Inflammopharmacology 15 (2007) 246–251
0925–4692/07/06246-6
DOI 10.1007/s10787-007-1591-3

Inflammopharmacology

Review

Proteinase-activated receptors in the gastrointestinal system: a functional linkage to prostanoids

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Received 9 October 2006; accepted 15 November 2006

Abstract. Proteinase-activated receptors (PARs), G protein-coupled receptors, play critical roles in the alimentary system. Increasing evidence suggests that endogenous prostaglandins (PGs) mediate some of PARs' gastrointestinal functions. Systemic administration of the PAR1 agonist protects against gastric mucosal injury through PG formation in rats. PGs also appear to contribute, at least in part, to enhancement of gastric mucosal blood flow and suppression of gastric acid secretion by PAR1 activation. There is also evidence for involvement of PGs in modulation of gastrointestinal motility by PAR1 or PAR2. Importantly, modulation of ion transport by PAR1 or PAR2 in the intestinal mucosal epithelium is largely mediated by PGs. Studies using gastric and intestinal mucosal epithelial cell lines imply that the PAR1-triggered formation of PGs involves multiple signaling pathways including Src, EGF receptor trans-activation and activation of MAP kinases. Collectively, a functional linkage of PAR1 and/or PAR2 to PGs is considered important in the gastrointestinal system.

Key words: Proteinase-activated receptor (PAR) – Gastrointestinal system – Prostanoid – Prostaglandin E_2 – Epithelial cell

Introduction

Proteinase-activated receptors (PARs) belong to a large superfamily of G-protein-coupled seven trans-membrane domain receptors, and four PAR family members, ranging from PAR1 to PAR4, have been cloned so far (Ishihara et al., 1997; Kahn et al., 1998; Nystedt et al., 1994; Vu et al., 1991; Xu et al., 1998). Agonist proteinases for each PAR cleave the extracellular N-terminus of the PAR molecule and expose a new N-terminal domain, which binds to the extracellular second loop of the receptor itself as a tethered ligand, leading to intracellular signaling (Fig. 1). Alternatively, PARs, except for PAR3, can be also non-enzymatically activated by exogenously applied synthetic peptides as short as 5–6 amino acids based on the sequence of the tethered ligands (Fig. 1) (Kawabata, 2002; Ossovskaya et al., 2004; Sekiguchi et al., 2004; Vergnolle, 2004). PAR1, PAR3 and PAR4, but not PAR2, are thrombin receptors, although some other proteinases are also capable of activating PAR1 and/or PAR4 (Kawabata, 2002; Oikonomopoulou et al., 2006; Ossovskaya et al., 2004; Sekiguchi et al., 2004; Vergnolle, 2004) (Fig. 1). On the other hand, multiple endogenous agonist proteinases for PAR2, such as trypsin, mast cell tryptase, coagulation factors VIIa and Xa, acrosin, kallikrein, etc. (Kawabata, 2002; Oikonomopoulou et al., 2006; Ossovskaya et al., 2004; Sekiguchi et al., 2004; Vergnolle, 2004) have been described (Fig. 1). Interestingly, PAR2 also appears to respond to some 'exogenous' proteinases including mite allergens, Arg-gingipains and cockroach proteinase (Kawabata, 2002; Ossovskaya et al., 2004; Sekiguchi et al., 2004; Vergnolle, 2004) (Fig. 1). PARs are widely distributed in the mammalian body, especially throughout the alimentary system, modulating gastrointestinal functions (Kawabata, 2002; Ossovskaya et al., 2004; Sekiguchi et al., 2004; Vergnolle, 2004). Interestingly, increasing evidence suggests involvement of endogenous prostaglandins (PGs) in some of biological events caused by activation of PARs. Since PGs are known to play central roles in regulation of gastrointestinal functions, here we focus on the cross talk between PARs and PGs, particularly prostanoid-dependent functions of PARs, in the alimentary system, extending the topics to the intracellular signaling mechanisms underlying PAR-triggered formation of PGs.

