]PGE2 binding when expressed in cultured cells. Honda et al. (1993) used the cDNA obtained by Sugimoto et al. (1992) as a probe and isolated a third clone that also encoded a protein that exhibited high-affinity [3H]PGE2 binding. These three clones encode proteins of different sequences consisting of 365, 405, and 513 amino acid residues, respectively, that generate different signals on agonist binding. Analysis of the ligand-binding specificities as well as second-messenger generation suggested that these three clones correspond to the pharmacologically defined EP3, EP1, and EP2 receptor subtypes, respectively. Genetic loci for the mouse EP2 and EP3 genes, ptgerep2 and ptgerep3, respectively, were mapped to the centromeric region of chromosome 15 and the distal end of chromosome 3, respectively (Taketo et al., 1994). Ligand-binding properties of the recombinant EP receptors, as well as other prostanoid receptors as examined by their expression in cultured cells, are shown in table 7 and are discussed in section II.B.2.

b. EP3 isoforms. When Namba et al. (1993) used cDNA cloning of an EP receptor from a bovine adrenal gland cDNA library, they found that the EP3 mRNA undergoes alternative splicing in this tissue to produce at least four EP3 isoforms (EP3a, EP3b, EP3c, and EP3d). The deduced amino acid sequences of these isoforms are identical from the amino terminus to the tenth intracellular amino acids after the seventh transmembrane domain but differ thereafter (fig. 2). Perhaps not surprisingly, because they share the same transmembrane and extracellular structures, the four isoforms showed almost identical ligand-binding specificities. Yet, as discussed in section II.B.3, these isoforms couple to different G-proteins to induce different signaling in the cells.

The presence of the EP3 isoforms is not limited to the cow, because at least three EP3 isoforms, EP3a, EP3b, and EP3c, have been identified in human, rat, and mouse tissues. These isoforms differ in their transmembrane and extracellular domains, but their intracellular G-protein-coupling properties and sensitivity to agonist-induced desensitization vary. The EP3a isoform, which is most abundant in the bovine adrenal gland, couples to G1 more efficiently and is more sensitive to desensitization than the other isoforms (Sugimoto et al., 1992; Negishi et al., 1993). The third isoform (EP3c) can couple to G3 in addition to G1 and, at higher agonist concentrations, tends to stimulate adenylate cyclase (Irie et al., 1993).

C. FP Receptors

1. Functional studies. a. SELECTIVE AGONISTS AND ANTAGONISTS. While PGF2a is a potent FP receptor agonist, it is not very selective, having appreciable agonist activity at FP and TP receptors. However, two compounds in particular, fluprostenol and cloprostenol, both PGF2a analogues synthesized by ICI in the 1970s, have proved to be at least as potent as the parent compound at FP receptors but have much reduced agonist activity at other prostanoid receptors (Dukes et al., 1974; Coleman, 1987). Of these compounds, fluprostenol is by far the most selective and in fact is probably the most selective agonist so far described at any prostanoid receptor. Although cloprostenol is a highly selective FP receptor agonist, it also has some, albeit weak, agonist activity at EP receptors. Although there are various other potent FP receptor agonists, all of which are analogues of PGF2a, such as prostalene, fenprostalene, and tiaprost (Witherspoon et al., 1975; Jackson and Jessup, 1984), none is as selective as fluprostenol or cloprostenol. Although a few compounds have been reported to exhibit FP receptor-blocking activity, none has held up under close scrutiny. Thus, the N,N-dimethylamino and dimethylamido ana-
logues of PGF2α (Maddox et al., 1978) and an acetylenic analogue, K 10136 (Ceserani et al., 1979), have all been reported to block responses to PGF2α on preparations containing FP receptors, but all three appear to be agonists (Coleman et al., 1990). In no study reported to date has any evidence for a subdivision of FP receptors emerged. This may be a reflection of the small number of selective FP agonists available and the absence of any antagonists. Quantitative data for some selective FP receptor agonists are summarised in table 8.

b. DISTRIBUTION AND BIOLOGICAL FUNCTIONS. FP receptors have been demonstrated to exist in a variety of different tissues from a range of different species. One tissue in which they are particularly prevalent is the corpus luteum, where they mediate luteolysis. Indeed, they appear to be present in the corpora lutea of all species investigated, including human. Because of their presence in this tissue, and their role in a fundamental stage of the female reproductive cycle, there was excitement initially in the potential of FP agonists as agents to control human fertility (Dennefors et al., 1983). However, this was never to be because in humans, unlike many other animal species, prostanooids are not central to the destruction of the corpus luteum, and PGF2α and its analogues proved ineffective luteolytics. In a variety of other species, such as rat, hamster, cow, sheep, pig, and horse, PGF2α and analogues are highly effective (Cooper et al., 1979) and have been marketed as agents to synchronize the oestrus cycles of farm animals to facilitate animal husbandry.

