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THROMBOXANE, PROSTAGLANDIN AND LEUKOTRIENE RECEPTORS

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INTRODUCTION

Thromboxanes (TX), prostaglandins (PG), and leukotrienes (LT) (eicosanoids) are metabolites of the the 20-carbon fatty acid arachidonic acid. Current evidence supports potential pathophysiologic roles for these autocoids in many diseases (1–3). While the synthesis and metabolism of these autocoids have been studied extensively in normal and pathologic states, the study of their receptors has lagged far behind. With the development of drugs that will be targeted to these receptors, there is a pressing need to improve our understanding of the fundamental characteristics of these important receptors.

PROSTAGLANDIN H_2 AND THROMBOXANE A_2 RECEPTORS

Introduction

Prostaglandin H_2 is the precursor for thromboxane A_2. Both are labile and have half-lives of approximately 5 min and 30 sec, respectively. They appear to share the same pharmacological properties in virtually every tissue in which they have been studied. Their major pharmacological actions are constriction of vascular and bronchial smooth muscle and aggregation of platelets (4, 5). Because they share these pharmacological properties, they presumably share a common receptor, which has been named the TXA_2/PGH_2 receptor.

Because PGH_2 and TXA_2 are so labile, stable structural analogs have been synthesized that have been used as pharmacological tools to provide evidence

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for the presence of the receptors in many tissues. Availability of numerous receptor antagonists has facilitated the study of these receptors (6, 7). In the main, these compounds have been synthesized as potential therapeutic agents but have also provided valuable tools for the study of the receptors.

DISTRIBUTION AND CLASSIFICATION OF TXA_2/PGH_2 RECEPTORS Pharmacological evidence for the presence of TXA_2/PGH_2 receptors exists for many diverse tissues and cells (Table 1). Although most of the studies have focused on cells of mammalian origin, evidence for the presence of putative TXA_2/PGH_2 receptors has been obtained even in amphibia (8, 9). Studies of the platelet TXA_2/PGH_2 receptors have shown significant species differences. Of those species studied, the guinea pig platelet TXA_2/PGH_2 receptor (5, 10) appears to most closely resemble the human, whereas the dog, rat, and rabbit receptors differ considerably from the human and each other (5, 11). The vascular TXA_2/PGH_2 receptors have not been characterized as extensively as have the platelet receptors. While quantitative differences have been found among the species or vessels so far studied, there do not appear to be major differences. Whether major differences do exist, is still an open question worthy of pursuit. Of the various blood vessels that have been studied, the canine saphenous vein appears to be one of the most sensitive to TXA_2/PGH_2 mimetics (12, 13).

Although platelet TXA_2/PGH_2 receptors have species differences the platelet and vascular TXA_2/PGH_2 receptors appear different irrespective of species. Early studies of the platelet and vascular receptors using single compounds, different species, or both provided evidence for subclasses of receptors (14–16), while other studies provided ambiguous evidence (17). Mais et al (13, 18, 19), in a series of experiments using structural analogs of 13-azapinane TXA_2 and isolated saphenous veins and platelets from canine and human sources, provided unequivocal evidence for two subclasses of TXA_2/PGH_2 receptors. They found that the canine and human saphenous vein TXA_2/PGH_2 receptors were similar but the human platelet and vascular TXA_2/PGH_2 receptors were significantly different (13, 18, 19). They named the platelet receptors $[TXA_2/PGH_2]_\alpha$, α for aggregation, and the vascular receptors, $[TXA_2/PGH_2]_\tau$, τ for tone. Within the subclass of $[TXA_2/PGH_2]_\alpha$ receptors may be further subclasses (20). Whether other subclasses of TXA_2/PGH_2 receptors exist, irrespective of species differences, remains to be determined. Indeed, preliminary evidence indicates that pulmonary TXA_2/PGH_2 receptors represent still another subclass (21, 22).

Second Messenger Systems

The second messenger system(s) for TXA_2/PGH_2 receptors has been studied most extensively in the platelet. TXA_2/PGH_2 and their stable mimetics induce

Table 1 Distribution of TXA_2/PGH_2 receptors

Tissue/cell	Pharmacologic studies[a]	Radioligand binding studies[b]	References
Platelets			
Human, rabbit, rat, guinea pig	+	+	(see 5 for review)
Vascular			
Dog saphenous vein	+	−	8, 9
Rat aorta	+	−	10
Smooth muscle cells	+	+	60
Endothelial cells	+	+	61
Rabbit aorta	+	−	12
Human placenta	+	+	73
Human umbilical vessels	+	−	74, 75
Guinea pig trachea	+	−	12
Human monocytes	+	−	76, 77
Rat glomerular mesangial cells	+	−	78
Toad bladder epithelial cells	+	−	9

[a]Plus sign indicates pharmacologic studies were performed using agonists and antagonists to provide evidence for the presence of the receptors.

[b]Plus sign indicates radioligand binding studies were performed; minus sign means no radioligand binding studies were performed.

shape change, secretion, aggregation, exposure of fibrinogen receptors, and phosphorylation of myosin light chains and a 40-kD protein in platelets (5, 20).

