MINIREVIEW

Enterokinase (43728)

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Abstract. **Enterokinase is a glycoprotein and is now designated enteropeptidase (E.C.3.4.4.8.). It is present in the duodenal and jejunal mucosa. Pancreatic proteolytic enzymes are secreted as proenzymes. Enterokinase converts trypsinogen to trypsin in the duodenal lumen. Duodenopancreatic reflux of duodenal enterokinase may be important in the pathogenesis of experimental and clinical acute pancreatitis. Congenital enterokinase deficiency is a distinct clinical entity characterized by diarrhea, failure to thrive, hypoproteinemia, and edema. Acquired enterokinase deficiency may occur in some diffuse small bowel diseases. Steatorrhea of celiac sprue may be due partly to the fact that deficiency of secretin and cholecystokinin may interfere with the action of enterokinase. The interrelationship between secretin, cholecystokinin, enterokinase, and bile salts is not completely understood.** [P.S.E.B.M. 1994, Vol 206]

It has been known for a long time that pancreatic enzymes are secreted in an inactive state (1, 2). In 1899, Schepovalnikoff (3) working in Pavlov's laboratory discovered that duodenal secretions activated the pancreatic proteolytic enzymes. Pavlov considered this activating factor in the intestinal secretions "an enzyme of enzymes" and named it enterokinase (4).

It was shown by Kunitz (5) that crystalline trypsinogen was completely converted into trypsin by enterokinase (EK) at a pH of 5.2–6.0. He established that the action of EK under those conditions was that of a typical enzyme; EK acted as a simple catalytic unimolecular enzyme. He also purified EK from the fluid contents of pig duodenum by fractional precipitation with ammonium sulfate (6). It was shown (6) that EK contained protein, carbohydrate, and amino sugar. EK was further purified by ethanol fractionation. The chemical analysis showed that it contained neutral sugars like fructose, mannose, and galactose, and the aminosugars, glucosamine and galactosamine. It was concluded that EK was a glycoprotein (7, 8).

EK is now designated enteropeptidase (E.C.3.4.4.8.). It was shown by Gyorgy *et al.* (9, 10) that EK, in addition to having the property of converting trypsinogen to trypsin, also was capable of counteracting the action of trypsin inhibitor on trypsin. However, other investigators have shown no effect of EK on the trypsin inhibitor (11). It seems that EK acts as a peptidase and splits off a Valyl peptide from the amino end of trypsinogen (12). Kinetics of the trypsinogen activation by EK have been worked out in detail (13). It has been shown in rat and humans that bile acids are important in the activation of trypsinogen by EK; disturbance of trypsinogen activation has been seen in patients with intrahepatic biliary atresia (14). It is interesting to note that endocytosis of EK by rat hepatocytes has been shown both *in vitro* and *in vivo* experiments (15).

In humans, enterokinase is present in the mucosa of first and second portion of the duodenum, and intraluminal EK does not appear to originate by simple desquamation or turnover of the intestinal mucosa (16).

The origin and cellular localization of EK has gen-

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erated some controversy. While it is generally agreed that EK is a brush border enzyme (17–23), the exact site of action of EK on trypsinogen is in dispute. According to one view (17), EK activates trypsinogen on the surface membrane of the small bowel mucosal cell rather than in the lumen. However, other workers (24) have suggested that EK is released by the action of bile salts into the lumen and trypsinogen activation is an intralumenal event. It is interesting to note that EK has been localized in the biliary tract by immunohistochemical methods (25). Long-term pancreatic-biliary diversion from the duodenum in the rats results in persistent loss of mucosal enterokinase. It seems that pancreatic-biliary secretions are important in the maintenance of duodenal mucosal EK activity (26). The EK activity has been localized to the villi; none was found in the crypts or Brunner glands (22). EK activity was also released from the duodenal mucosa by papain, trypsin, and chymotrypsin (21). Secretin and pancreozymin also release EK from the duodenal mucosa; the action of secretin and pancreozymin is independent of bile salt concentration and trypsin content in the duodenal lumen (27, 28). EK consists of two subunits linked by a disulfide bond. The light chain is the actual catalytic subunit, whereas the heavy chain anchors EK to the brush border membrane (29). EK probably lies on the same phylogenetic branch as the blood-clotting factors, since they have many common properties. α-Keto derivatives of amino acids are effective inhibitors of EK and trypsin (30, 31).

Role of EK in Pancreatitis

The exact pathogenesis of acute pancreatitis is not clear. Many theories (e.g., one which envisages the presence of a common channel between the common bile duct and pancreatic duct, and one which suggests that acute pancreatitis results from obstruction to the pancreatic duct [33] in the presence of actively secreting pancreas) have been put forward. Duodenopancreatic reflux of duodenal contents has been implicated in experimental and clinical pancreatitis (34–38). Duodenopancreatic reflux of EK-rich duodenal contents causes intrapancreatic activation of trypsinogen thereby initiating acute pancreatitis. Slow, low-pressure intraductal injection of EK in dogs (39, 40) and rats (41–43) causes hyperamylasemia and acute pancreatitis (Fig. 1 and 2). Intraductal EK also markedly increased the pancreatic protein content probably by increasing the proteolytic enzyme content (44). Concomitant intraductal administration of 5-azacytidine and cycloheximide prevented the effect of EK on pancreatic protein and amylase content (44, 45). Inhibition of protein synthesis by 5-azacytidine and cycloheximide may be the mechanism of protective effect of these agents in EK-induced experimental acute pancreatitis (43). It seems that EK plays an important role in acute experimental and clinical pancreatitis.