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General functions of the PAR receptor family in the gastrointestinal system

PARs, particularly PAR1 and PAR2, modulate a variety of alimentary functions such as glandular exocrine secretion, epithelial ion transport, and smooth muscle motility (Table 1). There is also evidence that PAR1 and/or PAR2 are protective/anti-inflammatory in the stomach (Table 1), although they might also be involved in colonic inflammation and visceral pain (Kawabata, 2002; Kawabata et al., 2006a; Kawabata et al., 2006b; Kawao et al., 2004; Vergnolle, 2004). In this context, PARs are now considered key regulatory molecules in the gastrointestinal system in both health and disease. Interestingly, some of gastrointestinal functions of PAR1 or PAR2 are attributable to endogenous PGs, although activation of capsaicin-sensitive sensory neurons is also one of the important mechanisms for PAR2-triggered gastrointestinal events including gastric mucosal protection (Kawabata, 2002; Kawabata et al., 2001a; Sekiguchi et al., 2004). Of note is that stimulation of PAR1 or PAR2 actually causes formation of PGs in certain gastrointestinal tissues/cells (Kong et al., 1997; Kubo et al.; Sekiguchi et al., 2005; Toyoda et al., 2003).

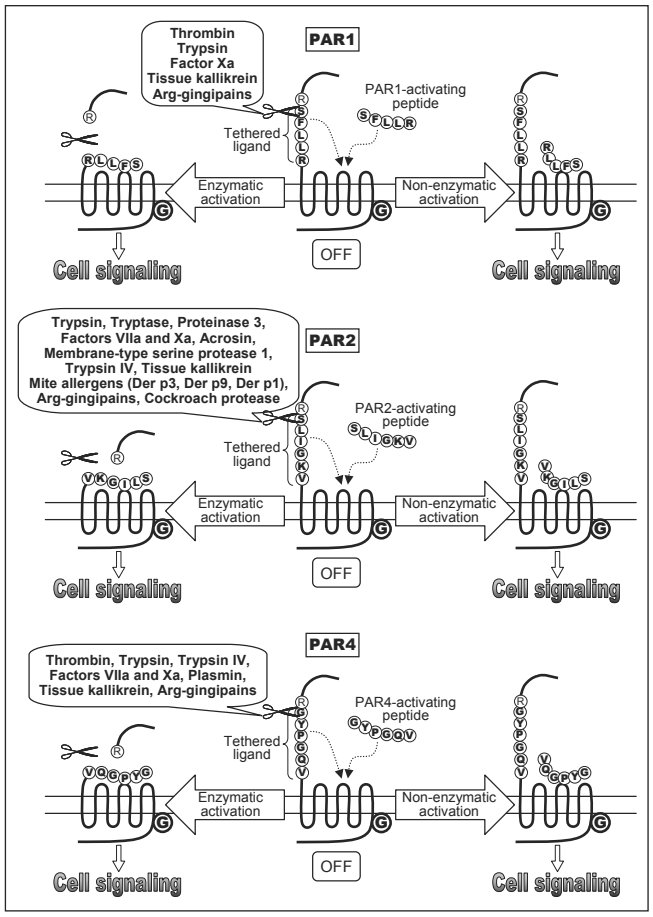

Fig. 1. Mechanisms for enzymatic or non-enzymatic activation of human PAR1, PAR2 and PAR4. Activation mechanisms for PAR3 are not shown, since PAR3 might function only as a co-factor for PAR1 (Nakanishi-Matsui et al., 2000).

Prostanoid-dependent functions of PAR1 in gastric mucosa

Messenger RNAs for PAR1 and PAR2 are abundant in both the gastric mucosa and smooth muscle of the rat (Nishikawa et al., 2002). PAR1 and PAR2 play multiple roles in the gastric mucosa in rats and/or mice, being primarily protective (Kawabata et al., 2001a; Kawabata et al., 2004c). However, the underlying mechanisms for the gastric mucosal protection caused by PAR1 and PAR2 activation are largely different; i.e. the protective effect of PAR1 agonists is dependent on PGs formation (Kawabata et al., 2004c), whereas PAR2 agonists elicit gastric mucus secretion and mucosal protection by activating sensory neuron, an effect being independent of PGs (Kawabata et al., 2001a; Kawabata et al., 2005). COX-1-derived PGs appear to predominantly mediate the PAR1-triggered gastric mucosal protection, since a COX-1 inhibitor, SC-560, but not a COX-2 inhibitor, NS-398, abolished the protective effect (Kawabata et al., 2004c). The PAR1-activating peptide, TFLLR-NH_2, when administrated systemically, increases gastric mucosal blood flow (GMBF), an effect being suppressed, in part, by indomethacin, a non-selective COX inhibitor (Kawabata et al., 2004c). In the isolated gastric artery, PAR1 stimulation with TFLLR-NH_2 actually causes endothelium-dependent relaxation through COX products in addition to endothelium-derived hyperpolarizing factor (EDHF) and NO (Kawabata et al., 2004b). The PAR1 agonist also strongly suppresses carbachol-evoked gastric acid secretion, an effect being abolished by pretreatment with indomethacin (Kawabata et al., 2004c). Our immunohistochemical study using rat and human gastric tissues has revealed that PAR1 and/or COX-1 are abundant in the small blood vessels and muscularis mucosae that project into the mucosal layer (Kawabata et al., 2004c). Taken together, it is hypothesized that thrombin, possibly derived from the blood stream during gastric mu-

