Epithelial effects of proteinase-activated receptors in the gastrointestinal tract

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The intestinal epithelium plays a crucial role in providing a barrier between the external environment and the internal milieu of the body. A compromised mucosal barrier is characteristic of mucosal inflammation and is a key determinant of the development of intestinal diseases such as Crohn's disease and ulcerative colitis. The intestinal epithelium is regularly exposed to serine proteinases and this exposure is enhanced in numerous disease states. Thus, it is important to understand how proteinase-activated receptors (PARs), which are activated by serine proteinases, can affect intestinal epithelial function. This review surveys the data which demonstrate the wide distribution of PARs, particularly PAR-1 and PAR-2, in the gastrointestinal tract and accessory organs, focusing on the epithelium and those cells which communicate with the epithelium to affect its function. PARs have a role in regulating secretion by epithelia of the salivary glands, stomach, pancreas and intestine. In addition, PARs located on subepithelial nerves, fibroblasts and mast cells have important implications for epithelial function. Recent data outline the importance of the cellular site of PAR expression, as PARs expressed on epithelia may have effects that are countered by PARs expressed on other cell types. Finally, PARs and their ability to promote epithelial cell proliferation are discussed in terms of colon cancer.

Key words: proteinase-activated receptors - epithelium - secretion - inflammation - cancer

The epithelia lining mucosal surfaces are well situated to act as sensors of the external environment, as transducers of luminal stimuli and as effectors of host defense against luminal pathogens. In the case of the gastrointestinal tract, the epithelium must have apical receptors to sense the luminal environment, particularly enteric flora, second messenger systems to relay luminal signals and an array of responses to integrate luminal stimuli with underlying elements including the mucosal immune system, the enteric nervous system and the mucosal and submucosal vascular and lymphatic systems. This capability must be integrated with the epithelium's role in the digestive, absorptive and secretory processes of normal physiological function.

The gastrointestinal epithelium is repeatedly, if not continuously, exposed to an array of proteinases involved in digestion and host defense. The discovery of proteinase-activated receptors (PARs) in the gastrointestinal tract suggests, however, that proteinases may act as signaling molecules in addition to their role as agents of protein breakdown. The pharmacology and physiology of PARs have been the subject of recent comprehensive reviews (Macfarlane et al. 2001, Hollenberg & Compton 2002, Ossovskaya & Bunnett 2004). Therefore, this review will focus on PARs expressed on the gastrointestinal epithelium and on cells which interact with the epithelium to regulate its function in health and disease.

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Distribution of PARs in gastrointestinal tract

PARs have been demonstrated in every region of the gastrointestinal tract and accessory organs including the salivary glands (Kawabata et al. 2000a, b), stomach (Nystedt et al. 1995, Kawabata et al. 2001, Kawao et al. 2002), intestine (Kong et al. 1997, Corvera et al. 1997, 1999, Green et al. 2000, Buresi et al. 2003, Seymour et al. 2003), pancreas (Bohm et al. 1996, Kawabata et al. 2002) and liver (Bohm et al. 1996, Fiorucci et al. 2004). The bulk of evidence points toward expression of PAR-1 and PAR-2, although PAR-3 and PAR-4 may also be present (Toyoda et al. 2003, Mule et al. 2004). In all regions of the gastrointestinal tract, PARs are expressed on epithelia and thus are in a position to respond to luminal or mucosal serine proteinases. Furthermore, as detailed below, PARs are found on cell types that have been demonstrated to affect epithelial function, including enteric neurons, fibroblasts and immune cells.

Effect of PARs on epithelial function

Salivary glands - PAR-2 has been demonstrated by immunohistochemistry to exist on parotid gland acinar epithelium (Kawabata et al. 2002). Activation of PAR-2 has been shown to stimulate the secretion of mucin in a non-cholinergic, non-adrenergic and tyrosine kinase-dependent manner (Kawabata et al. 2000a). In addition, activation of PAR-2 in vivo stimulates the secretion of salivary amylase through a mechanism that does not involve capsaicin-sensitive nerves (Kawabata et al. 2002). There is no evidence that PAR-1 is involved in salivary epithelial function as selective PAR-1-activating peptides did not stimulate secretion (Kawabata et al. 2000b). The

endogenous activator of salivary PAR-2 has not been identified.