In some rodents, but not guinea pig, and in humans, there are contractile FP receptors on the myometrium (Whalley and White, 1980; Senior et al., 1992). Interestingly, it is reported that in dogs, far from being the innocuous agents that they are in farm animals, FP agonists are lethal. In both dogs and cats, it is probably relevant, therefore, that there are FP receptors on airway smooth muscle, where they mediate contraction (Coleman, 1987); FP receptors have not been reported in the airways of any other species.

One tissue that has proved most useful in the study of FP receptors is the iris sphincter muscle from both cat and dog, where FP receptors mediate contraction (Coleman, 1983, 1987). In this tissue, FP receptors exist as homogeneous populations, and thus, much of the func-

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**Table 8**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Equieffective concentration (PGF2α = 1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluprostenol</td>
<td>0.2-1.0</td>
<td>Dong and Jones, 1982; Coleman, 1983; Woodward et al., 1990</td>
</tr>
<tr>
<td>Cloprostenol</td>
<td>0.3-0.5</td>
<td>Coleman, 1983</td>
</tr>
<tr>
<td>Prostalene</td>
<td>0.7-2.7</td>
<td>Coleman, 1983</td>
</tr>
</tbody>
</table>

* Data obtained on dog and cat iris sphincter and Swiss 3T3 cells.
tional work on FP receptors has been carried out on iris sphincter. The presence of FP receptors in ocular tissue has had important pharmacological consequences because PGF2α and various analogues such as latanoprost (Camras et al., 1992) have proven effective in lowering intraocular pressure in various species, including human, and are used to treat glaucoma. However, the exact site of action of these prostanoids in the eye to produce this effect is a matter of debate.

RNA blot analysis of mouse FP receptors showed two major hybridization bands at 2.3 and 6 kb, and, as expected, the highest expression was observed in the pregnant mouse ovary. Expression was also prominent in kidney and significant in lung, heart, and stomach. In situ hybridization analysis in ovary showed that the mRNA expression was observed exclusively in the large luteal cells of corpus luteum, whereas no labeling was found in any stages of ovarian follicle. This distribution of the receptor expression appears consistent with the known actions of PGF2α including its effects on luteolysis (Horton and Poyser, 1976), contraction of mesangial cells in kidney glomerulus (Mené et al., 1987), and bronchoconstriction (Wasserman, 1975).

2. Ligand-binding studies. A large number of studies have been performed on PGF-specific binding sites in membranes of corpora lutea from various species, including rat (Wright et al., 1979), rabbit (Losert et al., 1979), sheep (Kuehl, 1976; Hammarström et al., 1976), cow (Kimball and Lauderdale, 1975; Powell et al., 1976; Lin and Rao, 1978), and horse (Kimball and Wyngarden, 1977; Lin and Rao, 1978), preparations known to contain functional FP receptors. These all show high-affinity (<10 nM) binding for [3H]PGF2α and highly selective displacement by unlabeled PGF2α, and also in some studies by the potent and highly selective FP receptor agonist, fluprostenol (Kimball and Lauderdale, 1975; Kimball and Wyngarden, 1977; Lin and Rao, 1978; Wright et al., 1979). Significantly, the relative potencies of the prostanoids studied in inhibiting [3H]PGF2α binding to the luteal binding sites are remarkably similar to their relative agonist potencies at FP receptors (Coleman et al., 1990).

When expressed in COS cells, a recombinant FP receptor bound [3H]PGF2α with a Kd of 1.32 nM, and this [3H]PGF2α binding was displaced by unlabeled PGs in the order of PGF2α = 9α,11β-PGF2 > PGF1α > PGD2 > STA2 > PGE2 > iloprost. The affinity and specificity of this binding are in good agreement with the FP receptor characterized previously in corpus luteum membranes (Powell et al., 1974).

3. Second-messenger studies. FP receptor-mediated luteolysis is associated with an elevation of intracellular free Ca2+, which appears to be of intracellular origin (Behrman et al., 1985). Furthermore, Raymond et al. (1983) have shown that PGF2α induces PI turnover in isolated luteal cells. As noted in section II.C.1.b, PGF2α derivatives have been used to lower intraocular pressure in the treatment of glaucoma (Camras et al., 1989; Woodward et al., 1989). The order of potency of various prostanoids in lowering intraocular pressure is the same as that for contraction of the cat iris sphincter and for activation of Ca2+ mobilization in Swiss mouse 3T3 cells (Woodward et al., 1990; Woodward and Lawrence, 1994) where PGF2α acts as a mitogen (Jimenez de Asua et al., 1981; Nakao et al., 1993). The effect of PGF2α to induce Ca2+ mobilization in murine fibroblasts occurs in conjunction with formation of IP3 and is pertussis toxin insensitive (Gusovsky, 1991; Nakao et al., 1993; Quarles et al., 1993). These findings suggest that FP receptors can interact with a member of the G protein family to activate phospholipase C, leading ultimately to an IP3-mediated mobilization of Ca2+ from intracellular pools. However, there also appears to be a secondary phase of Ca2+ release by PGF2α-treated murine fibroblasts that is likely due to extracellular Ca2+ entry (Nakao et al., 1993).