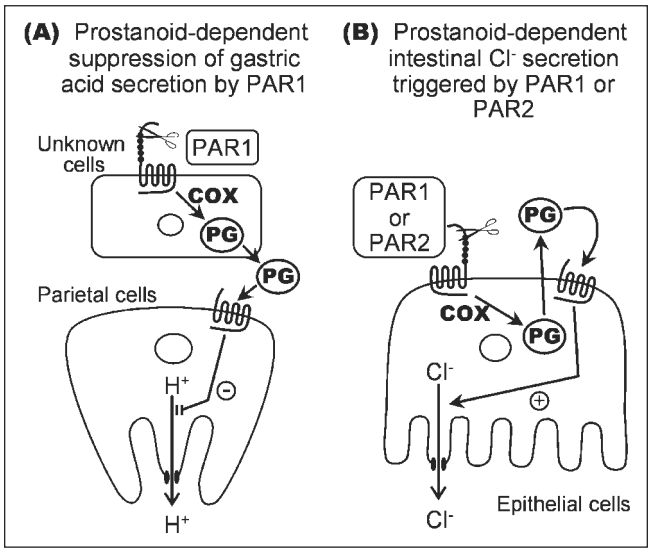

Fig. 2. Prostaglandin-dependent gastrointestinal functions of PAR1 and/or PAR2. (A) Activation of PAR1, present in 'unknown cells' (most probably muscularis mucosa cells), suppresses acid secretion by parietal cells in the gastric mucosa. (B) Upon activation, PAR1 and/or PAR2 trigger Cl^- secretion via PG formation in autocrine and/or paracrine manners.

cosal injury, might stimulate 'unknown cells' that are most probably muscularis mucosa cells expressing both PAR1 and COX-1, as described above, and subsequently cause formation of PGs, leading to suppression of acid secretion by parietal cells in the gastric mucosa (Fig. 2A). It is to note that, like PAR1, PAR2 also modulates gastric mucosal circulation and suppresses gastric acid secretion, whereas the underlying mechanisms do not involve endogenous PGs (Kawabata, 2002; Kawabata et al., 2001a; Kawabata et al., 2003; Nishikawa et al., 2002; Sekiguchi et al., 2004).

PAR1-triggered PGE_2 formation and the underlying cell signalling mechanisms in gastric mucosal epithelial cells

Although we failed to visualize expression of immunoreactive PAR1 in the mucosal epithelial cells of rat and human gastric tissues (Kawabata et al., 2004c), functional PAR1 was detectable in the rat normal gastric mucosal epithelial RGM1 cell line (Sekiguchi et al., 2005; Toyoda et al., 2003) and in the human gastric carcinoma MKN-1 cell line (Miyata et al., 2000). Interestingly, PAR1 stimulation caused delayed (after