Stomach - Both PAR-1 and PAR-2 have been shown to affect gastric mucosal function. Activation of PAR-1 appears to be cytoprotective in a gastric epithelial cell line (Toyoda et al. 2003) and in a rat model (Kawabata et al. 2004). In both studies, the protective effect was dependent upon cyclooxygenase activity. In contrast, other studies have shown that PAR-1 activation can cause plasma extravasation in the stomach (De Garavilla et al. 2001), suggestive of a pro-inflammatory effect of PAR-1. The difference may lie in the model used, with one being an assessment of the effect of PAR-1 activation on HCl-ethanol-induced damage in the rat (Kawabata et al. 2004) and one being an assessment of PAR-1 effects in normal mouse stomach (De Garavilla et al. 2001). The stimulation of plasma exudation by PAR-1 activation in the mouse is dependent upon sensory afferent nerves. Thus, the evidence to date suggests that epithelial PAR-1 may be protective in the stomach, through the stimulation of prostaglandin synthesis, whereas neuronal PAR-1 may promote neurogenic gastric mucosal inflammation.

PAR-2 activation has also been shown to be protective in the stomach. In this case, the effect may be to stimulate capsaicin-sensitive nerve-dependent release of calcitonin gene-related peptide and neurokinins and/or the stimulation of mucin secretion (Kawabata et al. 2001). Others have shown that activation of capsaicin-sensitive afferents in the stomach confers protection against injurious agents (Whittle et al. 1992, Morris et al. 1993).

In addition to their roles as a determinants of mucosal integrity, PAR-1 and PAR-2 have also been shown to stimulate pepsinogen secretion in the stomach (Fiorucci et al. 2003, Kawao et al. 2003). A clear mechanism for this secretory effect has yet to be elucidated, although in the case of PAR-2, its expression on chief cells suggests that the effect of activation may be direct (Kawao et al. 2002, Fiorucci et al. 2003).

Intestine - PARs have numerous effects on intestinal epithelial function. Indeed, one PARs may have several different effects depending upon the site of expression. For example, PAR-1 is expressed on both the basolateral and apical surfaces of SCBN, a duodenal crypt cell line. Activation of basolateral PAR-1 stimulates apically directed chloride secretion in a calcium-dependent manner (Buresi et al. 2001). This effect is due to a Src-kinase-associated epidermal growth factor receptor kinase transactivation which stimulates MAP kinase-induced activation of phospholipase A_2. The subsequent liberation of arachidonic acid stimulates chloride secretion via a mechanism dependent upon both cyclooxygenase (COX)-1 and COX-2 (Buresi et al. 2002). In contrast, activation of apical PAR-1 in the same cell line activates a different signaling pathway, which is not Src-dependent, leading to apoptosis and increases in epithelial monolayer permeability (Chin et al. 2003). Since these results were obtained from a cell line, caution must be exercised when extrapolating the data to an integrated system like the whole intestine, but these studies clearly illustrate the point that polarity of PAR expression can have profound effects in terms of the cellular response.

PAR-2 is also expressed on intestinal epithelial cells (Kong et al. 1997, Green et al. 2000). Our preliminary data suggest that activation of epithelial PAR-2 also stimulates ion transport in cell line models (van der Merwe & MacNaughton, unpublished data). Activation of PAR-2 in isolated segments of rat jejunum stimulates chloride secretion in a manner independent of enteric nerves, suggesting that the effect is directly on the epithelium (Vergnolle et al. 1998). Similarly, PAR-2 activation of segments of human colon in vitro results in neurally independent chloride secretion (Mall et al. 2002).

While it is clear that PARs are expressed on the intestinal epithelia of several species, there is growing evidence that activation of PARs on other cell types can affect epithelial function. We have shown that PAR-1 is ex-pressed on submucosal secretomotor neurons in mouse colon, and that its activation leads to the suppression of neurally evoked chloride secretion (Buresi et al. 2003). PAR-2 is also found on enteric nerves in pig (Green et al. 2000) and guinea pig (Reed et al. 2003) ileum. PAR-2 activation on enteric nerves may change the electrophysiological properties of these neurons, either causing excitation (Gao et al. 2002) or enhancing excitability by other activators (Reed et al. 2003).

In addition to enteric nerves, other cell types within the mucosal lamina propria can affect epithelial function. Subepithelial fibroblasts can respond to secretagogues with the production and release of secretory prostaglandins (Berschneider & Powell 1992). We have recently demonstrated that intestinal myofibroblasts of the type juxtaposed to secretory epithelia express PAR-1, and that PAR-1 activation leads to the expression of COX-2 and subsequent upregulation of PGE_2 secretion (Seymour et al. 2003). Experiments to conclusively demonstrate that fibroblast PAR-1 stimulates chloride secretion have yet to be performed.

