Intestinal Epithelial Secretory Function: Role of Proteinase-Activated Receptors

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ABSTRACT

The ability of enterocytes to secrete electrolytes and water into the intestinal lumen represents a critical feature of mucosal defense. During disease, this function may be altered and may initiate or exacerbate pathological conditions. Although many of the intracellular mechanisms linking stimulation to secretion have been elucidated, novel pathways continue to be revealed. These pathways provide potential for therapeutic manipulation of cellular function. In addition, the importance of the microenvironment surrounding enterocytes is increasingly being acknowledged, and the interactions between epithelial cells and their milieu are proving to be essential to the regulation of secretory function, both in health and disease. In this way, epithelial ion transport functions can be modulated by mediators released from neighboring nerves, inflammatory cells, and pathogens, or by endocrine factors. Much interest has recently been elicited by the discovery that proteinases can regulate cellular functions through the activation of proteinase-activated receptors (PARs). Because of the abundance of proteases within the gastrointestinal tract, particularly in the setting of development, inflammation, and healing, it is likely that PARs have an important role to play in these processes. PARs have been localized to a variety of cell types in the gastrointestinal tract, and have been shown to influence epithelial secretory function on several levels. In this review, we discuss the mechanisms by which proteases and PARs regulate intestinal secretory function, and the manner in which these modulations might contribute to inflammatory processes. Drug Dev. Res. 59:386–394, 2003.

THE ROLE OF THE INTESTINAL EPITHELIUM IN HOST DEFENSE

The gastrointestinal epithelium represents the first line of defense against ingested bacteria, antigens, drugs, and toxins. An important aspect of intestinal barrier function is the ability of a subpopulation of enterocytes to secrete electrolytes such as chloride in a basolateral to apical direction, promoting the movement of water into the lumen. The movement of chloride and water into the lumen of the intestinal crypts prevents their colonization by bacteria and slows the translocation of antigens into the lamina propria [Cirino et al., 1996; Asfaha et al., 2001].

Responsiveness of the epithelium to secretagogues can be significantly altered during pathological conditions. Excessive secretion due to a hyperresponsiveness to secretagogues, as exemplified by the response to cholera toxin, can result in diarrhea leading to excess water and electrolyte loss. Conversely, the inability to secrete chloride and water due to a hyporesponsiveness to secretagogues, exemplified by...
models of intestinal inflammation [MacNaughton et al., 1998; Asfaha et al., 2001; Freeman and MacNaughton, 2000] and as observed in human inflammatory bowel disease [Archampong et al., 1972; Hubel and Renquist, 1990; Sandle et al., 1990], may allow the translocation of bacteria, antigens, or toxins across the epithelium and into the lamina propria where they may then trigger, exacerbate, or prolong inflammation. Furthermore, defective chloride secretion in the gut, such as that which occurs in congenital deficiencies such as cystic fibrosis, leads to intestinal obstruction and malabsorption [O’Loughlin et al., 1991; Grubb and Gabriel, 1997].

REGULATION OF CHLORIDE SECRETION

Many of the intracellular mechanisms regulating chloride secretion by intestinal epithelial cells have been characterized. While the details are beyond the scope of this review, of relevance are the second messenger systems that regulate apical chloride secretion. Typically, chloride secretion in the gut is mediated by intracellular signaling pathways involving either an increase in intracellular calcium levels through the action of phospholipase C and inositol triphosphate, or an increase in cyclic AMP (cAMP) through the action of adenyl cyclase. These signaling systems can be modified by mediators originating from nerves, immune cells, myofibroblasts, or other neighboring cells, and responsiveness to these mediators may be altered during disease states [reviewed in Barrett and Keely, 2000]. For example, various reactive oxygen species, platelet activating factor, lipooxygenase metabolites of arachidonic acid, and certain cytokines including IL-1, all induce chloride secretion. Interestingly, the secretion elicited by each of these factors is sensitive to inhibition by cyclooxygenase inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDs). This observation suggests that arachidonic acid metabolites, such as the prostaglandins, may act as final common mediators of many stimulants of intestinal ion transport such as the prostaglandins, may act as final common mediators of many stimulants of intestinal ion transport [Hinterleitner and Powell, 1991; Montrose et al., 1999]. This has implications for the mediation of epithelial secretory function by PARs as discussed below.