Table 1. PARs-mediated responses in the alimentary system

Tissue or cell type	Receptor	Effect
Gingiva		
Oral epithelial cells (KB)	PAR1, PAR2	↑ $[Ca^{2+}]_{in}$, ↑ IL-6 (Lourbakos et al., 2001)
Human gingival fibroblasts	PAR1	↑ $[Ca^{2+}]_{in}$ (Tanaka et al., 2003)
Salivary glands		
Rat sublingual gland	PAR2	↑ Mucin secretion (TK-dependent) (Kawabata et al., 2000b)
Rat parotid gland	PAR2	↑ Amylase secretion (NO-dependent) (Kawabata et al., 2002)
Stomach		
Rat stomach	PAR1	Mucosal protection, ↓ Acid secretion (PGs-dependent) (Kawabata et al., 2004c), ↑ Pepsinogen secretion (Kawao et al., 2003)
	PAR2	Mucosal protection, ↑ Mucus secretion (sensory neuron-dependent) (Kawabata et al., 2001a), ↓ Acid secretion (Nishikawa et al., 2002), ↑ Pepsinogen secretion (Kawao et al., 2002)
Mouse stomach	PAR2	Mucosal protection (sensory neuron-dependent) (Kawabata et al., 2005)
Rat gastric epithelial cells (RGM1)	PAR1	↑ PGE_2 release (Sekiguchi et al., 2005; Toyoda et al., 2003), ↑ Mucus secretion (Sekiguchi et al., 2005; Toyoda et al., 2003)
Pancreas		
Dog pancreatic duct epithelial cells	PAR2	↑ Cl^- and K^+ secretion ($[Ca^{2+}]_{in}$ ↑-dependent) (Nguyen et al., 1999)
Rat pancreas	PAR2	↑ Amylase secretion (NO-dependent) (Kawabata et al., 2002)
GI motility		
Mouse GI transit (in vivo)	PAR1, PAR2	Facilitation of transit (L-VDCC-dependent) (Kawabata et al., 2001b)
Mouse gastric longitudinal muscle	PAR1, PAR2	Contraction (PGs and sensory neuron-dependent) (Sekiguchi et al., 2006), Relaxation (SK-dependent) (Cocks et al., 1999b; Sekiguchi et al., 2006)
Mouse ileal longitudinal muscle	PAR1	Contraction (PGs-dependent), Relaxation (partially SK-dependent) (Sekiguchi et al., 2006)
Rat esophageal muscularis mucosa	PAR1	Contraction (L-VDCC-dependent) (Kawabata et al., 2000a)
	PAR4	Relaxation (Kawabata et al., 2000a)
Rat gastric longitudinal muscle	PAR2	Contraction (PGs, L-VDCC and TK-dependent) (Saifeddine et al., 1996)
Rat duodenal longitudinal muscle	PAR1	Contraction (L-VDCC, PKC and TK-dependent) (Kawabata et al., 1999)
		Relaxation (SK-dependent) (Kawabata et al., 1999)
Rat colonic longitudinal muscle	PAR1, PAR2	Contraction (tachykinins and ACh-dependent) (Mule et al., 2004),
		Relaxation (NO-dependent), Contraction (tachykinins-dependent) (Mule et al., 2003)
	PAR4	Contraction (tachykinins and ACh-dependent) (Mule et al., 2004)
GI secretion		
Human intestinal epithelial cells (SCBN)	PAR1	↑ PGE_2 and PGF_2 release, ↑ Cl^- secretion (Buresi et al., 2002; Buresi et al., 2001)
Rat small intestinal enterocytes (hBRIE 380)	PAR2	↑ PGE_2 release, ↑ IP_3 formation (Kong et al., 1997)
Rat jejunum	PAR1	↑ Cl^- secretion (Buresi et al., 2002)
	PAR2	↑ PGE_2 release (Kong et al., 1997), ↑ Cl^- secretion (PGs-dependent) (Vergnolle et al., 1998)
Mouse distal colon	PAR2	↑ Na^+ absorption, ↑ K^+ and Cl^- secretion (Cuffe et al., 2002)

$[Ca^{2+}]_{in}$, intracellular Ca^{2+} level; TK, tyrosine kinase; PGs, prostaglandins; GI, gastrointestine; L-VDCC, L-type voltage-dependent Ca^{2+} channels; NO, nitric oxide; ACh, acetylcholine; PKC, protein kinase C; SK, small conductance Ca^{2+}-activated K^+ channels.

