0031-6997/94/4602-0205$03.00/0
PHARMACOLOGICAL REVIEWS
Copyright © 1994 by The American Society for Pharmacology and Experimental Therapeutics
Vol. 46, No. 2
Printed in U.S.A.

PHARMACOLOGICAL REVIEWS

VIII. International Union of Pharmacology Classification of Prostanoid Receptors: Properties, Distribution, and Structure of the Receptors and Their Subtypes

ROBERT A. COLEMAN,[1] WILLIAM L. SMITH,[2] AND SHUH NARUMIYA[3]

[1]Glaxo Research and Development, Ltd., Ware, Hertfordshire, England; [2]Department of Biochemistry, Michigan State University, East Lansing, Michigan, USA; and [3]Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto 606, Japan

I. Introduction 206
A. Historical background 206
B. Studies of receptor identification and classification 206
1. Functional studies 206
2. Radioligand-binding studies 207
3. Second-messenger studies 207
4. Molecular biology 208
II. Types, subtypes, and isoforms of prostanoid receptors 211
A. DP receptors 211
1. Functional studies 211
a. Selective agonists and antagonists 211
b. Distribution and biological function 211
2. Ligand-binding studies 211
3. Second-messenger studies 211
B. EP receptors 212
1. Functional studies 212
a. Selective agonists and antagonists 212
i. EP_1 receptors 212
ii. EP_2 receptors 212
iii. EP_3 receptors 212
iv. EP_4 receptors 213
b. Distribution and biological functions 213
2. Ligand-binding studies 214
a. EP_1 receptors 214
b. EP_2 receptors 215
c. EP_3 receptors 215
3. Second-messenger studies 215
a. EP_1 receptors 215
b. EP_2 receptors 215
c. EP_3 receptors 216
4. Molecular biology 216
a. EP receptor subtypes 216
b. EP_3 isoforms 217
C. FP receptors 217
1. Functional studies 217
a. Selective agonists and antagonists 217
b. Distribution and biological functions 218
2. Ligand-binding studies 219
3. Second-messenger studies 219
4. Molecular biology 219
D. IP receptors 219
1. Functional studies 219

Downloaded from by guest on July 31, 2017

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

a. Selective agonists and antagonists ... 219
b. Distribution and biological functions ... 220
2. Ligand-binding studies ... 220
3. Second-messenger studies ... 221
4. Molecular biology ... 221
E. TP receptors ... 221
1. Functional studies ... 221
a. Selective agonists and antagonists ... 221
b. Distribution ... 222
2. Ligand-binding studies ... 222
3. Second-messenger studies ... 223
4. Molecular biology ... 223
III. Conclusions ... 224
IV. References ... 224

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, PGE_1 and $PGF_{1\alpha}$. 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 PGA_2 to PGH_2. Of these, PGA_2, PGB_2, and PGC_2 are prone to extraction artifacts (Schneider et al., 1966; Horton, 1979). PGG_2 and PGH_2 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-eicosatrienoic 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, TXA_2 (Hamberg et al., 1975) and PGI_2 (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

* Abbreviations: PG, prostaglandin; TX, thromboxane; PGI_2, prostacyclin; G_s, stimulatory G-protein; G_i, inhibitory G-protein; G_q, pertussis toxin-insensitive G-protein; RCCT, rabbit cortical collecting tubule.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

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 β_1 and β_2. 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 D_2, E_2, $F_{2\alpha}$, I_2, and TXA_2, 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 to this day no other examples of a family of hormones that demonstate 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 EP_1, EP_2, EP_3, and EP_4. 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 EP_1, EP_2 (or EP_4), and EP_3. 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 [^{3}H]PGs E_1, E_2, or $F_{2\alpha}$, 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 [^{3}H]PGs D_2 and I_2, 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 unlabeled 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 PGE_2-binding sites was accompanied by a reduction in PGE_2-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

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

TABLE 1
*Classification and nomenclature of prostanoid receptors, with selective agonists and antagonists, and system of response transduction**

Receptor/subtype	Selective agonists	Selective antagonists	Transduction system
DP	BW 245C, ZK110841, RS 93520	BW A868C, AH6809†	↑cAMP via G_s
EP			
EP_1	Iloprost,‡ 17-phenyl PGE_2, sulprostone‖	AH6809,[7]§ SC-19220	↑Intracellular Ca^{2+}
EP_2	Butaprost, AH13205, misoprostol‖	None	↑cAMP via G_s
EP_3	Enprostil, GR63799, sulprostone,¶ misoprostol,** M&B 28767††	None	↓cAMP via G_i ↑PI turnover via G_q
EP_4	None	AH22921,‡‡ AH23848‡‡	↑cAMP via G_s?
FP	Fluprostenol, cloprostenol, prostalene	None	↑PI turnover via G_q
IP	Cicaprost, iloprost, octimibate	None	↑cAMP via G_s
TP	U44069, U46619, SQ 26655, EP 011, I-BOP	AH23848,§§ GR32191, EP 092, SQ 29548, ICI 192605, L-655240, BAY u 3405, S-145, BM 13505	↑PI turnover via G_q

* For more detailed information, see relevant section in text.
† Also EP_1 receptor-blocking drug.
‡ Also IP receptor agonist.
§ Also DP receptor-blocking drug.
‖ Also EP_3 receptor agonist.
¶ Also EP_1 receptor agonist.
** Also EP_2 receptor agonist.
†† Also TP receptor agonist.
‡‡ Also TP receptor-blocking drug.
§§ Also EP_4 receptor-blocking drug.