The original studies in platelets provided evidence that activation of the TXA_2/PGH_2 receptor resulted in a decrease in PGE_1- or PGI_2-stimulated adenylate cyclase activity (23–25). The decrease in adenylate cyclase activity is probably not a direct effect but is mediated by secretion of adenosine diphosphate (ADP) and/or increases in intracellular calcium (26). Furthermore, TXA_2/PGH_2 mimetics failed to lower basal adenylate cyclase activity (27). Indeed, more recent studies have failed to demonstrate an effect of a thromboxane mimetic on cyclic AMP accumulation (28).

Several studies have demonstrated that TXA_2/PGH_2 mimetics increase intracellular free calcium (29) and calcium mobilization (30, 31). The source of the calcium is uncertain, but current evidence suggests the calcium is derived from an extracellular source (32) and/or the dense tubular system in platelets (33) and possibly mitochondria in vascular tissue (34).

Stimulation of the platelet TXA_2/PGH_2 receptor also results in activation of phospholipase C and the subsequent formation of inositol trisphosphate and diacylglycerol (35–38). Inositol trisphosphate may be responsible for the

increase in intracellular free calcium concentrations (36). Endogenous synthesis of TXA_2 may mediate the increase in intracellular free calcium induced by inositol trisphosphate (28, 39). The diacylglycerol that is formed in conjunction with calcium stimulates protein kinase C.

The TXA_2/PGH_2 receptor appears to be coupled to its second messenger(s) via a guanine nucleotide binding protein. Activation of the receptor results in stimulation of guanosine triphosphatase (GTPase) activity (40, 41). Guanosine 5'-0-2-thiophosphate (GDPβS), an inhibitor of G proteins, blocked a thromboxane-mimetic–induced formation of phosphatidic acid and platelet secretion (28, 42). However, cholera and pertussis toxins failed to block the phosphoinositide hydrolysis (28, 42). Thus, the current evidence supports the hypothesis that a guanine nucleotide binding protein couples the receptor to the second messenger system(s) but that the protein is not G_s or G_i.

Interestingly, Johnson et al (43) reported that the calcium channel agonist Bay K 8644 stereoselectively competed for binding with [^{3}H]-U46619, a TXA_2/PGH_2 mimetic (44), in washed human platelets. This evidence raises the possibility that the TXA_2/PGH_2 receptor is somehow related to a calcium channel.

The second messenger system in vascular smooth muscle cells and other tissues or cells has not been studied extensively. In vascular tissue and smooth muscle cells, thromboxane mimetics increase intracellular free calcium (45) and $^{45}Ca^{++}$ fluxes (46–48). Thromboxane mimetics also increase $^{45}Ca^{++}$ fluxes in toad bladder epithelial cells (49). Much remains to be learned about the second messenger systems for TXA_2/PGH_2 receptors.

Radioligand Binding Studies

Several [^{3}H]- or [^{125}I]-labelled agonists and antagonists for TXA_2/PGH_2 receptors have been synthesized recently and used for radioligand binding studies (20, 50–56). Most of the studies have been conducted in either washed human platelets or platelet membranes. While the K_d values for the ligands have differed, the B_{max} values reported for most of the ligands have been in the range of 1500 to 2500 sites/platelet (50–56). Using subcellular fractionation and radioligand binding studies, the receptor has been localized to the plasma membrane/dense tubular system in the platelet (57, 58). Radioligand binding studies have also demonstrated the presence of the putative TXA_2/PGH_2 receptor in dog (59) and guinea pig platelets (10), cultured rat vascular smooth muscle cells (60), and endothelial cells (61) (see Table 1).

DESENSITIZATION The platelet TXA_2/PGH_2 receptor undergoes homologous desensitization (62, 63). Desensitization apparently results in a de-

crease in the number of available binding sites (64, 65). The mechanisms responsible for the desensitization of these receptors are currently unknown.

Purification and Biophysical Properties of TXA_2/PGH_2 Receptors

While progress has been made toward the purification of the platelet TXA_2/PGH_2 receptor, the receptor has yet to be purified to homogeneity from any source. Several photoaffinity ligands have been synthesized that should facilitate purification of the receptor (66–68). The receptor has been solubilized and its hydrodynamic properties determined (69, 70). The molecular weight determined from hydrodynamic properties is 140 to 180 kd (70). In addition, photoaffinity labeling of washed human platelets and SDS-PAGE studies of the receptor have been conducted, and several specifically radiolabelled bands have been found with M_r values of 43, 39, and 27 kd (D. E. Mais & P. V. Halushka, unpublished observations). In addition, isoelectric focussing of the radiolabelled bands has yielded pI values in the acid range (71). As might be expected, the TXA_2/PGH_2 receptor also apparently is a glycoprotein [(72) D. E. Mais & P. V. Halushka, unpublished observations].

PROSTACYCLIN RECEPTORS

Introduction

Prostacyclin (PGI_2) is believed to be an important cellular protective compound and is synthesized and physiologically active in many tissues. PGI_2 produces vasodilation, inhibition of platelet function and secretion in the intestine, and it may be important in the modulation of platelet-vascular interactions [see (79) for review]. Presently, a wealth of literature describes the synthesis, physiology, and metabolism of PGI_2, a summary of which is beyond the scope of this review. PGI_2 is a labile compound under physiological conditions and has a half-life of approximately 3 min. A wide range of structurally similar and stable analogs to prostacyclin have been developed, several of which have proven useful in the examination of PGI_2 receptors of various tissues [for a complete review of PGI_2 analogs, see (80)].