18-h stimulation) formation of PGE_2 in RGM1 cells (Sekiguchi et al., 2005; Toyoda et al., 2003). Toyoda et al. (Toyoda et al., 2003) have also revealed delayed secretion of mucus by RGM1 cells after PAR1 stimulation, although systemic administration of the PAR1 agonist failed to cause secretion of gastric mucus in anesthetized rats *in vivo* (Kawabata et al., unpublished data). Our cell signaling study has shown involvement of multiple signaling pathways including Src, EGF receptor trans-activation and activation of MAP kinases in the PGE_2 formation caused by PAR1 activation in RGM1 cells (Sekiguchi et al., 2005). Briefly, upon activation, PAR1 causes prompt activation of Src and the MEK/ERK pathway and also trans-activation of EGF receptors, leading to up-regulation of heparin-binding EGF (HB-EGF) and persistent activation of EGF receptors and the MEK/ERK pathway. These signaling mechanisms appear to be essential for up-regulation of COX-2 followed by delayed PGE_2 formation in RGM1 cells (Fig. 3A). Although specific inhibitors of COX-1, protein kinase C (PKC) and p38 MAP kinase also abolish the PAR1-triggered PGE_2 formation (Sekiguchi et al., 2004), the precise mechanisms by which these signaling molecules contribute to the PGE_2 formation have yet to be clarified. Thus, PAR1 triggers PG formation both in laboratory animals *in vivo* and in the cultured cells *in vitro*, whereas there is some discrepancy between the *in vivo* and *in vitro* findings; i.e., different time-courses of PGE_2 formation (rapid *in vivo* vs. slow *in vitro*) and distinct COX isoforms involved (COX-1 *in vivo* vs. both COX-1 and COX-2 *in vitro*).

Prostanoid-dependent ionic transport by PAR1 or PAR2 and the underlying cell signalling mechanisms in intestinal epithelial cells

PAR1 and/or PAR2 regulates ion transport in the intestinal mucosa predominatly through PG formation (Buresi et al., 2002; Buresi et al., 2001; Cuffe et al., 2002; Vergnolle et al., 1998). In the monolayers of SCBN, a non-transformed human duodenal epithelial crypt cell line, basolateral application of PAR1 agonists causes rapid Cl^- secretion to the apical direction (Buresi et al., 2002; Buresi et al., 2001) (Fig. 2B). This effect of the PAR1 agonists is dependent on endogenous PGs, on the basis of the results from inhibition experiments (Buresi et al., 2002). Although PAR1 stimulation actually enhances release of PGE_2 and $PGF_{2\alpha}$ in SCBN cells, these PGs do not appear responsible for the PAR1-triggered Cl^- secretion (Buresi et al., 2002) (Fig. 3B). In the tissues of mouse distal colon, basolateral application of PAR2 agonist, but not thrombin, causes Cl^- and K^+ secretion, suggesting involvement of PAR2, but not PAR1, PAR3 and PAR4, in the regulation of colonic ion transport (Cuffe et al., 2002). Nonetheless, endogenous PGs might not be involved in the PAR2-triggered colonic ion secretion in the mouse, since indomethacin inhibited the effect of trypsin, but not PAR2-activating peptides (Cuffe et al., 2002). In contrast, there is evidence that activation of basolateral PAR2-like receptors regulates jejunal ion transport in the rat (Vergnolle et al., 1998). In hBRIE 380 cells, a polarized, differentiated enterocyte cell line from rat small intestine, trypsin or a PAR2-activating peptide enhances IP_3 formation and causes PGE_2 formation (Kong et al., 1997). Because trypsin is capable of producing those effects at the physiological concentration of pancreatic trypsin, the luminal trypsin in the intestine is considered to regulate functions of the intestinal epithelium via PGE_2 formation by stimulating PAR2 (Kong et al., 1997). Collectively, epithelial PAR1 and/or PAR2 appear to regulate intestinal ion transport mainly through PG formation in autocrine and/or paracrine manners (Fig. 2B).

Cell signaling mechanisms for PG formation triggered by PAR1 or PAR2 in the intestinal epithelial cells largely remain unknown. Buresi et al. (Buresi et al., 2002) have studied signal transduction mechanisms by which PAR1 triggers prostanoid-dependent Cl^- secretion in SCBN cells. Interestingly, as described for the signaling mechanisms underlying the PAR1-triggered PGE_2 formation in RGM1 cells (Fig. 3A), the regulation of ion transport by PAR1 in SCBN cells also appears to involve Src, EGF receptor trans-activation and activation of MAP kinases (Buresi et al., 2002) (Fig. 3B). Phosphorylation of cytosolic phospholipase A_2 ($cPLA_2$) by ERK is considered essential for the PG-dependent effect of the PAR1 agonist in SCBN cells (Buresi et al., 2002) (Fig. 3B), while a $cPLA_2$ inhibitor does not prevent PAR1-triggered PGE_2 formation in RGM1 cells (Sekiguchi et al., 2005). Involvement of constitutively expressed COX-2 in addition to COX-1 is also demonstrated in the prompt prostanoid-dependent secretion of Cl^- in response to PAR1 stimulation in SCBN cells (Buresi et al., 2002) (Fig. 3B), while transcriptional up-regulation of COX-2 is critical for the delayed PGE_2 formation in RGM1 cells (Sekiguchi et al., 2005) (Fig. 3A). Thus, in spite of some common pathways involved, signal transduction mechanisms for PG formation in RGM1 and SCBN cells are essentially different.