PGF2α (1 to 1000 nM) added to COS cells transfected with a recombinant murine FP receptor evoked a concentration-related formation of inositol-1,4,5-trisphosphate, and the response reached a plateau of 180% of the control at 1 μM (Sugimoto et al., 1994a,b,c). These results indicate that the recombinant receptor also couples to activation of phospholipase C, resulting in a consequent Ca2+ mobilization.

4. Molecular biology. Sugimoto et al. (1994a,b,c), using bovine corpus luteum and mouse ovary, isolated a cDNA for the mouse FP receptor by homology-based polymerase chain reaction and cross-hybridization. The mouse FP receptor is a rhodopsin-type protein of 366 amino acids homologous to other prostanoid receptors. High homology is observed to the mouse TP receptor and EP1 receptor, 39.3 and 39.8% in total sequences, respectively.

D. IP Receptors

1. Functional studies. a. SELECTIVE AGONISTS AND ANTAGONISTS. PGI2 itself has been tested as an inhibitor of platelet aggregation for the treatment of thrombotic diseases and as a vasodilator for the treatment of vascular occlusive diseases. The chief problems with PGI2 itself are that it is chemically unstable, and thus, has too short a duration of action, and that it does not discriminate between platelet and vascular IP receptors. Both PGE1 and its 6-keto analogue are moderately potent IP receptor agonists, which are both more stable than PGI2, but suffer from the same vascular side effects. It is possible that PGI2 may also suffer from limited selectivity of action, but because of its short duration of action, non-vascular side effects are not limiting. A great deal of effort has been directed toward trying to identify more stable and more platelet-selective analogues. Although increased stability was relatively simple to achieve in all of the early analogues, it was at the expense of potency, and simple carbon analogues of PGI2, such as carbacy-
clin, are considerably weaker IP agonists than the parent compound. The first compound to combine chemical stability with high IP receptor agonist potency was the Schering compound, iloprost (Schrörr et al., 1981), which is at least as potent as PGI2 at IP receptors but is far more stable and has an extended duration of action in vivo (Skuballa et al., 1985). However, like the parent compound, iloprost fails to distinguish between platelet and vascular IP receptors, and therefore, anti-platelet activity is at the expense of profound vasodepression. Although in most respects iloprost is more IP receptor selective than is PGI2, particularly in their respective EP3 and TP receptor agonist activities, it is less selective in terms of EP1 receptor agonist activity. At EP1 receptors it is approximately equipotent with PGE2, albeit a partial agonist, and is at least 20-fold more potent than PGI2 (Sheldrick et al., 1988). A further development from Schering was cicaprost (Stürzebecher et al., 1985), which is slightly more potent than iloprost as an IP receptor agonist but with little or no agonist activity at any other prostanoid receptor at which it has been tested. There are now many other PGI2 analogues that are potent IP receptor agonists, but none has been tested in as comprehensive a way as iloprost and cicaprost. One other compound worthy of note is octimibate (Merritt et al., 1991), a compound with little obvious structural resemblance to PGI2, yet one that exhibits modest agonist activity at primate IP receptors. Interestingly, this compound is much weaker at IP receptors from other species, which is suggestive of species variability among IP receptors. However, even with octimibate, there is no indication of any difference between vascular and platelet IP receptors within a species. Quantitative data for some selective IP receptor agonists are summarised in table 9.

Although an enormous amount of chemistry has been undertaken to make analogues of PGI2, no IP receptor-blocking drug has yet been identified. One compound of interest is (5Z)-6a-carba-PGI2 (Corsini et al., 1987), which behaves as a full IP receptor agonist on human platelets and rabbit isolated mesenteric artery. There are many other PGI2 analogues that are potent IP receptor agonists, but none has been tested in as comprehensive a way as iloprost and cicaprost. One other compound worthy of note is octimibate (Merritt et al., 1991), a compound with little obvious structural resemblance to PGI2, yet one that exhibits modest agonist activity at primate IP receptors. Interestingly, this compound is much weaker at IP receptors from other species, which is suggestive of species variability among IP receptors. However, even with octimibate, there is no indication of any difference between vascular and platelet IP receptors within a species. Quantitative data for some selective IP receptor agonists are summarised in table 9.

Although an enormous amount of chemistry has been undertaken to make analogues of PGI2, no IP receptor-blocking drug has yet been identified. One compound of interest is (5Z)-6a-carba-PGI2 (Corsini et al., 1987), which behaves as a full IP receptor agonist on human platelets and rabbit isolated mesenteric artery. Therefore, this compound appears to possess a low efficacy at IP receptors and may represent a starting point toward the development of IP receptor-blocking drugs. Finally, one compound reported to be an IP receptor-blocking drug is another PGI2 analogue, FCE 22176 (Fassina et al., 1985). This compound antagonises the contractile effects of PGI2 on guinea pig tracheas, but this preparation does not contain IP receptors, contraction being mediated by EP1 and TP receptors (Coleman and Kennedy, 1985). It is more likely, therefore, that FCE 22176 is an EP1 receptor-blocking drug.