Regulation of epithelial chloride secretion is also under tight neural control, especially through the action of submucosal secretomotor neurons of the enteric nervous system. Specifically, release of vasoactive intestinal polypeptide or acetylcholine stimulates chloride secretion through activation of cAMP- and Ca++-dependent pathways respectively. In addition, the rate of chloride secretion can be influenced by a variety of neurally derived mediators including opiates, gamma-aminobutyric acid (GABA), calcitonin gene related peptide (CGRP), substance P, and serotonin [Keast et al., 1986; Cooke, 1992; MacNaughton et al., 1996, 1997; Green et al., 2000]. Thus, substances that affect enteric neural function also have the potential to alter epithelial secretion of chloride and water.

PROTEINASES AND EPITHELIAL FUNCTION: MICROENVIRONMENT AND NEUROIMMUNE CONTROL SYSTEMS

How can the secretory function of enterocytes be regulated by serine proteinases and PARs? Serine proteinases often have activities related to inflammation, tissue remodeling, and healing [reviewed in Macfarlane et al., 2001]. The PARs in different tissues might thus respond selectively to site-targeted proteinases, either in the setting of tissue injury or in the course of development [Werb, 1997]. The gastrointestinal (GI) tract is exposed to a particularly large array of proteinases, whether in physiological or pathological settings, due to its proximity to digestive enzymes, proteinases from pathogens and proteinases derived from inflammatory cells or the microvasculature. Proteinases such as thrombin and mast cell tryptase are abundant in the gastrointestinal mucosa and submucosa during inflammatory conditions where hemorrhage, vascular disruption, and immune cell infiltration are evident [reviewed in Vergnolle, 2000]. Luminal trypsin may also act on enterocytes via PAR2 localized to the apical surface [Kong et al., 1997] or may access the basolateral surface of the epithelium, as well as other cell types, as a result of increased epithelial permeability. Other proteinases, such as plasmin, granzyme A, and cathepsin G have also been shown to cleave and activate PARs, albeit less potently [Suidan et al., 1994, 1996; Molino et al., 1995; Vouret-Craviari et al., 1995]. In addition, it has been shown that allergic house dust mite proteinases are capable of cleaving and activating PARs to affect epithelial function in the respiratory tract [Sun et al., 2001; Asokananthan et al., 2002b]. Some bacterial proteinases have also been demonstrated to activate PARs situated on oral epithelial cells, and to affect secretion of inflammatory mediators [Lourbakos et al., 2001]. Regulation of epithelial function by PAR1 may furthermore be influenced by proteinases, such as neutrophil elastase, proteinase 3, and cathepsin G, that are able to cleave PAR1 downstream of the thrombin cleavage site, resulting in the deactivation of the receptor, and rendering it unresponsive to further activation by thrombin.

Thus, a variety of proteinases may be in a position to act on PARs and to influence enterocyte secretory function on several levels, either by direct interaction with the epithelium, or by altering the release of secretagogues from other cell types. Although PAR
activation by a protease is an irreversible event, and resensitization is dependent on mobilization of intracellular stores and on synthesis of new receptors, the turnover of PARs in native tissue is quite rapid and supports a role for these receptors in the regulation of ion transport [Bohm et al., 1996; Cocks et al., 1999a,b; Mall et al., 2002].