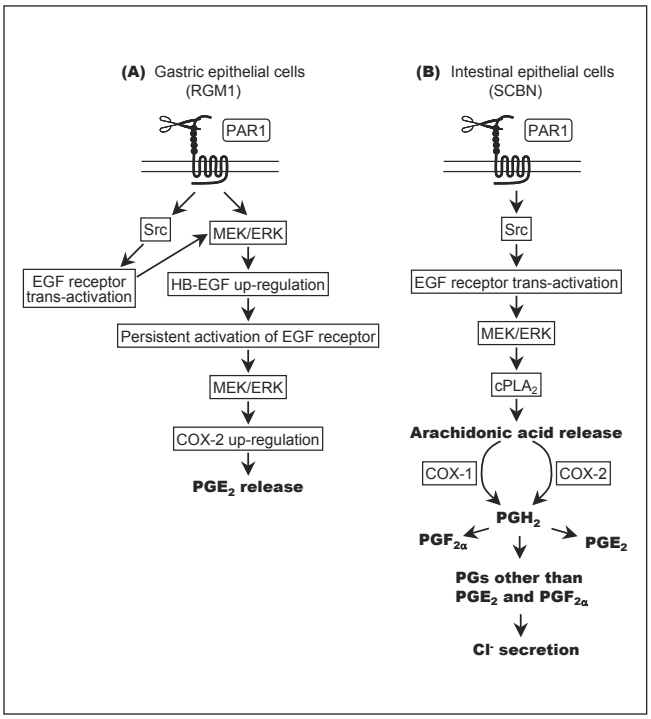

Fig. 3. Signal transduction mechanisms for PAR1-triggered PG formation in gastric and intestinal epithelial cells.

Involvement of prostanoids in modulation of gastrointestinal motility by PAR1 or PAR2

Modulation of gastrointestinal smooth muscle motility by PAR1, PAR2 and PAR4 has been described *in vitro* (Cocks et al., 1999b; Kawabata et al., 2000a; Kawabata et al., 1999; Mule et al., 2003; Mule et al., 2004; Saifeddine et al., 1996; Sekiguchi et al., 2006) as well as *in vivo* (Kawabata et al., 2001b) (Table 1). The underlying mechanisms for the motility modulation by PARs vary with species and the parts of the gastrointestinal tract. Involvement of contractile PGs has been shown in PAR2-mediated contraction in the gastric longitudinal smooth muscle of rats (Saifeddine et al., 1996) and PAR1- and PAR2-mediated contraction in the gastric and ileal longitudinal smooth muscle of mice (Sekiguchi et al., 2006). The contractile PGs involved in the effects of PAR agonists have yet to be identified.

Prostanoid-dependent functions of the PAR receptor family in tissues/cells other than the alimentary system

In the respiratory system, PAR2 is abundantly expressed in the epithelium, and upon activation, causes PGE_2 release, leading to airway smooth muscle relaxation. (Cocks et al., 1999a; Kawabata et al., 2004a; Kawao et al., 2005). In the guinea-pig gallbladder, stimulation of PAR1 and PAR2, but not PAR4, causes smooth muscle contraction via formation of endogenous PGs (Tognetto et al., 2000). In the rat urinary bladder, activation of PAR2 stimulates release of PGs from the mucosal layer and thereby contracts the smooth muscle (Nakahara et al., 2003). In the human keratinocyte cell line, HaCaT, PAR2 activation modulates skin pigmentation through direct action on keratinocyte uptake of melanosomes and through release of PGE_2 and $PGF_{2\alpha}$ that function as paracrine factors, leading to stimulation of melanocyte dendricity (Scott et al., 2004).

Conclusion

In conclusion, endogenous PGs are considered to mediate many of the biological functions of PARs, particularly PAR1 and PAR2, in the gastrointestinal systems as well as other organs. To clarify the functional and molecular linkage between PARs and PGs would be useful for understanding of roles for PARs in the pathogenesis of various diseases.

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