Despite considerable effort directed toward developing platelet-selective analogues of PGI2, there is no clear example of success, and there is no convincing evidence that IP receptors can be subclassified; the only differences that exist probably result from species variants of IP receptor rather than from true subtypes.

b. DISTRIBUTION AND BIOLOGICAL FUNCTIONS. PGI2 is synthesised primarily by the vascular endothelium, and it plays an important inhibitory role in local control of vascular tone and platelet aggregation (Moncada, 1982). It is not surprising, therefore, that IP receptors are localised in both blood platelets and vascular smooth muscle (Oliva and Nicosia, 1987). However, it has been observed that, whereas arterial homogenates appear to generate substantial amounts of PGI2, venous homogenates do not, generating, instead, PGE2 (Skidgel and Prinz, 1978). It is interesting, therefore, that vascular IP receptors appear to be confined to the arterial side of the circulation, and although venous smooth muscle does possess inhibitory prostanoid receptors, they are primarily of the EP type (Coleman et al., 1990).

IP receptors are also found in other tissues, particularly in sensory afferent nerves, where they mediate an excitatory response (Birrell and McQueen, 1993). PGI2 is a potent hyperalgesic agent, and IP receptors have been implicated in this action.

Northern blot analysis using the IP receptor cDNA revealed that its mRNA is most abundantly expressed in thymus, followed by spleen, heart/aorta, and lung, in this order (Namba et al., 1994). In the thymus, this receptor is expressed almost exclusively in the medulla, suggesting a novel immunomodulatory role for this prostanoid.

2. Ligand-binding studies. IP receptors are widely distributed on blood platelets, vascular smooth muscle, and nerve cells, and binding studies have been performed on membrane preparations from each (Schafer et al., 1979; Sieg et al., 1979b; Schilling and Losert, 1980; Schilling and Prior, 1980; Blair and MacDermot, 1981; Town et al., 1982; Leigh et al., 1984; Lombroso et al., 1984), variously using [3H]PGE2, [3H]PGI2, and [3H]iloprost as radioligand. Binding studies have also been performed on guinea pig lung homogenates, using [3H]PGI2 as ligand (MacDermot et al., 1981). In each case, the radioligand had high affinity (<10 nM), and in competition studies, a clear rank order of competitive potency was obtained: iloprost ≈ PGI2 > PGE2 > PGD2, PGE2, PGF2α.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Equieffective concentration (PGI2 = 1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbacyclin</td>
<td>3.5–30</td>
<td>Whittle and Moncada, 1985; Armstrong et al., 1989</td>
</tr>
<tr>
<td>Iloprost†</td>
<td>0.4–3.5</td>
<td>Casals-Stenzel et al., 1983; Stürzebecher et al., 1985; Humbles et al., 1991; Armstrong et al., 1989</td>
</tr>
<tr>
<td>Cicaprost</td>
<td>0.05–1.2</td>
<td>Stürzebecher et al., 1985; Armstrong et al., 1989; Humbles et al., 1991</td>
</tr>
<tr>
<td>Octimibate</td>
<td>29</td>
<td>Merritt et al., 1991</td>
</tr>
</tbody>
</table>

* Data obtained on human and rat platelets and bovine coronary artery.
† Also a potent EP1 receptor agonist (see table 3).
with PGE\textsubscript{1} being of the order of 10-fold less potent than 
PGL\textsubscript{2} (Coleman et al., 1990). This profile corresponds to
that associated with functional activity at IP receptors.

With the murine IP receptor expressed in cultured
Chinese hamster ovary cells, \textsuperscript{3}H iloprost binds with a
$K_d$ of 4.5 nM, and this binding is inhibited by various 
PGI analogues and other prostanoids in the order: cica-
prost > iloprost > PGE\textsubscript{1} > carbacyclin \Rightarrow PGD\textsubscript{2}, STA\textsubscript{2}, 
PGE\textsubscript{2} > PGF\textsubscript{2a}. Thus, the affinity of iloprost and the
specificity of the binding for the recombinant mouse IP 
receptor agree well with the previous characterization of 
the receptor in platelets and other sources (Leigh et al., 
1984; Armstrong et al., 1989; Hashimoto et al., 1990).