**MUCOSAL DISTRIBUTION OF PARs AND CONTROL OF CHLORIDE SECRECTION**

**PAR1**

PAR1 has been localized to various epithelia and its activation has been demonstrated to induce the secretion of growth factors and cytokines in airway and corneal epithelia [Asokananthan et al., 2002a; Shimizu et al., 2000; Lang et al., 2003]. Furthermore, a role for PAR1 in electrolyte transport has been suggested, and may occur via the release of arachidonic acid metabolites, which are known to stimulate secretion. As mentioned above, arachidonic acid metabolites and the cyclooxygenase (COX) pathways are essential to the mechanism of action of many secretagogues. The induction by PARs of a COX-dependent pathway leading to the release of prostaglandins has been reported in several systems including platelets, Chinese hamster ovary cells, murine tracheal smooth muscle, and human urothelial carcinoma cells [Winitz et al., 1994; Seiler et al., 1995; Lan et al., 2001; McHowat et al., 2001]. In addition, we have recently shown that an intestinal myofibroblast cell line is capable of responding to thrombin with increased expression of COX-2 and synthesis of PGE2. This effect of thrombin had both PAR1-dependent and independent components [Seymour et al., 2003]. The ability of mediators such as PGE2 that are released from neighboring cells to modulate epithelial secretory function has been well described [Berschneider and Powell, 1992; Powell et al., 1999], and now seems to be a component of the response to mucosal activation of PAR1 also.

In the GI tract, PAR1 has been localized to smooth muscle cells, myenteric neurons [Kawabata et al., 1999; Gao et al., 2002], and endothelial cells of the lamina propria and submucosa [Wang et al., 2002]. Recently, PAR1 was also found to be expressed in a non-tumorogenic, chloride-secreting intestinal epithelial cell line, SCBN [Buresi et al., 2001]. Furthermore, we have shown that exposure of the basolateral (but not apical) SCBN cell membrane to thrombin or PAR1-activating peptides results in calcium-dependent chloride secretion. Cross-desensitization between thrombin and PAR1-activating peptides strongly suggests that the effect is mediated by PAR1 [Buresi et al., 2001]. Interestingly, PAR1 activation results in a novel stimulus-secretion coupling pathway, wherein EGF receptor transactivation and Src activation precede a MEK-ERK1/2 dependent activation of cytosolic PLA2 and COX-1/COX-2 dependent chloride secretion [Buresi et al., 2002] (Fig. 1). Although it is a novel finding that a mitogenic signaling pathway is involved in stimulus-secretion coupling in the intestine, PARs have been linked to mitogen-activated protein (MAP) kinase pathways in other systems. Thrombin is mitogenic in many cells and furthermore induces interleukin-8 production in endothelial cells via activation of PAR1 and subsequent activation of MAP kinases [L'Allemain et al., 1991; Molloy et al., 1996; Takata et al., 2001].

The signaling mechanisms by which GPCRs such as the PARs activate the MAP kinase pathway is complex and not fully understood, but much evidence has accumulated for the transactivation of a receptor tyrosine (tyr) kinase, and in particular, the epidermal growth factor (EGF) receptor. The mechanism by which GPCR signaling leads to the transactivation of the EGF receptor has been hypothesized to occur in a number of ways and is reviewed elsewhere [Gschwind et al., 2000].

![Proposed model of PAR1 induced chloride secretion in intestinal epithelial cells](image)

**Fig. 1.** Proposed model of PAR1 induced chloride secretion in intestinal epithelial cells. PAR1 activation by thrombin (or by PAR1-activating peptides) leads to increased intracellular Ca++, likely through a PLC/IP3/DAG mechanism. This leads to a Src-associated EGF receptor transactivation, which triggers a Raf-dependent activation of the ERK1/2 MAP kinase pathway. ERK1/2 mediates PLA2 activation and subsequent liberation of arachidonic acid from the plasma membrane. Arachidonic acid metabolism by cyclo-oxygenase (COX) results in a prostanoid (PG)-dependent increase in apically directed chloride secretion. The chloride channel involved is not known, but is unlikely to be the cystic fibrosis transmembrane conductance regulator (CFTR). With data from [Buresi et al., 2001, 2002; Dery et al., 1998].
PGE2 in a variety of systems [Laporte et al., 1999; Mall et al., 2002] may represent a control mechanism for dampening the stimulation [Buresi et al., 2003]. While the nature of dependent secretion stimulated by electrical field in the mouse distal colon resulted in a decrease in neurally depolarized porcine ileum have been shown to express PAR2 and agonists of the receptor stimulate active anion secretion by a neurogenic mechanism. This effect can furthermore be modulated by prostanoids and opioids [Green et al., 2000]. In addition, PAR activation in cultured neurons results in release of substance P and CGRP, both of which modulate intestinal ion transport [Perdue et al., 1987; Cooke, 1992; Cox and Tough, 1994; Kawabata et al., 2001; Coelho et al., 2002]. PAR2 activation may also occur on fibroblasts and inflammatory cells, enhancing the release of factors that regulate epithelial secretory function. For instance, PAR2 activation of primary gastrointestinal mucosal myofibroblasts stimulates PG E2 synthesis, which would then be able to stimulate chloride secretion from nearby epithelial cells [Seymour et al., 2001].

