Pancreatic protease-activated receptors: friend and foe

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PAR2 activation may protect the acinar cell by enhancing secretion, but may still leave you in pain

Two regulatory elements of the exocrine pancreas, one new and one old, may interact to regulate previously undescribed physiological and pathological responses of the pancreas. The new targets are protease-activated receptors (PARs); the old elements are the serine proteases trypsin and trypsinase, which are the most potent agonists of these G-protein-coupled receptors.¹ The PAR family of G-protein-coupled receptors exhibits a distinct activation mechanism. Limited cleavage of the extracellular domain of PARs by serine proteases uncovers a peptide on the N-terminus that interacts with another extracellular region to activate the receptor. PAR isoforms exhibit a distinct tissue distribution and are selectively activated by specific classes of proteases. For example, the PAR2 isoform that is found in the pancreas on epithelial cells and nerves is selectively activated by trypsin and trypsinase. While the role of the PAR2 in health and disease is currently being elucidated, recent studies have suggested that it might participate in pancreatic responses.

Previous findings by Nguyen *et al*² could be relevant to the effects of pancreatic PAR2 under physiological and pathological conditions. They showed that activation of the pancreatic ductal cell PAR2 by trypsin results in activation of electrolyte secretion. The enhanced chloride secretion was mediated by PAR2-dependent increases in cellular calcium. This study suggests that if activated trypsin was released from the pancreatic acinar cells (which might occur even with physiological stimulation), it could stimulate fluid and electrolyte secretion from small duct cells and help clear activated enzymes from the pancreatic duct. It could also function as a back-up mechanism should the major mechanism of ductal secretion, cystic fibrosis transmembrane conductance regulator, fail. This study found that PAR2 was localised to the basolateral membrane, and trypsin generated by acinar cells would not normally reach this domain. However, both tight junction disruption and basolateral exocytosis from the acinar cell could be

features of acute pancreatitis, and could allow access of acinar cell trypsin to the basolateral domain of duct cells. Alternatively, activation of mast cells has been reported in both acute and chronic pancreatitis. The release of tryptase by mast cells, a potent activator of PAR2, might also allow activation of the receptor on the basolateral membrane. The possible distribution of PAR2 receptors on the apical membrane of acinar and duct cells, as has been reported in other epithelial cells, warrants further studies.

In the setting of acute pancreatitis, Namkung *et al*³ have demonstrated a role of the PAR2 receptor in cellular and in vivo models of pancreatitis using PAR2 agonists. Activation of acinar cell PAR2 receptor was reported to enhance cytosolic calcium signalling. In the context of acute pancreatitis, the effects of PAR2 activation on the acinar cell seemed to be protective, but it may have additional deleterious effects (discussed below). Complementary studies by Sharma *et al*⁴ demonstrated a worsening of severity in response to caerulein-induced pancreatitis in mice with genetic deletions of PAR2. Notably, pretreatment with an agonist of PAR2 reduced some, but not all parameters of severity in the caerulein pancreatitis model. Interestingly, PAR2 ligand pretreatment did not affect trypsin activation, but did seem to reduce the translocation of extracellular-signal regulated kinase (ERK)1/2 to the nucleus in acinar cells. Since this nuclear translocation may mediate inflammatory responses, its inhibition of ERK translocation could limit pancreatic injury. Another study by this group demonstrated that a PAR2 agonist caused both amylase secretion and increased cytosolic calcium.⁵ It may be relevant to the mechanism of PAR2 signalling that pretreatment of acinar cells with caerulein extinguished the ability of acinar cells to respond to a subsequent PAR2 activation with an increase in intracellular calcium.

Previous studies have suggested that PAR2 activation would stimulate secretion from the pancreatic acinar cell.⁶ In this issue of *Gut*, Singh *et al*⁷ (see page 958)

have extended this observation by examining the effects of PAR2 activation on the inhibited secretion observed in acute pancreatitis. Importantly, the inhibition of secretion observed with high concentrations of caerulein is believed to be a key feature of acute pancreatitis. The study confirmed, by serum amylase, oedema, pancreatic trypsin content and histology, that acute caerulein-induced pancreatitis was enhanced in the PAR2 knock-out compared with the control. Then, several notable effects of PAR2 stimulation on amylase secretion were described. First, 6 h after a single in vivo treatment with caerulein hyperstimulation, acini demonstrated a normal biphasic secretory response when exposed to increasing concentrations of caerulein. Second, acini obtained both after the in vivo caerulein hyperstimulation and in a model of arginine-induced pancreatitis demonstrated enhanced amylase secretion after PAR2 stimulation. In summary, this study confirms that PAR2 deletion worsens pancreatitis responses in the caerulein model and demonstrates that under the conditions observed early in the course of two distinct acute pancreatitis models, PAR2 stimulation can enhance amylase secretion. The authors conclude that the protective effects of PAR2 may be due, in part, to its stimulation of secretion and removal of activated enzymes from the acinar cell. They suggest that this may represent a mechanism for acinar cells to sense and protect themselves from protease activation.