3. Second-messenger studies. As discussed in section
II.D.1.b, IP receptors are found principally in the arterial 
vasculature and circulating platelets, where along with 
TP receptors they participate in the reciprocal regulation 
of cAMP levels (Gorman et al., 1977a, 1978; Schafer et 
al., 1979; Siegl et al., 1979a,b; Halushka et al., 1989). IP 
receptors appear to couple to adenylate cyclase via $G_\text{i}$ 
(Siegl et al., 1979b; Hashimoto et al., 1990; Ito et al., 

Although cAMP generation is generally regarded as 
the sole signal transduction system for IP receptors, 
there have been several reports of PGI\textsubscript{2} and its analogues 
inducing increases in intracellular [Ca\textsuperscript{2+}] and evoking 
smooth muscle contraction (Whittle et al., 1979; Watan-
abe et al., 1991b; Lawrence et al., 1992; Vassaux et al., 
1992). Although these findings suggest the possibility 
that IP receptors may couple through calcium mobilisa-
tion, it is by no means certain that all of these effects 
are actually mediated by IP receptors and, even if they 
are, that the "excitatory effect" does not result from the 
release by the IP agonist of another mediator.

The recombinant IP receptor from mouse, when ex-
pressed in Chinese hamster ovary cells, stimulates aden-
ylate cyclase. The $EC_{50}$ of iloprost in this response was 
0.1 nM, which is 10-fold lower than that found for the p-
815 cells, from which the IP receptor was cloned. This 
suggests that the receptor-G-protein coupling is more 
efficient in the Chinese hamster ovary cell system. In 
addition to this well-known effect on adenyl cyclase, the 
recombinant receptor mediated phosphatidylinositol 
turnover at the higher iloprost concentrations, with an 
$EC_{50}$ of 100 nM; at 1 $\mu$M iloprost, IP\textsubscript{3} formation was 
observed 30 s after the agonist addition and reached a 
maximum about 3-fold over the control at 2 min.

cDNA for the mouse IP receptor by polymerase chain 
reaction-based homology screening from the library of 
mouse mastocytoma p-815 cells, which express a high 
amount of the receptor. It is a protein of 417 amino acids 
with a calculated $M_\text{r}$ of 44,722, and the amino acid 
sequence in the transmembrane domains shows the high-
est homology, 39%, to the EP\textsubscript{2} receptor; the homology to
other receptors is 32, 28, and 32% to EP\textsubscript{3}, EP\textsubscript{1}, and TP, 
respectively.

There have been some concerns regarding the exist-
ence of subtypes or isoforms of the PGI receptor. How-
ever, there has been no evidence for the presence of 
homologous molecules or alternatively spliced variants 
of the cloned receptor.

E. TP Receptors

1. Functional studies. a. SELECTIVE AGONISTS AND 
ANTAGONISTS. The situation with regard to the availa-
bility of TP receptor-active agents is unlike that for 
other prostanoid receptors in that there are few selective 
agonists, but many antagonists, and these are of a wide 
variety of chemical types. The only selective TP receptor 
agonists that have been at all widely used are the 9,11-
epoxymethano and 9,11-methanoepoxy analogues of 
PGH\textsubscript{2}, U44069 and U46619 (Malmsten, 1976). Of these 
two, U46619 is the more selective, and the most fre-
cently used, and appears to have an agonist profile 
similar to that of TX\textsubscript{A}\textsubscript{2} itself (Coleman et al., 1981b). 
Although other TP receptor agonists [e.g., 9,11-azo PGH\textsubscript{2} 
(U-57093), PGF\textsubscript{2a} acetal and EP 011] have been 
developed and used experimentally, they are not only less 
selective but in most cases also less potent (Corey et al., 
1975; Portoghese et al., 1977; Wilson and Jones, 1985).
However, there are exceptions, such as EP171, SQ 26655, 
I-BOP, and STA\textsubscript{2} (Sprague et al., 1985; Wilson and 
Jones, 1985). Of these compounds, EP171 has a unique 
profile, being exceptionally long acting.

The many antagonists that exist are of a wide range of 
structural types, some more or less closely related to 
TX\textsubscript{A}\textsubscript{2}, some loosely prostanoid in nature, and others that 
are of quite different structural origin. Some of the earliest TP receptor-blocking drugs identified on blood 
platelets were TX\textsubscript{A}\textsubscript{2} analogues, such as 9,11-azaprosta- 
5,13-dienoic acid, 9,11-epoxyimino-5,13-dienoic acid, 
carbathomoxane, and pinane TX (Gorman et al., 
1977a; Fitzpatrick et al., 1978; Nicolaou et al., 1979), but 
these compounds behaved as agonists on vascular prepa-
trations. This probably does not constitute evidence for 
receptor subtypes but, rather, reflects the fact that these 
compounds are partial agonists, and the TP receptors in 
smooth muscle appear to be more efficiently coupled 
than those in platelets. Other compounds that, although 
broadly prostanoid in nature, are less closely related to 
TX\textsubscript{A}\textsubscript{2} include the 7-oxabicyclo [2.2.1] heptane analogues, 
such as SQ 29548 (Sprague et al., 1980), AH19437, 
AH23848, and vapiprost (Coleman et al., 1981a; Brittain 
et al., 1985; Lumley et al., 1989), EP 045 and EP 092 
(Jones and Wilson, 1980; Armstrong et al., 1985), and 
ICI 192605 (Brewster et al., 1988). In addition to such 
prostanoid-related compounds, there are others that bear 
no obvious structural resemblance to the prostanoids 
but, nevertheless, are potent TP receptor-blocking drugs. 
Examples of such compounds are trimetoquinol, the ben-
zylsulfonamide, daltroban, the indole-2-propanoic acid, L-655240 (Hall et al., 1987; Lefer, 1988), and BAY u 3405 (Rosentretreter et al., 1989). Each of these compounds is a relatively highly potent antagonist, with \( pA_2 \) values in the range 7.0 to 9.0, but for only some is there any information regarding their TP receptor selectivity. Thus, it is clear that only AH19437, AH23848, vapiprost, EP 045, EP 092, ICI 192605, and BAY u 3405 are without antagonist activity at DP, EP\(_1\), EP\(_2\), EP\(_3\), FF, or IP receptors (Coleman and Humphrey, 1993), although AH23848 does have some additional weak antagonist activity at EP\(_4\) receptors (Coleman et al., 1994). Quantitative data for some selective TP receptor agonists and antagonists are summarised in Table 10.