Second Messenger System

PGI_2 causes a concentration-dependent increase in intracellular levels of cyclic AMP in platelets via activation of adenylate cyclase (81). Through this rise in cyclic AMP, which is believed to produce a decrease in the intracellular concentration of calcium (82), PGI_2 causes its cellular effects. The coupling of the PGI_2 receptor to adenylate cyclase appears to be via a G_s

Table 2 Distribution of PGI_2 receptors in the cardiovascular system

Tissue/cell	Pharmacologic studies[a,b]	Radioligand binding studies[b]	References
Human platelets	+	+	84–86, 93
Human platelet membranes	+	+	87, 89, 90, 108
Canine, porcine, equine, guinea pig, ovine, rat, and rabbit platelets	+	+	89, 112, 114, 121 (review)
NCB-20 cells	+	+	88, 90–92
Human uterus	−	+	95
Guinea pig lung	+	+	109
Porcine aorta	−	+	97
Human pulmonary artery	+	−	110
Human umbilical artery	+	−	111
Bovine coronary artery	+	+	96, 117
Canine renal vasculature	+	−	118 (review)
Canine coronary artery	+	−	119
Canine basilar artery	+	−	120
Rabbit mesenteric artery	+	−	115, 116
Rabbit aorta	+	−	116
Rabbit heart	+	−	113
Guinea pig heart	+	−	113

[a]Pharmacological studies are representative reports.
[b]Plus sign indicates studies performed; minus sign indicates studies not performed.

binding protein (83). A search for other potential second messengers for this receptor has yet to be performed.

Radioligand Binding Studies

The development of stable PGI_2 analogs has facilitated characterization of the receptor for PGI_2. Table 2 summarizes selected studies that support the existence of putative PGI_2 receptors on cells and tissues of various types.

The first reports characterizing PGI_2 receptors on platelets used either [^{3}H]-PGE_1 (84) or [^{3}H]-PGI_2 (85). PGE_1 and PGI_2 are believed to interact at the same receptor (86). Both studies reported the existence of two classes of PGI_2/PGE_1 receptors: a high-affinity class of receptors with a K_d value in the nanomolar range and a low-affinity class with a K_d value in the micromolar range. A series of prostaglandins was used to displace the ligand from its receptor. Both studies found a correlation between the ability of the various prostaglandins to displace the ligand and their ability to stimulate adenylate cyclase. The rank order for both was $PGI_2 \geq PGE_1 > PGE_2 >$ 6-keto $PGF_{1\alpha}$ (a stable, inactive metabolite of PGI_2). Both studies concluded that PGI_2 and

PGE_1 interacted at the same receptor via interaction with the high-affinity sites. This interaction may produce a sixtyfold amplication from binding to intracellular response (87). The results also raise the possibility of spare receptors for PGI_2 on the platelet surface (87), a concept supported by data from a hybrid neuronal cell line (NCB-20) (88).

Rat, canine, and bovine platelets also possess PGI_2 receptors (89). However, only in the bovine platelets were two classes of receptors found.

The NCB-20 cell line has also been used to investigate the binding and mechanism of action of PGI_2. PGI_2 bound with high affinity to crude membrane preparations of these cells and stimulated adenylate cyclase. The affinity of these receptors agreed well with the EC_{50} values for activation of adenylate cyclase. The K_d values have been reported to range from 5 to 16 nM. The EC_{50} value for the activation of adenylate cyclase ranged from 25 to 86 nM (88–91).

In addition to [^{3}H]-PGI_2 and [^{3}H]-PGE_1, [^{3}H]-iloprost, a stable analog of PGI_2, has also been used to examine the putative PGI_2 receptors. Iloprost binds stereoselectively to the PGI_2 receptor in both platelets and the NCB-20 cell line. The inactive stereoisomer of iloprost, ZK36375, was unable to displace [^{3}H]-iloprost (90).

Recent evidence has raised the possibility that the platelet receptor for PGI_2 is different from the receptor for PGE_1 and PGE_2 (93, 94). Although PGE_1 and PGE_2 were capable of displacing [^{3}H]-PGI_2 from platelets, their respective IC_{50} values were too high to be of physiological significance.

A recent study using the technique of quantitative light microscopic autoradiographic analysis examined the potential presence of PGI_2 binding sites on nonpregnant human uterus (95). Specific binding sites were found on the uterine myometrium and vascular smooth muscle but not on the endometrium or vascular endothelium.

There have been very few radioligand binding studies that characterized the PGI_2 receptor on vascular smooth muscle. The binding of [^{3}H]-PGI_2 to bovine coronary artery and pig aortic membranes have shown similar binding characteristics (96, 97).

REQUIREMENTS FOR HIGH-AFFINITY BINDING To date, there has been very little progress on understanding the importance of the relationship between the structure of PGI_2 and its ability to interact with its receptor. However, certain aspects of the molecular structure seem to be pivotal for receptor-ligand interactions [for a detailed review of structure activity relationships, see (80, 98)]. The C-1 carboxyl group, the C5–6 *Z* double bond, the 11-OH group and the 15-OH group in the S configuration are required for high-affinity binding.

