

Multiple signalling options for prostacyclin

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ABSTRACT

The fate of a cell following stimulation by the prostanoid prostacyclin is cell specific, depending not only on the ability of prostacyclin to activate the cell surface prostacyclin (IP) receptor and regulate its coupling to various G proteins, but also on its ability to act intracellularly via the nuclear peroxisome proliferator-activated receptor family (PPAR). This review will highlight the different signalling options available to prostacyclin, and discuss the consequences for cell responses.

INTRODUCTION

Prostanoids are produced by the action of cyclo-oxygenase (COX) enzymes which convert arachidonic acid to cyclic endoperoxides (prostaglandin G₂ and prostaglandin H₂) and additional enzymes which produce prostaglandin D₂, prostaglandin E₂, prostaglandin F_{2α}, prostaglandin I₂ (prostacyclin, PGI₂), or thromboxane A₂ in a tissue-dependent fashion^[1,2]. The enzyme responsible for prostacyclin synthesis is prostacyclin synthase (PGIS) which is localised to the endoplasmic reticulum in endothelial cells and to the nuclear and plasma membranes in smooth muscle cells^[3]. In many systems, prostacyclin is the primary product of COX-2^[4], and both COX-1 and COX-2 can be found not only in the endoplasmic reticulum, but also in the nuclear envelope^[5]. Prostacyclin produced near the plasma membrane can readily diffuse or be transported out of the cell to act locally in an autocrine or a paracrine fashion by stimulating the seven transmembrane IP receptor on the cell surface^[2]. In contrast, prostacyclin

produced near the nuclear membrane has ready access to cytoplasmic and perinuclear peroxisome proliferator-activated receptors (PPARs)^[6], and will therefore influence the behaviour of the cell in which it is produced (ie, an intracrine effector). Although there is evidence for localisation of prostaglandin E₂ receptors to the nuclear membrane^[7,8], no such evidence for IP receptors has been presented. Thus, the final outcome for a cell will be determined by the balance between these two apparently independent autocrine/paracrine and intracrine pathways.

An additional third signalling option is available for prostacyclin, since it is also a ligand for a novel type of prostacyclin receptor found in the central nervous system^[9,10]. It is possible that this prostacyclin receptor mediates a neuronal cytoprotective action of prostacyclin^[11]. Unfortunately, the precise cellular localization, protein structure, and cell signalling properties of this novel prostacyclin receptor remain unknown.

AUTOCRINE/PARACRINE SIGNALLING BY PROSTACYCLIN

The IP receptor is a seven transmembrane receptor which couples primarily to G_s to activate adenylyl

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cyclase, but may also couple to other G proteins^[12,13]. Prostacyclin itself is rarely used experimentally, due to its marked biological and chemical instability^[14] and its poor selectivity for IP receptors^[15]. Therefore, characterization of IP receptor signalling has predominantly focussed on the use of prostacyclin mimetics such as cicaprost, iloprost, carbacyclin, and prostaglandin E₁^[15]. However, none of these agonists are specific for IP receptors: the affinity of cicaprost for human IP (hIP) receptors is merely 3-fold higher than for the prostaglandin E₂ EP₄ subtype of receptor, which also couples to G_s^[16], and only 17-fold higher than for the EP₃ receptor in the mouse^[17]. Iloprost is equipotent at both human and mouse IP and EP₁ receptors, and carbacyclin and prostaglandin E₁ show even greater affinity for EP₃ than for IP receptors^[16,17].

The poor selectivity of IP agonists combined with the lack of an IP antagonist, has meant that precise characterization of cellular and physiological responses mediated by IP receptors is problematical. However, the generation of mice lacking IP receptors (IP-KO mice) has provided some clarity (eg, platelet and vascular IP receptors are identical^[18]) and presented new ideas (eg, IP-KO mice show reduced responses to inflammatory pain^[18] and enhanced airway inflammatory responses^[19]). More specifically, combining knowledge obtained from IP-KO mice, with the results of PGIS-deficient or PGIS-overexpression in mice, has brought new insights into the complex role played by prostacyclin, particularly in the field of vascular physiology.

Mice lacking IP receptors are more susceptible to thrombosis^[18] and injury-induced vascular proliferation^[20], and gene transfer of PGIS into rat carotid arteries prevents neointimal formation after carotid balloon injury^[21,22]. The ability of prostacyclin to modulate platelet-vascular interactions *in vivo* and to specifically limit platelet and vascular tissue responses to thromboxane A₂ may account for adverse cardiovascular effects associated with selective COX-2 inhibitors^[20,23].