### Table 10

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>( pA_2 )</th>
<th>References</th>
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<tbody>
<tr>
<td>AH19437</td>
<td>5.9-6.6</td>
<td>Coleman et al., 1981a,b</td>
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<tr>
<td>AH23848</td>
<td>7.8-8.3</td>
<td>Brittain et al., 1985</td>
</tr>
<tr>
<td>GR32191 (vapiprost)</td>
<td>8.2-8.8</td>
<td>Lumley et al., 1989</td>
</tr>
<tr>
<td>EP 045</td>
<td>5.9-7.1</td>
<td>Jones et al., 1981</td>
</tr>
<tr>
<td>EP 092</td>
<td>7.2-8.4</td>
<td>Armstrong et al., 1985</td>
</tr>
<tr>
<td>SQ 29548</td>
<td>7.8-9.1</td>
<td>Oglister et al., 1985</td>
</tr>
<tr>
<td>ICI 129505</td>
<td>8.2-8.4</td>
<td>Brewster et al., 1988</td>
</tr>
<tr>
<td>L-655240</td>
<td>8.0-8.4</td>
<td>Hall et al., 1987</td>
</tr>
<tr>
<td>BAY u 3405</td>
<td>8.1-8.9</td>
<td>McKenniff et al., 1991</td>
</tr>
<tr>
<td>S-145</td>
<td>8.5-9.0†</td>
<td>Hanasaki and Arita, 1988</td>
</tr>
<tr>
<td>BM 13505 (daltroban)</td>
<td>6.7-7.9</td>
<td>Lumley et al., 1989</td>
</tr>
</tbody>
</table>

* Data obtained on platelets and vascular smooth muscle from a range of species.

† \( K_i \) values from binding experiments on platelets from rat, rabbit, and human.

be pathological or pathophysiological in nature. TP receptors do not appear to be widely distributed in cells other than smooth muscle and platelets, but they are present in myofibroblasts (Coleman et al., 1989) and may well play a role in wound healing and scar formation. They also exist in mesangial cells in the kidney, where they mediate an increase in filtration rate (Scharschmidt et al., 1986), and in epithelium of the gastrointestinal tract, where they mediate an increase in secretion (Bunce and Spraggs, 1987; Clayton et al., 1988).

Recently, Namba et al. (1992) examined the tissue distribution of TP receptor mRNA by Northern blot analysis in various mouse tissues. This study revealed that the TP receptor is expressed most abundantly in thymus, followed by spleen, lung, kidney, heart, and uterus, in that order. Traces of TP receptor mRNA were observed in brain. In a similar analysis of human tissues (Hirata et al., 1991), placenta and cultured megakaryocytic leukaemia cells showed higher expression than lung. Thus, along with placenta and platelet precursor cells, thymus is one of the richest tissues in receptor expression. Ushikubi et al. (1993) examined a thymic cell population expressing the receptor by means of radioligand binding and found that the thymic lymphocytes express TP receptors at a density comparable to that found in human platelets. TP receptors are most highly expressed in immature thymocytes, such as CD4^-8^- and CD4^-8^+ cells, but the numbers decrease during T-cell development. They also found that the addition of a TP agonist induced apoptotic cell death of these immature thymocytes and that this action was antagonized by a TP receptor antagonist. These results, together with the finding that TX synthase is rich in dendritic cells and macrophages in thymus (Nüsing et al., 1990; Homod-Larache et al., 1985), led these authors to suggest that TXA\(_2\) is released from these stromal cells and acts on thymocytes under some physiological conditions. Thus, in addition to its well-known actions in cardiovascular and respiratory systems, TP receptors may play a role in thymocyte differentiation and development.