Mast cells express PAR-1 and PAR-2 (D'Andrea et al. 2000, Gordon et al. 2000), at least under some circumstances. Mucosal mast cells in the intestine are an important source of mediators which can stimulate intestinal epithelial chloride secretion (McKay & Perdue 1993). While the effect of PAR activation on mast cell mediated secretion has not been investigated, the demonstration of such a relationship would have important implications for mast cell-associated conditions such as intestinal anaphylaxis.

There has been increased interest in the role of PAR-2 as a mediator of neurogenic inflammation in the intestine (Steinhoff et al. 2000). Related to this is the recognition that PAR-2 can mediate hyperalgesia of the intestine (Coelho et al. 2002). Both of these situations point to a relationship between PAR-1 and capsaicin-sensitive afferent activation and indeed this relationship has been demonstrated experimentally (Steinhoff et al. 2000, De Garavilla et al. 2001, Vergnolle et al. 2001). We have evidence that capsaicin-sensitive extrinsic sensory afferent neurons can, through local axon reflexes, stimulate chloride secretion in guinea pig ileum in vitro (Vanner & MacNaughton 1995). More recent evidence implicates an interaction of the cannabinoid CB_1 receptor and the

capsaicin receptor, TRPV1 (MacNaughton et al. 2004). It is interesting to speculate that PAR-2 could modulate an interaction among the CB_1 receptor and capsaicin-sensitive afferents in the regulation of intestinal epithelial secretory function.

Pancreas - In the pancreas, PAR-2 is expressed on both acinar cells (Kawabata et al. 2002) and duct epithelium (Nguyen et al. 1999), where its activation stimulates amylase secretion and electrolyte transport, respectively. Some forms of pancreatitis are characterized by premature activation of trypsinogen to trypsin, which activates PAR-2, suggesting that PAR-2 may play a role in pancreatic inflammation. Indeed, PAR-2 expression increases in the pancreas during a model of acute pancreatic lesion (Olejar et al. 2001). However, there is no direct evidence to implicate PAR-2 in the development of pancreatitis, either clinically or in animal models.

PARs, epithelial cell proliferation and cancer

It has long been known that activation of PAR-1 and PAR-2 can stimulate second messenger pathways leading to cellular proliferation and differentiation (Dery et al. 1998). Substances which promote proliferation and differentiation are often associated with the potential to cause uncontrolled cellular growth. Recently, both PAR-1 and PAR-2 have been associated with the development of cancer in numerous models, including gastrointestinal epithelia. For example, abnormal PAR-1 expression is found in oral squamous cell carcinoma (Liu et al. 2001) and colon cancer cell lines (Darmoul et al. 2003). PAR-1 is also associated with tumour cell differentiation (Rudroff et al. 2002) and adhesion (Rudroff et al. 2001) in pancreatic cancer. PAR-1 appears to promote invasion signals in some cancer cells through activation of a Rho-A-dependent pathway (Nguyen et al. 2002).

Similarly, there is recent evidence linking PAR-2 with cancers of epithelial tissue in the gastrointestinal tract. In the colon, activation of PAR-2 leads to proliferation of several colonic epithelial cancer cell lines (Darmoul et al. 2001), which in the case of the DLD-1 colonic carcinoma cell line is mediated by a MEK-MAP kinase signal transduction pathway (Jikuhara et al. 2003). Likewise, PAR-2 has been implicated in cell proliferation, invasion and fibrosis in pancreatic cancer (Ikeda et al. 2003, Ohta et al. 2003, Shimamoto et al. 2004).

Conclusion

The wide distribution of PARs on gastrointestinal epithelia and on cells which affect epithelial function, along with the fact that gastrointestinal epithelia are exposed to serine proteinases under both physiological and pathophysiological conditions, suggests that PARs will be implicated as important modulators of health and disease in the gastrointestinal tract. Pathological conditions characterized by inflammation, abnormal secretion or uncontrolled proliferation may ultimately be shown to have a substantial component related to activation of PARs by serine proteinases. Advancing our knowledge of epithelial PARs will provide the impetus for the development of pharmacological agents to manipulate PARs to determine the course of diseases of the gut.

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