A few reports have also examined ionic requirements of the receptor for

optimal binding. Divalent cations are required for the interaction of PGI_2 with its receptor (99). However, the apparent increase in affinity may have been due to an effect on the three-dimensional solution structure of PGI_2 rather than an effect on the receptor. To date, little else is known regarding the optimal requirements for high-affinity binding to the PGI_2 receptor.

DESENSITIZATION The PGI_2 receptor can be desensitized. In a series of experiments employing the NCB-20 cell line, researchers showed that pre-exposure of the cells to carbacyclin (a stable PGI_2 analog) caused an increase in the K_{act} value for adenylate cyclase activity (88, 100). The K_d value of the high-affinity receptor increased from 18 nM to 50 nM, with an approximately 50% decrease in the B_{max} value after exposure of the cells to carbacyclin. Additionally, this desensitization process may be reversible (101).

Pretreatment of isolated human platelets with iloprost resulted in desensitization, as assessed by a decreased activation of adenylate cyclase (83, 102). This desensitization was accompanied by a decrease in the number of receptors without a change in their affinity. Of greater interest was the finding that this desensitization resulted in decreased adenylate cyclase activation in response to adenosine, PGD_2, forskolin, NaF, and GTP. Both studies showed that the desensitization resulted in decreased ADP-ribosylation of a 45-kd protein induced by cholera toxin. Thus, iloprost caused heterologous desensitization that occurred at the level of the G_s subunit of adenylate cyclase.

Receptor Subtypes

Recent evidence suggests PGI_2 receptor subtypes may exist on vascular smooth muscle and platelets. The majority of this work was done employing carbacyclin. Carbacyclin is reportedly more efficacious than PGI_2 in stimulating adenylate cyclase in platelets compared with smooth muscle (103). Wilkens & MacDermot (104) reported on the actions of a putative competitive PGI_2 receptor antagonist, FCE22176, on human platelets and NCB-20 cells. They found FCE22176 to be a competitive antagonist to the vascular effects of PGI_2 (105); however, FCE22176 behaved as an agonist in platelets and the NCB-20 cells (104). FCE22176 stimulated adenylate cyclase with EC_{50} values of 174 nM in human platelets and 193 nM in NCB-20 cells. Additionally, FCE22176 inhibited the binding of [^{3}H]-iloprost to both cell types, with IC_{50} values of 280 nM for platelets and 400 nM for NCB-20 cells. The potential existence of PGI_2 receptor subtypes is of interest and deserves further investigation.

Two types of receptors for PGI_2/PGE_1 may exist on the platelet membrane (106). Kinetic evidence, gained using cyclic AMP accumulation, indicated the presence of a high-affinity stimulatory receptor followed by a lower-affinity inhibitory receptor. The development of selective receptor antagonists should greatly facilitate the identification of potential receptor subtypes.

Purification of PGI_2 Receptors

To date, only two studies have determined the molecular weight of the PGI_2 receptor. By using membranes prepared from the NCB-20 cell line and the technique of radiation inactivation, Leigh et al (91) estimated the molecular weight of the PGI_2 receptor complex to be 83 kd.

The isolation and purification of the PGI_2/PGE_1 receptor from platelet membranes has been reported (107). A molecular weight of 190 kd, with two subunits of 85 kd and 95 kd for the receptor, were reported. The purified receptor had a high-affinity site with a K_d value of 9.8 nM and a low-affinity site with a K_d value of 700 nM for [^{3}H]-PGE_1, values similar to those reported for the intact receptor. Additionally, this group reintroduced the isolated receptor into platelets that had been stripped of the receptor. Upon reinsertion, the ability of PGE_1 and PGI_2 to stimulate adenylate cyclase was restored. In contrast to the findings mentioned previously (93, 94), the isolated receptor was shown to interact with both PGI_2 and PGE_1. Presently, there are no other reports of the isolation and purification of PGI_2 receptors.

PGE RECEPTORS

Introduction

The pharmacology of PGE is quite diverse. Cardiovascular effects include inhibition of platelet aggregation and contraction or relaxation of vascular and nonvascular smooth muscle. In the kidney, PGE_2 can decrease vasopressin-stimulated water reabsorption and enhance sodium excretion (122).

Two subclasses of PGE receptors in smooth muscle have been proposed (123–126). EP_1 receptors mediate contraction in tissues such as guinea pig ileum longitudinal muscle; guinea pig, dog, and rat fundus; and bullock iris sphincter muscle. EP_2 receptors mediate relaxation in tissues such as cat trachea and guinea pig ileum circular muscle. Certain tissues such as guinea pig trachea and dog saphenous vein contract or relax depending on the tone of the preparation.