relationship between PGEs and adenylyl cyclase was reported in platelets (Shio and Ramwell, 1972). PGE_1 caused an elevation of platelet cAMP, but PGE_2 caused a reduction. Interestingly, this distinction was reflected in the effects of PGE_1 and PGE_2 on platelet aggregation. PGE_1 inhibited aggregation, whereas PGE_2 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 PGE_1 and PGE_2 were seen to exert marked effects on levels of cAMP, both stimulatory and inhibitory, $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$ 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 ^{3}H-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 EP_1 receptor, the mouse EP_1, EP_2,

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

TABLE 2

Glossary of the chemical names of prostanoid agonists and antagonists quoted as code numbers in this review

Code	Chemical name
AH6809	6-isopropoxy-9-oxoxanthene-2-caboxylic acid
AH13205	*trans*-2-[4-(1-hydroxyhexyl)phenyl]-5-oxocyclo pentaneheptanoic acid
AH19437	1α(Z),2β,5α(±)-methyl,7-2-(4-morpholinyl)-3-oxo-5(phenylmethoxy)cyclopentyl-5-heptenoate
AH22921	[1α(Z),2β,5α]-(±)-7-[5-[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-5-heptenoic acid
AH23848	[1α(Z),2β,5α]-(±)-7-[5-[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid
AY23626	11-deoxy-prostaglandin E_0
BAY u 3405	3(*R*)-3-(4-fluorophenylsulphonamido)-1,2,3,4-tetrahydro-9-carbazole propanoic acid
BW 245C	5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin
BW A868C	3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin
EP 011	17,18,19,20-tetranor-16-*p*-fluorophenoxy-9,11-etheno PGH_2
EP 045	(±)-5-*endo*-(6′-carboxyhex-2′Z-enyl)-6-*exo*[N-(phenylcarbamoyl)hydrazono-methyl]-*bicyclo*[2.2.1] heptane
EP 092	9α,11α-ethano-ω-heptanor-13-methyl-13-phenyl-thio-carbamoyl-hydrazino-prosta-5Z-enoic acid
EP171	10a-homo-15S-hydroxy-9α,11α-epoxy-16*p*-fluorophenoxy-ω-tetranor-5Z,13E-dienoic acid
FCE 22176	(+)-13,14-didehydro-20-methyl-carboprostacyclin
GR32191	[1*R*-[1α(Z)2β,3α,5α]]-(+)-7-[5-[[(1,1′-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid
GR63799	[1*R*-[1α(Z),2β(*R**),3α]-(−)-4-benzoylamino)phenyl-7-[3-hydroxy-3-phenoxypropoxy)-5-oxocyclopentyl]-4-heptenoate
I-BOP	[1*S*-(1α2β(5Z),3α(1E,3*S**),4-α)]-7-[3-(3-hydroxy-4-(4′-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1]heptan-2-yl]-5-heptenoic acid
ICI 192605	4(Z)-6-(2-*o*-chlorophenyl-1,3-dioxan-*cis*-5-yl) hexenoic acid
K 10136	13,14-didehydro-20-methyl $PGF_{2\alpha}$
L-655240	3-[1-(4-chlorobenzyl)-5-fluoro-3-methyl-indol-2-yl]2,2-dimethyl-propanoic acid)
M&B 28767	15S-hydroxy-9-oxo-16-phenoxy-ω-tetranorprost-13E-enoic acid
N-0164	Sodium *p*-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl] phenyl phosphonate
ONO-3708	(9,11)-(11,12)-dideoxa-9α,11α-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epi-TXA_2
RS 93520	Z-4-{(C3′*S*,1*R*,2*R*,3*S*,6*R*)-2C3′-cyclohexyl-3′-hydroxyprop-1-ynyl)-3-hydroxybicyclo[4.2.0]oct-7-ylidene} butyric acid
S-145	(1*S*,2*R*,3*R*,4*R*)-(5Z)-7-(3-[phenyl,sulphonyl-amino-bicyclo[2.2.1] hept-2-yl)hept-5-enoic acid
SC-19220	1-acetyl-2-(8-chloro-10,11-dihydodibenz[b,f][1,4]oxazepine-10-carbonyl) hydrazine
SQ 26655	(1*S*-(1a,2b(5Z),3a(1E,3*s**),4a))-7-(3-(3-hydroxy-1-octenyl)-7-oxabicyclo(2.2.1)hept-2-yl)-5-heptenoic acid
SQ 29548	[15-[1α,2β(5Z),3β,4α]-7-[3-[2-(phenylamino)-carbonyl]hydrazino] methyl]-7-oxobicyclo-[2,2,1]-hept-2-yl]-5-heptenoic acid
STA_2	9α,11α-thia-11a-carba-prosta-5Z,13E-dienoic acid
U44069	9α,11α-epoxymethano-15S-hydroxy-prosta-5Z,13E-dienoic acid
U46619	11α,9α-epoxymethano-15S-hydroxy-prosta-5Z,13E-dienoic acid
ZK110841	9-deoxy-9β-chloro-16,17,18,19,20-pentanor-15-cyclohexyl-$PGF_{2\alpha}$

and EP_3 receptors, the rat EP_3 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 EP_2, EP_3, and TP receptors on chromosomes 15, 3, and 10, respectively. On the other hand, as shown

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.

WHAT WILL YOU BUILD? | sales@docketalarm.com | 1-866-77-FASTCASE

fastcase®
Smarter legal research.