In addition to its effects on cells that regulate epithelial secretory function, PAR2 may also be expressed and activated on the epithelium itself to directly stimulate chloride secretion [Kong et al., 1997; Mall et al., 2002] (Buresi, unpublished observations). Indeed, PAR2 is expressed in human colonic epithelium and mediates intestinal electrolyte secretion induced by the release of mast cell tryptase [Mall et al., 2002]. The signaling mechanisms involved in the regulation of chloride secretion by epithelial PAR2 have not been fully elucidated. However, all of the PARs cloned to date appear able to couple to both Gα and Gβγ [Hollenberg, 1999], and thus receptor agonism typically results in Ca2+ mobilization and activation of protein kinase C (PKC). Concurrently, coupling to Gα proteins leads to inhibition of adenyl cyclase and hence suppression of cAMP [Hung et al., 1992;
Baffy et al., 1994; Benka et al., 1995]. Hence, as for several of the examples discussed above, PAR2 mediated effects often demonstrate a calcium dependency. Furthermore, luminal trypsin is able to activate PAR3 at the apical surface of enterocytes to stimulate secretion of eicosanoids. These, in turn, may regulate multiple cell types through paracrine and autocrine processes [Kong et al., 1997].

An anti-secretory role for PAR2 has also been reported. Activation of PAR2 inhibited amiloride-sensitive ion transport in monolayers of bronchial epithelial cells [Danahay et al., 2001]. Furthermore, in the human colonic carcinoma cell line, T84, trypsin causes an inhibition of forskolin stimulated chloride secretion [Danahay et al., 2000]. The effects of trypsin appear to be mediated at the basolateral membrane where trypsin causes an initial activation followed by an inactivation of the K⁺ conductance. These effects are mirrored by a transient increase and subsequent decrease in Isc.

**Other PARs**

PAR1 has been detected in the stomach and small intestine [Ishihara et al., 1997] and PAR4 is expressed in high levels in the small intestine and to a more moderate extent in the colon [Xu et al., 1998], including on the epithelium [Ferazzini et al., 2003]. A potential role for these PARs in the regulation of intestinal ion transport has yet to be examined, however.

Pharmacological evidence suggests that even more PARs exist, but have yet to be cloned [Vergnolle et al., 1998]. The structure-activity relationship (SAR) data obtained with a spectrum of PAR-APs continue to provide evidence for the existence of novel PARs in target tissues such as the endothelium and the gastrointestinal tract [Tay-Uyboco et al., 1995; Vergnolle et al., 1998; Roy et al., 1998]. Indeed, the presence of a “PAR3-like” receptor, that is pharmacologically distinct from PAR1 or PAR2, and that regulates intestinal transport via a cyclo-oxygenase-dependent mechanism, has been demonstrated in rat jejunum [Vergnolle et al., 1998], but has yet to be fully characterized.