Second Messenger System

Smith et al have proposed that PGE receptors are coupled to guanine nucleotide (N) regulatory proteins, the type of which determines the biological response (127, 128). PGE receptors coupled to N_s proteins mediate stimulation of adenylate cyclase; PGE receptors coupled to N_i, inhibition of adenylate cyclase; and PGE receptors coupled to N_p, stimulation of Ca^{2+} mobilization perhaps through turnover of phosphoinositides (127). The PGE receptor has been partially purified from the canine renal medulla (129). The digitonin-solubilized receptor is associated with a guanine nucleotide regulatory protein that can be dissociated from the receptor by solubilization in the detergent CHAPS. The G regulatory protein is presumably of the N_i type, since binding

is stimulated by GTP and PGE_2 inhibits cyclic AMP formation in this region of the kidney (129). In the rabbit cortical collecting tubule, PGE_1 in low concentrations inhibits arginine vasopressin (AVP)-induced water reabsorption but at higher concentrations stimulates water reabsorption (130). If inhibition of AVP-induced water reabsorption is through inhibition of adenylate cyclase, low concentrations of PGE_1 might stimulate the PGE-N_i receptor complex, while higher concentrations stimulate the PGE-N_s receptor complex. In membranes prepared from rabbit cortical collecting tubules, low concentrations (0.1–100 nM) of PGE_2 inhibited AVP-induced increases in cyclic AMP that had been blocked by pertussis toxin pretreatment (131). This result suggests PGE can act through receptors coupled to an inhibitory guanine nucleotide regulatory protein. PGE receptors in the human platelet may also be of the N_i and N_s type. PGE produces activation of adenylate cyclase at low concentrations but inhibition at high concentrations (106).

In the adrenal medulla, PGE_2 does not activate or inhibit adenylate cyclase and pertussis toxin does not inhibit PGE_2 binding (132). Therefore, the PGE_2 receptor is associated with a pertussis-toxin–insensitive GTP-binding protein and is not coupled to adenylate cyclase. In cultured bovine adrenal chromaffin cells, PGE_2 produces a concentration-dependent formation of inositol phosphates, presumably through activation of phospholipase C, that correlates well with catecholamine release (133). This system may represent the PGE-N_p receptor complex. Until the PGE receptor can be isolated from tissue that exhibits apparent differences in the second messenger system and the structure of these receptors determined, the mechanism of the varied effects of PGE attributed to specific receptor stimulation will remain in question.

Radioligand Binding Studies

A comprehensive listing of binding studies in a variety of tissues can be found in the review by Robertson (134). PGE binding has been demonstrated in tissues where PGE stimulates adenylate cyclase, such as in the platelet (84, 86), glucose-responsive beta cells (HIT) (135), and hamster (136) and rat (137) adipocytes. In the hamster and rat adipocytes, the binding of PGE was enhanced by GTP (136, 137).

The binding of PGE to its receptor can also be reduced by guanine nucleotides. PGE_1 binding in the frog erythrocyte is reduced by GTP or its analogs (138). The PGE_2 receptor from porcine brain has been solubilized and separated from its GTP-regulatory component (139). Binding of PGE to the isolated receptor was not inhibited by GTP until the GTP regulatory protein was added back and the complex reconstituted.

DESENSITIZATION Agonist-induced down-regulation of PGE_2 receptors by 16,16'-dimethyl-PGE_2 has been demonstrated in the rat renal medulla (140),

where the number of PGE_2 receptors decreased by 40%. Down-regulation was reversible and demonstrated agonist-induced cycling of the receptors. Rat hepatic PGE receptors exhibit down-regulation in vivo after administration of the 16,16'-dimethyl-PGE_2 (141). Receptor density was decreased but affinity remained unchanged. This down-regulation was associated with a significant decrease in PGE_1-stimulated adenylate cyclase activity. Up-regulation of the rat hepatic PGE receptor was also demonstrated in vivo (142). Treatment with aspirin and indomethacin increased receptor density. There was no change in adenylate cyclase activity, which suggests the increase in receptor density reflects the presence of spare receptors.

PGE receptors have also been reported in the human myometrium (143, 144) and in cultured rabbit endometrial cells (145). The affinity and number of receptors did not change during the menstrual cycle and are similar before and during labor (143). The receptors in the rabbit endometrial cells are linked to stimulation of adenylate cyclase and exhibit up-regulation and down-regulation (145). PGE receptors were also reported in bovine (146, 147) and human corpus luteum (148), where stimulation of adenylate cyclase may be responsible for the luteotropic effects of PGE (148).

Purification of PGE_2 Receptors

PGE_1 and PGE_2 also display specific binding to particulate fractions of the bovine, ovine, and human adrenal medulla (132) with K_d values in the low nanomolar range. A putative PGE_2 receptor has been partially purified from the bovine adrenal medulla (149). It was characterized as a glycoprotein with an apparent M_r value of 110 kd estimated from size-exclusion gel chromatography. However, the PGE receptor isolated from the canine renal medulla was reported to be a glycoprotein with a M_r value of 65 kd by the same method (129). The differences in molecular weights of these receptors may indicate structural differences or differences in associated G proteins.

$PGF_{2\alpha}$ RECEPTORS

Introduction

Definitive pharmacological evidence for the presence of specific $PGF_{2\alpha}$ receptors is yet to be established. An N-dimethylamine analog of $PGF_{2\alpha}$ has been reported to antagonize the pulmonary effects of $PGF_{2\alpha}$ while leaving the responses to the TXA_2 mimetic U46619 intact (150). Although there was no antagonism of U46619, the effects on PGD_2-elicited responses were not studied. This putative, selective $PGF_{2\alpha}$ antagonist is interesting in that it lacks the carboxyl group thought to be required for interaction at prostanoid receptors.