2. **Ligand-binding studies.** Ligand-binding studies with TP receptors are very much more numerous than those with any of the other prostanoid receptors, and there has also been a greater variety of ligands, both agonist and antagonist, used. Such studies have been performed predominantly on membrane preparations from platelets, but vascular and bronchial smooth muscle have also been used. Agonist ligands include \(^{3}H\)U44069 (Armstrong et al., 1983), \(^{3}H\)U46619 (Kattelman et al., 1986), and \(^{125}I\) -BOP (Morinelli et al., 1990), the latter having a particularly high affinity of 2 nM, compared with values of about 100 nM for the other two compounds. Antagonist ligands used in earlier studies were \(^{3}H\)13-azaprostanolic acid (Hung et al., 1983) and \(^{125}I\)13-aza,16-p-hydroxyphenylpinane TxA\(_2\) (Mais et al., 1985a,b; Halushka et al., 1986), but both of these compounds have relatively
low affinities, of the order of 100 and 20 nM, respectively. More recently, a variety of radiolabeled TP receptor-blocking drugs have been developed as ligands, and these have very much higher affinities for TP receptors; examples of these ligands are [³H]SQ 29548 (Hedberg et al., 1988), [³H]GR32191B (Armstrong et al., 1993), and [¹²⁵I]S-145 (Kishino et al., 1991), all of which have Kd values of <10 nM; for [¹²⁵I]S-145, Kishino et al. (1991) reported Kd values of 0.2 and 0.4 nM, respectively, at TP receptors on membranes prepared from platelets and vascular smooth muscle.

Ligand-binding properties of recombinant TP receptors have been examined following receptor expression in cultured COS cells. The human and mouse receptors showed specific binding of the selective TP receptor ligand, [³H]S-145, with a Kd of 1.2 and 3.3 nM, respectively; this binding is displaced selectively by various TP analogues in the order of S-145 > ONO-3708 > STA2 > U46619. Other PGs, such as PGD₂, also displace the binding, but their potencies are more than two orders of magnitude less than those of the above compounds.

3. Second-messenger studies. Studies of the relationship between TP receptor agonists and antagonists and second-messenger generation have been conducted largely on platelets and vascular smooth muscle cell preparations. In the case of platelets, there appear to be two separable effects with different pharmacologies. S-145 and U44619 both cause an initial platelet shape change, but only U44619 causes platelet aggregation (Arita et al., 1989; Hansaki and Arita, 1988); in fact, S-145 antagonises platelet aggregation mediated through TP receptors. S-145 causes an increase in the cytosolic Ca²⁺ concentration which is independent of extracellular Ca²⁺ (Nakano et al., 1988) and which does not involve changes in IP₃ levels (Arita et al., 1989), suggesting that platelet shape change involves mobilization of Ca²⁺ from intracellular stores via an IP₃-independent process. Platelet aggregation itself involves mobilization of Ca²⁺ from intracellular pools but in association with activation of phospholipase C and consequent production of IP₃ (Arita et al., 1989; Brass et al., 1987; Pollock et al., 1984; Sage and Rink, 1987; Siess et al., 1986; Watson et al., 1985); platelet aggregation also requires extracellular Ca²⁺. In platelets, the effects of U44619 are not inhibited by pertussis toxin (Brass et al., 1987). This finding, in combination with results indicating that TP receptor occupancy leads to phospholipase C activation, suggest that platelet TP receptors are coupled in part to a pertussis toxin-insensitive G-protein of the Gₐ family. In support of this suggestion, Shenker et al. (1991) showed that antibodies specific for the Gₐ family block U44619-induced phospholipase C activity; moreover, Knezevic et al. (1993) showed that TP receptors purified by affinity chromatography are associated with two G-proteins; one is a Gₐ (Mₐ 42,000) and another has a higher Mₐ (85,000). Overall, these studies suggest that platelet TP receptors couple directly to Gₐ to cause phospholipase C activation, IP₃-dependent Ca²⁺ mobilization, and activation of protein kinase C through diglyceride formation and further that TP receptors couple to a second G-protein. It is not clear whether coupling to the Mₐ, 85,000 G-protein is involved in IP₃-independent Ca²⁺ mobilization and/or a Ca²⁺ channel that mediates the entry of extracellular Ca²⁺.

U44619 is well-known to cause contraction of vascular smooth muscle strips (Dorn et al., 1992; Yamagishi et al., 1992). Treatment of cultured smooth muscle cells with U44619 causes an increase in IP₃, the mobilization of intracellular Ca²⁺, and phosphorylation of myosin light chain kinase (Dorn et al., 1992; Miki et al., 1992; Yamagishi et al., 1992). TP receptor-blocking drugs inhibit the Ca²⁺ mobilization caused by U44619. These results suggest that smooth muscle contraction occurring in response to agonists involves interaction with a G-protein coupled to phospholipase C; the parallel to second-messenger generation associated with TP receptor-mediated platelet aggregation is obvious. Vascular endothelial cells also contain TP receptors, and treatment of bovine aortic endothelial AG4762 cells with U44619 causes increases in intracellular Ca²⁺ concentrations. The mechanism underlying TP receptor-mediated Ca²⁺ by endothelial cells is not yet known.