Prostacyclin has an important therapeutic role in the treatment of pulmonary hypertension^[24]; a condition in which patients have reduced IP receptor expression in the remodelled pulmonary arterial smooth muscle^[25]. IP receptor-deficient mice produce a more severe vascular remodelling response and a greater degree of pulmonary hypertension in response to hypoxia^[25]. Furthermore, selective pulmonary overexpression of PGIS in transgenic mice protects

against the development of hypoxia-induced pulmonary hypertension^[26].

CELL TYPE SPECIFIC SIGNALLING OF IP RECEPTORS

While the IP receptor would be expected to couple through adenylyl cyclase to provide an antiproliferative, antithrombotic, or hyperalgesic response, the IP receptor can couple to other G proteins, but does so in a highly cell-specific manner. As with other G_s-coupled receptors, the IP receptor couples readily to G_q/phospholipase C pathways when stably expressed in Chinese hamster ovary (CHO) cells^[27,28], and in human embryonic kidney 293 (HEK 293) cells^[13,29]. IP agonists also increase phosphatidyl inositol turnover and mobilize intracellular calcium in transformed cell lines endogenously expressing IP receptors^[12]. Evidence for multiple G-protein coupling in native cells or tissues is harder to find, for although iloprost appears to activate phospholipase C in piglet cerebral microvascular smooth muscle cells^[30] and in isolated rat dorsal root ganglion cells^[31], we cannot yet definitively prove that this response is mediated through IP receptors. This is where studies on cells transfected to express cloned IP receptors become invaluable, because problems of agonist selectivity are minimized.

Recent studies have clearly shown that the cloned mouse IP (mIP) receptor, overexpressed in HEK 293 cells, couples to G_q and G_i in a protein kinase A-dependent manner, due to phosphorylation of the mIP receptor on Ser-357^[13]. In contrast, the cloned human IP (hIP) receptor couples independently to G_s and G_q and does not couple to G_i^[29]. While these latter studies with hIP support previous work with the human neuroblastoma SK-N-SH cell line^[32], and with the mIP expressed in CHO cells^[28] and in the rat/mouse neuroblastoma glioma NG108-15 cell line (unpublished observations), there was no previous evidence for mIP receptor switching coupling from G_s to G_q/G_i proteins. Furthermore, we have found no evidence for mIP receptor coupling to G_i in HEK 293 cells transiently expressing these receptors (unpublished observations). In our hands, hIP and mIP receptors couple independently to G_s and G_q (in a cell-specific manner) and never couple to G_i, and protein kinase A inhibitors enhance rather than inhibit mIP receptor coupling to the G_q/phospholipase C pathway^[32].

The concept of receptor switching arose in 1997

for the G_s -coupled β -adrenergic receptor overexpressed in HEK 293 cells, which produced G_i -dependent activation of extracellular signal-regulated kinases (ERK1/2) only after protein kinase A-dependent phosphorylation of the β -adrenergic receptor^[33]. More recently, this concept of β -adrenergic receptor switching G-protein coupling has been challenged, because β_2 -adrenergic receptors deficient in protein kinase A consensus sites were still able to activate ERK in HEK 293 cells^[34]. So why do β -adrenergic receptors behave so differently in the same cell line? Well, different isolates of HEK 293 cells have different cell signalling properties^[35]. Thus, although the mIP overexpressed in one isolate of HEK 293 cells can switch coupling from G_s to G_q and G_i , this phenomenon is not observed in all isolates of HEK 293 cells, and is not a consistent property of the mIP receptor in transformed cell lines. Hence, the emphasis herein on the cell-dependent nature of IP receptor coupling.

Further discrepancies concerning the structural features of IP receptors crucial for efficient coupling to G-proteins also exist. For example, the IP receptor contains an isoprenylation site at the end of the carboxy-terminal sequence, removal of which prevents coupling to adenylyl cyclase and phospholipase C pathways^[36]. However, we^[37] and others^[38] have evidence that the end of the carboxy-terminal tail of the IP receptor may not be essential for successful G-protein coupling.

INTRACRINE SIGNALLING BY PROSTACYCLIN

It had been recognized for some time that the actions of prostacyclin mimetics could not completely be accounted for by cell signalling via IP receptors. Three processes (adipocyte differentiation, embryo implantation, and apoptosis) have now been clearly identified to involve prostacyclin acting via nuclear PPARs, rather than IP receptors alone. When PGIS is localized to the nuclear membrane, it co-localizes with cytosolic phospholipase A_2 (cPLA₂) and COX-2^[39]. As a result, when cPLA₂ releases arachidonic acid from nuclear membranes, this substrate for COX-2 is immediately available for conversion to prostaglandin H₂ as the substrate for PGIS. Thus, prostacyclin can readily bind to perinuclear PPARs, causing their translocation to the nucleus and the formation of heterodimers with retinoic acid receptors to bind to the peroxisome proliferator response element^[40]. The PPAR family of receptors is comprised of PPAR α , PPAR δ , and PPAR γ , of which

carbacyclin, iloprost, and prostacyclin are agonists for PPAR α and PPAR δ , but cicaprost is not^[41-43].