$PGF_{2\alpha}$ produces bronchoconstriction in the cat (151) and dog (152) in vivo

and produces constriction of the isolated guinea pig trachea, fundus, and lung strip (153) and of other nonvascular and vascular smooth muscle preparations (126). The luteolytic activity of $PGF_{2\alpha}$ has also been studied extensively (154, 155).

Second Messenger System

The second messenger system activated by occupation of the PGF_{2a} receptor is still unknown. In cultured rabbit endometrial cells, PGF_{2a} exerts no effect on the levels of cyclic AMP or cyclic GMP (145); however, phosphatidic acid and phosphoinositide turnover can be stimulated by $PGF_{2\alpha}$ (156). Smith et al (127) have suggested that $PGF_{2\alpha}$ receptors are associated with a specific guanine nucleotide regulatory protein, N_p, linked to activation of phospholipase C.

Radioligand Binding Studies

Specific, saturable binding of $[^3H]$-$PGF_{2\alpha}$ has been demonstrated in a variety of tissues [for review, see (134)]. Much of the work has centered around tissues involved in reproduction. In the human myometrium, specific $PGF_{2\alpha}$ binding sites have been suggested (143), but the affinity of $[^3H]$-$PGF_{2\alpha}$ is lower than for PGE_1 and PGE_2; therefore, specific receptors for $PGF_{2\alpha}$ in the human myometrium are yet to be firmly established (144). However, in primary cultures of rabbit endometrial cells, up-regulation and down-regulation of $PGF_{2\alpha}$ receptors have been demonstrated (145). In the bovine corpora lutea, $PGF_{2\alpha}$ binds with high affinity (146, 157), and a putative receptor protein has been partially purified (157). The receptor protein was characterized as a nonglycosylated protein with a M_r value of 107 kD, as estimated by Sepharose 6B chromatography and density gradient centrifugation (157, 158). In the rat ovary, specific $PGF_{2\alpha}$ receptors were found only in the luteal cells (159). Receptor number cycled with progesterone levels with an apparent dependence on cyclooxygenase products, since cycling was inhibited by indomethacin (159).

A single class of high-affinity binding sites for $PGF_{2\alpha}$ has been demonstrated in the smooth endoplasmic reticulum fraction isolated from rat fibroblasts (160). The K_d value for $PGF_{2\alpha}$ was unchanged in rats with essential fatty acid deficiency; however, the number of binding sites increased fivefold. This apparent up-regulation of the PGF_{2a} receptor suggests a role for $PGF_{2\alpha}$ in proliferative processes of the skin.

PGD_2 RECEPTORS

Introduction

The pharmacology of PGD_2 was recently reviewed by Giles and Leff (161). A wide and varied range of activities has been reported for PGD_2, including

inhibition of platelet aggregation (84), bronchoconstriction in the cat (151) and dog (162), contraction of the guinea pig trachea (163) and rat fundus and rabbit jejunum, but relaxation of the rabbit stomach strip (164). In isolated vascular tissue, PGD_2 generally contracts tissue in the absence of tone (165, 166) but can relax precontracted tissues (165). Without selective PGD_2 receptor antagonists, the presence of specific PGD_2 receptors assessed from pharmacological studies alone will be difficult to establish.

Second Messenger System

In the platelet, PGD_2 inhibits aggregation via stimulation of adenylate cyclase and accumulation of cyclic AMP (84, 167). While PGE_1 and PGI_2 exhibit similar activity, PGD_2 appears to inhibit aggregation through its own receptor. The potency ratios of PGE_1 and PGI_2 in platelets from a wide range of species are similar, while the potency ratio of PGD_2 varies, which suggests a receptor distinct from that of PGE_1 and PGI_2 (167). The second messenger system responsible for the varied effects of PGD_2 in vascular and nonvascular smooth muscle is unknown.

Until radioligand binding studies are performed in these tissues and specific antagonists are available, establishing the presence of specific PGD_2 receptors will be difficult. Indeed, the pharmacological responses to PGD_2 in vascular and nonvascular smooth muscle may be a result of interactions of PGD_2 at other eicosanoid receptors (161).

Radioligand Binding Studies

Radioligand binding studies in the platelet have suggested a distinct receptor for PGD_2, although the receptor has yet to be isolated and purified. A single class of binding sites in the platelet has been reported (84, 168–170). Both PGE_1 and PGI_2 have very low affinity for the putative PGD_2 receptor (168, 170). The K_d values for PGD_2 range from 400 to 600 nM (168, 170), with a B_{max} value of 760 sites/platelet (168) or 787 fmol/mg protein (169). The PGD_2 receptor is reported to be regulated by agents that raise cyclic AMP levels in the platelet. Isoproterenol can raise the B_{max} value by 50% without altering the K_d value (169), which suggests regulation of the platelet PGD_2 receptor through cyclic-AMP–dependent mechanisms. Indeed, PGE_1 and PGI_2, which also raise cyclic AMP, can increase PGD_2 binding.