Signal transduction examined by expression of the recombinant murine TP receptors revealed that when stimulated by an agonist, the receptor evoked PI turnover and subsequent Ca²⁺ release, as suggested by studies with cells and membranes containing the native TP receptor (Arita et al., 1989).

4. Molecular biology. Ushikubi et al. (1989), using a TP receptor antagonist, S-145, as an affinity ligand, purified the TP receptor about 9000-fold to apparent homogeneity from solubilized membranes from human blood platelets. Based on partial amino acid sequences obtained from the purified protein, Hirata et al. (1991) cloned a cDNA for the human TP receptor from MEG-01 human megakaryocytic leukaemia cell and human placenta cDNA libraries. A mouse homologue was subsequently isolated by polymerase chain reaction and hybridization screening (Namba et al., 1992). The human and mouse TP receptors consist of 343 and 341 amino acids, respectively, and are 76% identical in the overall sequences. The receptors have an N-terminal extracellular portion of <30 amino acids, and the fourth and 16th asparagine in the sequence suggests that these hydrophobic amino acids form a hydrophobic pocket for the hydrophobic ring structure of TxA₂ (Yamamoto et al., 1993). The cytoplasmic loops are short, and there are two conserved potential protein kinase C phosphorylation sites in the second cytoplasmic

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loop, which together with several serine and threonine residues in the C terminus, may be involved in phosphorylation-mediated receptor desensitization (Okwu et al., 1992). Indeed, desensitization of TP receptors has been reported (Murray and FitzGerald, 1989). The carboxy terminal cytoplasmic tail is also short, and there is no cysteine residue in this region, suggesting that the C termini of these receptors are not membrane tethered by myristoylation but present free in the cytoplasm.

There has been some controversy regarding the existence of TP receptor subtypes (Halushka et al., 1989; Ogletree and Allen, 1992). To clarify this issue, Nüssing et al. (1993) cloned and characterized the gene for the TP receptor from a human genomic library. The gene is present as a single copy per haploid, spans 15 kb and contains three exons divided by two introns. The first intron is 6.3 kb long and exists in the 5’-noncoding region, 83 bp upstream from the ATG start site. Intron 2 is 4.3 kb long and is located at the end of the sixth putative transmembrane domain and separates it from the downstream coding region. When reverse transcription and polymerase chain reaction analysis using primers flanking intron 2 was performed on poly(A)+ RNA from MEG-01 cells, human platelets, and meanagial cells, it amplified only a single DNA fragment having the expected size of 173 bp. This suggests that no alternative splicing of the coding region occurs and that only one type of TP receptor protein is formed from this gene in these cells. Although these findings cannot exclude the presence of a less homologous TP receptor, they provide support for the existence of a single form of the TP receptor in both platelets and smooth muscle cells. The chromosomal localization of this receptor gene was assigned to 19p13.3 of the human chromosome. The mouse TP receptor gene (Txa2r) has been mapped to chromosome 10 (Taketo et al., 1994).

III. Conclusions

The classification of prostanoid receptors was originally based solely on functional data, obtained by means of comparisons of rank orders of agonist activity, and where possible, also the effects of antagonists. A comprehensive classification was developed using this approach, which covered many, if not all, of the actions of prostanoids. Broadly speaking, the classification has also proved consistent with the developing knowledge of second-messenger coupling. Thus, DP, EP2, and IP receptors are coupled at least predominantly to Gαq and Gαi and Gαq and FP and TP largely to Gαq. The emerging molecular biology of the prostanoid receptors fully supports the functional classification, with EP1, EP2 (or EP4), EP3, FP, IP, and TP receptor cDNAs so far having been cloned. So far, no orphan prostanoid receptor cDNA has been cloned. Our understanding of EP3 receptors has been considerably expanded by the knowledge that splice variants exist, and these exhibit quite distinct character-

istics with respect to second-messenger coupling. Doubtlessly, the DP receptor will soon be cloned, and we will gain insight into whether there also exist subtypes of DP, FP, IP, and TP receptors. It is an exciting time for prostanoid research, but it is a sobering thought that when the vast numbers of prostanoid analogues were being synthesised in the 1970s and 1980s, little was known about the receptors at which prostanoids act. Without the benefits of our current level of understanding, it seems that much of this chemical effort was premature. Many of these compounds should now be reevaluated. During the next decade, we will learn a lot more about prostanoid receptors, their species variance, their structural diversity, their function, their distribution, and the potential for their therapeutic exploitation with agonists and antagonists.


WHITTLE, B. J. R., MONCADA, S., AND VANE, J. R.: Comparison of the effects of prostacyclin (PG\textsubscript{I}\textsubscript{2}), prostaglandin E\textsubscript{1} and D\textsubscript{2} on platelet aggregation in different species. Prostaglandins 16: 373-388, 1978.