**THE ROLE OF PROTEINASES AND SECRETORY FUNCTION IN INTESTINAL INFLAMMATION**

As we have discussed, proteases have the potential to influence secretory function through various mechanisms. These pathways may be substantially altered during inflammation and may thus impact significantly on epithelial barrier function in disease. Thrombin has been found in abundance in the lumen and lamina propria of the gastrointestinal tract during inflammatory conditions of the gut. Due to the vascular disruption, hemorrhage, and increased vascular permeability that are characteristic of inflammation, thrombin would be in a position to interact with many cell types in the lamina propria and submucosa. Thrombin has long been known to be involved in inflammation [Cirino et al., 1996; Dery et al., 1998] and has been implicated in various aspects of the pathogenesis of inflammatory bowel disease [Wakefield et al., 1989; Channonard et al., 1995; Thompson et al., 1995; Stadnicki et al., 1997; Carty et al., 2000; Swiatkowski et al., 2000; van Bodegraven et al., 2001]. Given that thrombin plays numerous roles in the inflammatory process in the intestine, it is not unreasonable to assume that it would also be in a position to affect epithelial secretory function in the inflamed gut.

Trypsin is an abundant digestive enzyme in the upper GI tract and so could readily interact with the luminal surface of enterocytes even under physiological conditions [Kong et al., 1997], and in circumstances where the epithelial barrier is disrupted and permeability is increased, trypsin would be allowed to access the intestinal mucosa. Furthermore, trypsin levels are increased during inflammation, and thus might contribute to altered secretory function under such conditions [reviewed in Vergnolle, 2000]. In addition, the regulation of ion secretion by this proteinase has been suggested to elicit either pro- or anti-inflammatory effects in epithelia of the pancreatic ducts, airways, and urinary tract [Bertog et al., 1999; Cocks et al., 1999a; Nguyen et al., 1999; Danahay et al., 2001]. It is unlikely that trypsin is the endogenous activator at all of these sites however, and in many tissues, the identities of proteases that stimulate PAR2 in vivo require further clarification [Bertog et al., 1999].

Tryptase is released during mast cell degranulation. These cells, of which there are many in the mucosa of the gut, may furthermore secrete mediators, such as TNF-α, that have been shown to upregulate PAR2 expression, and that are critically involved in inflammatory processes [Nystedt et al., 1996; Bischoff et al., 1999]. Indeed, an important characteristic of inflammatory bowel disease is mast cell infiltration, and mast cell regulation of intestinal ion transport is altered in patients with IBD [Levo and Livni, 1978; Crowe and Perdue, 1992].

Finally, numerous other proteases that act on PARs, including neutrophil elastase and cathepsin G, are also characteristic of inflammatory cell activity, and have been implicated in epithelial secretory function in the airway [Lundgren et al., 1994; Nadel et al., 1999]. More work needs to be done in order to determine the role of these proteases in intestinal epithelial function in inflammatory diseases of the intestine.
RELEVANCE AND FUTURE DIRECTIONS

In addition to their degradative functions in digestion, coagulation, and tissue remodeling, serine proteinases also act as signaling molecules via activation of PARs, and may thus affect a wide array of physiological functions. In this way, proteinases may act on enterocytes or on neighboring cells to alter secretory function and modulate inflammatory pathogenesis. In the mouse colon, activation of PAR1 may initially evoke chloride secretion but subsequently suppress responses to other secretagogues and thus contribute to the hyporesponsiveness seen in IBD. Elucidation of the mechanisms underlying the control of secretory function and responsiveness by PARs will lead to a better understanding of ion transport processes in mucosal tissues in general. Furthermore, the use of inhibitors of PAR1, or of signaling proteins, could lead to decreased secretory dysfunction during inflammatory conditions of the gut. In addition, elucidation of the intracellular stimulus-secretion coupling mechanisms that are induced by PAR activation is of importance. Given that PAR cleavage typically leads to increased intracellular calcium, it appears unlikely that the route of apical chloride secretion is through the cAMP-dependent CFTR. It is thus possible that stimulation of a calcium-dependent chloride channel by PAR activation represents a potential therapeutic strategy for cystic fibrosis. Knowledge of the signaling mechanisms underlying the regulation of chloride secretion by PARs will allow for the elucidation of potential therapeutic targets in the treatment of secretory dysfunction not only in the gastrointestinal tract, but in all mucosal surfaces.

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REFERENCES


Tsai W, Morielli AD, Peralta EG. 1997. The m1 muscarinic acetylcholine receptor transactivates the EGF receptor to modulate ion channel activity. EMBO J 16:4597–4605.