Shimizu et al have suggested PGD_2 acts as a neuromodulator (171), and in the rat brain, PGD_2 is the major prostaglandin produced (172). Specific binding of [^{3}H]-PGD_2 has been demonstrated in various regions of the rat brain, with the highest specific binding found in the pituitary, hypothalamus, and olfactory bulb (173). In a synaptic membrane preparation from whole brain, a K_d value of 28 nM and a B_{max} value of 124 fmol/mg protein have been reported (173). A similar K_d value of 33 nM was reported for the rat

diencephalon (174). Na^+ augments PGD_2 binding in synaptic membranes (173).

LEUKOTRIENE RECEPTORS

Introduction

The peptidoleukotrienes consist of a family of arachidonic acid metabolites that collectively make up the slow-reacting substances of anaphylaxis (SRS-A). Included in this group are the leukotrienes LTC_4, LTD_4, and LTE_4. In addition, under the more general term of *leukotrienes* is included the dihydroxy-eicosanoid LTB_4. The biosynthetic pathways to the leukotrienes have been well characterized and their description is outside the scope of this review (175–179).

The leukotrienes have profound effects on a wide variety of tissues, and these effects are mediated via specific membrane receptors [see Crooke et al (180) for review]. The effects of the leukotrienes on various tissues can be divided into effects on immunologic and smooth muscle systems. The smooth muscle system effects include those in the respiratory and cardiovascular systems and the gastrointestinal tract. These effects will be discussed briefly; more extensive reviews are found elsewhere (180, 181).

LTB_4 induces adhesion of neutrophils to the endothelium (182) and is a potent chemotactic and cytokinetic compound (183). In addition, LTB_4, LTC_4, and LTD_4 appear to play roles in interferon production (184).

LTC_4, LTD_4, and LTE_4 are potent smooth muscle contractors that produce prolonged contraction of smooth muscle from pulmonary (185, 186), reproductive (187), gastrointestinal (188), and vascular tissues (189). In addition, they induce mucus secretion and bronchoconstriction in a variety of species (190–192). In the heart, the leukotrienes exert a negative inotropic effect and, depending on the species, this effect may be direct through effects on calcium influx and/or indirect as a result of coronary artery vasoconstriction (193–195).

Second Messenger Systems

Early observations that LTD_4 and LTE_4 binding to receptors was regulated by guanine nucleotides and Na^+ suggested that these receptors were coupled to cyclic AMP generation (196), but this coupling does not now appear likely (180). Current evidence suggests that LTD_4 and LTE_4 bind to the same receptor, but this hypothesis remains to be proven. More recent work suggests an alternative mechanism for the signal transduction. LTD_4/LTE_4 receptors appear to be linked to the turnover of inositol phosphates via activation of phospholipase C (197, 198). Regulation of the activity of phospholipase C

appears to be coupled to the receptor via a guanine nucleotide binding protein (180). Since the response to LTD_4 is inhibited by pertussis toxin, the G protein may be of the G_i class (180). In a variety of tissues and cell culture systems, LTD_4/LTE_4 and agonist analogs produced a rapid increase in inositol phosphates after stimulation (197, 199, 200), an effect which was blocked stereoselectively by receptor antagonists. The agonist-induced increases in inositol phosphates correlated directly with the generation of cyclooxygenase products and smooth muscle contraction. Current data support the hypothesis that, at least for LTD_4/LTE_4 receptors, phosphoinositide hydrolysis with subsequent Ca^{+2} mobilization and generation of arachidonic acid metabolites constitute important transduction mechanisms.

Both LTB_4 and LTC_4 have been studied to a lesser degree but also appear to stimulate phosphoinositide turnover. In all cases, the nature of the G protein appears variable and tissue dependent, in particular with regard to pertussis toxin sensitivity (180, 201, 202). The potential role of cyclooxygenase products as mediators in the stimulation of LTB_4 and LTC_4 receptors remains uncertain.

Radioligand Binding Studies

Radioligand binding to a wide variety of tissues has been examined with all the leukotrienes. Nearly all the studies have used the tritiated form of the corresponding leukotriene, that is, $[^3H]$-LTB_4, $[^3H]$-LTC_4, and $[^3H]$-LTD_4. Recently, a potent tritiated LTD_4/LTE_4 antagonist, ICI 198,615, has been synthesized and is structurally quite different from the leukotrienes (203). It should solve the problems of metabolism that are associated with use of the tritiated leukotrienes.

Most radioligand binding studies have focused on LTD_4 receptors, and the data support the existence of specific receptors on plasma membranes in a wide variety of tissues (180, 197, 203–207). The binding of $[^3H]$-LTD_4 to these tissues or membrane preparations is saturable and stereoselective. The binding of agonists to the receptors is regulated by guanine nucleotides (197). A strong correlation exists between the binding affinities of various agonists and antagonists and their pharmacologic potencies (180, 197, 205–207). The effects of cations on binding have been examined (196) along with tissue and species variability (180). In addition, in the guinea pig, subtypes of LTD_4 receptors have been suggested based on differential effects on binding of cations, GTP, and sulfhydryl reagents (180).

High-affinity and stereoselective $[^3H]$-LTC_4 binding sites have also been reported in a variety of tissues (205, 208–211). LTD_4 does not appear to bind to these sites. Detailed pharmacologic and biochemical studies have shown that these sites are ubiquitously distributed in many tissues, including those with no known pharmacologic function for LTC_4 (180, 205, 212, 213).