The association between reduced pancreatic secretion and acute pancreatitis has been recognised. The aetiology of this decrease is likely to be both reduced secretion from the acinar cell and enhanced paracellular permeability. The latter allows contents of the pancreatic duct to leak into the interstitial space. Whether duct cell secretion is directly reduced during acute pancreatitis is unexplored, but likely. The mechanism behind the reduced acinar cell secretion remains unclear, but it has been linked by some researchers to the disruption of the apical actin cytoskeleton.⁸⁻⁹ Other studies have suggested that this reduced acinar cell secretion observed after caerulein hyperstimulation might involve complete inhibition of secretion from the apical pole of the acinar cell and a shift to secretion from the basolateral domain.¹⁰⁻¹¹ Gaisano *et al*¹¹ have suggested that the basolateral secretion might result from inactivation of a protein (Munc18c) that normally prevents the formation of a SNARE (soluble N-ethylmaleimide-sensitive factor attachment receptor) complex required for basolateral exocytosis of secretory granules. Why would inhibition of acinar cell secretion

Figure 1 Potentially protective and damaging effects of protease-activated receptor (PAR) 2 activation. The activation of this proteinase-activated receptor has the potential to both benefit and harm the pancreas. By enhancing secretion from both acinar and duct cells, PAR2 might reduce cell injury. However, its potential effects on blood pressure and vascular permeability, ability to activate some inflammatory cells and stimulation of nociceptive neural pathways could worsen some disease features.

worsen pancreatitis? The simplest explanation may be linked to the trafficking of proteases and other zymogens that are pathologically activated within the acinar cell during acute pancreatitis. Thus, studies by Grady *et al.*¹² and Chaudhuri *et al.*¹³ on isolated acini demonstrate that virtually all active enzymes are retained in the acinar cells after caerulein hyperstimulation. However, treatments that promote the secretion of proteases from the acinar cells (such as bombesin or increasing cellular cAMP) reduce or eliminate acinar cell injury. One possible conclusion is that the early induction of pancreatitis at the level of the acinar cell may require both pathological protease activation and retention of the activated enzymes within the acinar cell.

The study by Singh *et al.*⁷ raises a number of interesting mechanistic questions. First, does the trafficking of active enzymes parallel that of amylase? Thus, the compartment in which acinar cell protease activation takes place remain unclear, and it is conceivable that it might not follow amylase activation. Second, the mechanism of enhanced secretion induced by PAR2 agonists is unknown; it might prevent disruption of the apical actin cytoskeleton or enhance exocytosis from the basolateral domain. Finally, which cell-signalling pathway is responsible for the reversal of inhibited secretion by PAR2 activation? While candidates include the release of calcium from a distinct pool, disordering of trafficking or ERK1/2, recent studies suggest another possible mechanism. A report by Amadesi *et al.*¹⁴ shows that PAR2 agonists stimulate the generation of cAMP in neuronal cells. Since increased cAMP levels in acinar

cells have been shown to reverse both the inhibited secretion induced by caerulein hyperstimulation and injury, it should be considered as a candidate for mediating the PAR2 responses described by Singh *et al.*⁷

Finally, should PAR2 agonists be considered in a therapeutic context, such as in the prevention of endoscopic retrograde cholangiopancreatography-induced pancreatitis? While there is compelling evidence from several laboratories that acinar PAR2 activation reduces select local markers for pancreatitis, PAR2 activation at other sites may have unwanted and detrimental effects (fig 1). Thus, PAR2 activation can be pro-inflammatory in some tissues and directly activate some types of inflammatory cells. Further, activation of PAR2 receptors on endothelial cells is associated with hypotension.³ Finally, Hoogerwerf *et al.*¹⁵ demonstrated that activation of PAR2 receptors at nociceptive neurones supplying the pancreas caused pain in experimental pancreatitis models. Although it is possible that local administration of the PAR2 agonist into the pancreas might elicit protective responses, it could also stimulate neural pathways linked to pain. Together, these findings suggest that pancreatic PAR2 activation might be a multiedged sword. It could have an entirely protective effect when there are low levels of protease activation and only acinar and duct cells are stimulated, and might have both a locally protective and systemically damaging effects when there is more widespread PAR2 activation, such as in the setting of acute pancreatitis. If the model supported by Singh *et al.*⁷ is correct, a key

to translating the potentially protective effect of PAR2 activation into treatment may be elucidating the mechanism by which it enhances acinar cell secretion.

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