Adipocyte differentiation involves dual activation by prostacyclin of PPARs and cell surface IP receptors^[44]. Prostacyclin can be distinguished from prostaglandin E₂ by its action as an autocrine promoter and/or amplifier of terminal differentiation of preadipocytes, and this effect is mediated by cell surface IP receptors^[45]. But the stable prostacyclin analogue carbacyclin additionally regulates gene expression in preadipocytes and adipocytes in a way distinct from that elicited by its cell surface receptor. It was this study that first revealed an intracrine role for prostacyclin^[46].

For efficient embryo implantation in the mouse, COX-2, PGIS, and PPAR δ need to coexist at the implantation site^[39,47]. In COX-2-deficient mice, the compromised implantation process is rescued by the administration of carbacyclin to substitute for prostacyclin to activate PPAR δ . In contrast, cicaprost which only activates the cell-surface IP receptor, failed to restore embryo implantation.

As an alternative procedure to study the physiological role of prostacyclin, cell lines or transgenic mice have been generated which are either deficient in prostacyclin or overexpress PGIS in a cell-specific manner. The phenotype of PGIS deficient mice is not the same as that of IP-KO mice, in particular, they develop morphological abnormalities in the kidneys which are somewhat similar to those reported in COX-2-deficient mice, and have thickening of arterial and small blood vessel walls^[48]. The discovery that prostacyclin deficiency induces kidney damage which could not be improved by administration of an IP agonist, suggests that these morphological changes may be mediated by lack of stimulation of the PPAR δ receptor by prostacyclin.

When PGSI was first overexpressed in HEK 293 cells, these cells mysteriously died^[49]. Extensive investigation demonstrated that prostacyclin activation of PPAR δ (not PPAR α) was responsible for the decrease in cell viability, thus demonstrating the apoptotic activity of prostacyclin.

BLANCE BETWEEN AUTOCRINE/PARACRINE AND INTRACRINE SIGNALLING BY PROSTACYCLIN

We have seen above that prostacyclin acts solely on IP receptors to produce its characteristic antithrombotic and hyperalgesic effects, and acts solely

on PPAR δ to achieve successful embryo implantation. However, we need to stimulate both IP receptors and PPAR δ to achieve adipocyte differentiation. But what happens when signalling through the IP receptor and PPAR δ results in counter-regulatory responses?

For example, responses to hypoxia in IP-KO mice highlight the normal protective antiproliferative role of prostacyclin acting on IP receptors^[50,51], but it should be noted that PPAR δ is also expressed in vascular smooth muscle cells, and here they promote cell proliferation^[52]. Prostacyclin has also been proposed as an angiogenic factor, and may stimulate tumour angiogenesis via a nuclear site of action^[3]. However, when PGIS overexpression was selectively targeted to alveolar cells in transgenic mice, these animals became more resistant to tumour development, suggesting that prostacyclin plays a key role in preventing lung carcinogenesis^[53]. Here we have the potential conflict between the IP receptor-mediated antiproliferative action on vascular smooth muscle cells and the PPAR δ -mediated angiogenic response, and we have evidence for both pro- and anti-angiogenic activity for prostacyclin.

The case for counter-regulatory signals from prostacyclin in promoting or inhibiting apoptosis is even stronger. In HEK 293 cells lacking cell surface IP receptors and endogenous PGIS, overexpression of PGIS, but not COX-1 or COX-2, produced clear apoptotic morphological changes^[49]. Interestingly, it was also shown that overexpression of either COX-1 or COX-2 in bovine aortic endothelial cells, which constitutively express PGIS at relatively high levels, also increased apoptotic cell death. Hatae *et al*^[49] then raised the interesting question of why endothelial cells and vascular smooth muscle cells, which endogenously express PGIS, do not ordinarily undergo apoptosis. The answer proposed was that these cells also expressed IP receptors which protect against apoptosis by the generation of cyclic AMP. Thus, prostacyclin produces apoptosis mediated by nuclear PPAR δ , and inhibits apoptosis through activating cell surface IP receptors. The fate of a cell will therefore depend on the balance between these two signalling pathways stimulated by prostacyclin.

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