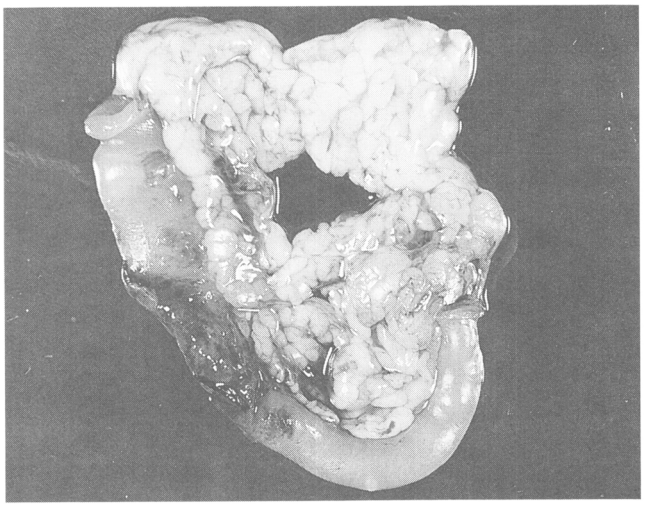

Figure 1. Rat pancreas showing edema and hemorrhage after intraductal injection of enterokinase.

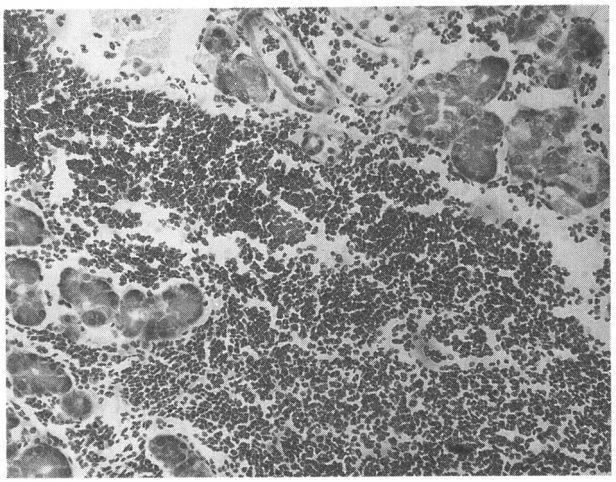

Figure 2. On microscopic examination there was pancreatic destruction, necrosis, and infiltration with inflammatory cells (H&E ×250).

EK Deficiency

As already mentioned, EK is responsible for activating pancreatic proenzymes. EK converts inactive trypsinogen to trypsin; trypsin, in turn, activates other proenzymes such as chymotrypsinogen, procarboxy peptidases, and proelastase (46). It would seem that deficiency of EK would result in marked malabsorption of protein causing impaired development and growth. The first case of congenital EK deficiency was reported in 1969 (47). Since then, more cases of EK deficiency have been reported (48–53). These children present with diarrhea, failure to grow, edema, vomit-

ing, hypoproteinemia, and anemia (54). EK is absent in the duodenal mucosa. It is known that pancreatic proteolytic enzymes are needed in the vitamin B_{12} absorption, the pancreatic proteolytic enzyme split off B_{12} from R-proteins thereby enabling B_{12} to attach to intrinsic factor. Vitamin B_{12} malabsorption has been documented in cases of chronic pancreatic insufficiency (55–57). In all the cases of EK deficiency with anemia, the hemoglobin level improved after pancreatic replacement therapy. It was suggested (58) that anemia in EK-deficient patients was due to vitamin B_{12} malabsorption and deficiency.

In diffuse diseases of the small bowel, acquired EK deficiency may be responsible for functional pancreatic insufficiency resulting in maldigestion and malabsorption. It has been reported that 10% of adults and 20% of children with celiac sprue have functional pancreatic insufficiency needing pancreatic replacement therapy in addition to gluten-free diet (59, 60). However in functional pancreatic insufficiency of celiac sprue, impaired release of secretin and cholecystokinin from the abnormal duodenal mucosa may be responsible for pancreatic maldigestion (61–64). EK concentration in the small bowel mucosa in various small bowel diseases has been measured (65). In addition to celiac disease, functional pancreatic insufficiency has been seen in cases of inflammatory bowel disease, chronic cholestatic diseases, and after gastrectomy (66). However, in most of these conditions pancreatic insufficiency is probably due to impaired secretin and cholecystokinin release. Impaired cholecystokinin release which is seen in celiac sprue (61, 66) is particularly interesting. Impaired cholecystokinin release in celiac sprue may cause gallbladder inertia and sluggish enterohepatic circulation of bile salts (67–69). It has been suggested that bile salts play an important role (14, 24) in the activation of trypsinogen by EK, and disturbance of trypsinogen activation has been seen in patients with intrahepatic biliary atresia. If this is true, then impaired cholecystokinin release in celiac sprue patients through gallbladder inertia may cause steatorrhea by interfering with the action of EK. However, other investigators have demonstrated that the increase in EK in the duodenal juice after secretin and pancreozymin stimulation is independent of bile salt concentration (27). The interrelationship between secretin, cholecystokinin, bile salts, and EK is not completely understood.

EK activity in the duodenal mucosa of the celiac patients is reported to be normal (52, 70, 71) but more and intensive studies are needed to settle this issue. It seems likely that in celiac patients impairment of secretin and cholecystokinin release interferes with the action of EK on trypsinogen rather than that the diseased mucosa of the celiac sprue patients is deficient in EK. EK activity in the duodenal mucosa was markedly reduced in cases of intractable diarrhea of infancy (71). It would be interesting to measure the EK concentration in the duodenal mucosa of patients with tropical sprue, radiation enteritis, Whipple's disease, etc. Primary and secondary EK deficiency may be an important cause of protein maldigestion and protein malabsorption. Congenital EK deficiency must be differentiated from isolated or congenital trypsinogen deficiency disease (72, 73).

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