Furthermore, binding and contractile potencies in guinea pig lung do not correlate with each other (205). Tissue distribution and effects of cations, GTP, and sulfhydryl agents have been examined (180). The possibility of subtypes is less compelling than for LTD_4 receptors. A physiological role for LTC_4 binding thus is not nearly as well defined as for LTD_4 receptors. The broad tissue distribution, absence of a pharmacologic effect in some tissues, and lack of an effect of GTP on binding are consistent with the notion that the LTC_4 binding site may not be of physiological importance in many systems. There has been one report of subcellular localization of LTC_4 binding sites (213). Conversion of LTC_4 to LTD_4 remains a technical problem, and the development of a high-affinity LTC_4 antagonist that can be radiolabelled should advance the field significantly.

Fewer studies have been carried out with binding of LTB_4 to its putative receptor. Pharmacologic studies strongly suggest the presence of specific LTB_4 receptors (183, 201), a presence also supported recently by corresponding binding data (214, 215). This binding has been shown to correlate well with potencies of analogs in chemotactic and white blood cell aggregation assays. Thus, pharmacologic response and binding agree well, but species differences have been noted (214). Recently, pertussis toxin has been shown to decrease binding affinity of [^{3}H]-LTB_4, which suggests that the LTB_4 receptor is coupled to a guanine nucleotide binding protein (202).

Purification and Biophysical Properties of Leukotriene Receptors

Biophysical characterization of the leukotriene receptors has been limited by the difficulties encountered in attempts to solubilize the receptors in active form. Nonetheless, there has been some success using digitonin in solubilizing LTD_4/LTE_4 receptors from guinea pig lung membranes (180). Depending on the source of the LTD_4 receptors, binding of [^{3}H]-LTD_4 is sensitive to reducing agents or N-ethylmaleimide, which suggests the presence of either free thiol or disulfide groups in the receptor (212). Size-exclusion gel chromatography studies suggest a molecular weight of the detergent-lipid-protein binding complex of between 240 and 500 kd, and the presence of sugars on the receptor is suggested by the retention of binding activity on wheat germ lectin columns (180).

While no reports have appeared on solubilization of LTB_4 and LTC_4 receptors, there have been reports of successful cross-linking of LTB_4 and LTC_4 receptors by using disuccinimidyl suberate and the appropriate tritiated leukotriene analogs (216, 217). For LTB_4 receptors, a broad band that was stereoselectively inhibited by LTB_4 analogs appeared at a molecular weight of 60 kd on the SDS-PAGE-autoradiogram (216). Both GTP and pertussis toxin, which decrease the affinity of the receptor for LTB_4, also reduced the

labelling of this protein band (216). Similar results have been observed for LTC_4 receptors in ileal smooth muscle cells, except two specifically labelled bands at 17 and 33 kD were observed. However, the much greater background compared to the cross-linking of LTB_4 receptors made interpretation more difficult (217).

SUMMARY AND FUTURE DIRECTIONS

Pharmacological, biochemical, and biophysical characterizations of the eicosanoid receptors are in their infancy. Progress has been retarded by a critical lack of the necessary tools. For example, with the exception of the leukotriene and thromboxane A_2 receptors, the other eicosanoid receptors have no high-affinity antagonists or photoaffinity ligands. In addition to facilitating the purification of the receptors, such antagonists and ligands will also aid in the identification of potential subclasses of receptors. None of the eicosanoid receptors has been purified to homogeneity, nor have their primary amino acid sequences been determined. Once the eicosanoid receptors are purified, the areas of structural homology can be determined. For the prostaglandins and thromboxanes, there are several functional group requirements for biological activity: the carboxylic acid at C-1, 5-6 *Z*, and 13, 14 *E* double bonds, the trans orientation of the α and ω side chains, and the 15-*S* hydroxyl group. Of great interest will be which amino acids or domains are conserved among the various receptors that confer these requirements for biological activity. Of similar interest will be to learn which domains or amino acids are required for the selectivity among the eicosanoid receptors. The application of the tools of modern molecular biology to this major class of receptors is desperately needed.

As improved ligands are developed for all the receptors, investigations need to be done to see if abnormalities exist in receptor number, receptor affinity, or receptor transducing systems, particularly in disease states, and to learn whether these abnormalities play pathogenic roles. So far, abnormalities in platelet TXA_2/PGH_2 (218) and PGI_2 (219) receptors have been reported in coronary artery syndromes. Abnormalities in the transducing system for the platelet TXA_2/PGH_2 receptor have also been reported (220). The potential hormonal and nonhormonal regulation of the various eicosanoid receptors is another area in need of further investigation.

There is also a need for further clarification of receptor subtypes and perhaps adoption of a unifying nomenclature for the receptors (126). The apparent species differences for the various eicosanoid receptors need to be further explored and defined. Another research area concerns whether eicosanoid receptors communicate with and/or modulate other receptors within the same cell (83, 102, 169).

Finally, two other groups of eicosanoids should also be mentioned for which putative receptors have been postulated to exist but for which no convincing data have been reported: the lipoxins (221) and the cytochrome P_{450} metabolites of arachidonic acid.

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