Regulation of Pancreatic Exocrine Secretion in Ruminants: A Review

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ABSTRACT Mechanisms regulating ruminant pancreatic exocrine function differ in some respects from those in nonruminants. This may affect the post-ruminal digestion of certain dietary nutrients such as starch. Ruminants do not exhibit clearly defined cephalic and gastric phases of pancreatic regulation, a likely consequence of the continuous nature of digesta flow from the rumen. Local neural reflexes and secretin-mediated exocrine responses may be more important than stimulation by cholecystokinin. Additionally, the ruminant pancreas may be stimulated by short-chain fatty acids produced in the rumen. A "ruminant phase" of pancreatic exocrine regulation has been proposed. The failure of cattle to digest efficiently starch in the small intestine may result from an asynchrony between delivery of starch to the intestines and pancreatic amylase release. J. Nutr. 122: 191-202, 1992.

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Although knowledge of the mechanisms regulating the synthesis of pancreatic enzymes and secretion of pancreatic fluid in most mammals, including humans, has increased markedly during the last two to three decades, our understanding of pancreatic exocrine regulation in ruminants is much less developed. The pancreas plays a central role in the digestion of ruminal fermentation products (including microbial cells) as well as ingested nutrients that escape fermentation in the rumen. This role warrants increased attention, given the development of modern-day feeding practices that stress the use of food concentrates that may result in large amounts of unfermented starch and protein leaving the rumen and entering the small intestine. A better understanding of the normal physiological mechanisms controlling pancreatic function in ruminants could lead to innovative methods of improving nutrient digestion and thereby increasing efficiency of growth, lactation or wool production.
Release of pancreatic islet hormones that function in a paracrine role also play an important part in local control of pancreatic secretion (2, 11, 12). Additionally, circulating nutrients absorbed from the gut may have both direct and indirect effects on pancreatic secretion (13—15), although this involvement is believed to be minor.

Neural control. The neural control of secretion occurs at several different levels, including both the central and peripheral nervous systems (16). Drugs that mimic the actions of acetylcholine also influence the rate and composition of pancreatic exocrine secretion (17, 18). The vagal nerve plays a major role in neural stimulation of pancreatic exocrine secretion in a wide variety of species [Fig. 1] (19). When the vagus trunks are cut or nerve impulse transmission blocked with cholinergic antagonists such as atropine, a decreased enzyme output from the pancreas is observed in both the basal and stimulated state; however, some basal level of secretion remains (20, 21). Conversely, administration of cholinergic agonists such as carbachol and methacholine increase secretion both in vivo and in vitro [Fig. 2] (17, 18, 22). A gastric phase of pancreatic secretion mediated by long vagovagal reflexes has also been described. Neural regulation of enzyme secretion from the pancreatic acinar cells is mediated via the release of acetylcholine at the terminal synapse near the exocrine cell (2, 16, 23). Other neurotransmitters released with acetylcholine such as peptidergic neurotransmitters [i.e., vasoactive intestinal polypeptide (VIP)] and catecholamines released from other nerve terminals may

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5Abbreviations used: CCK, cholecystokinin; PP, pancreatic polypeptide; VIP, vasoactive intestinal polypeptide; VFA, volatile fatty acids.
influence the response to acetylcholine (24–26). Acetylcholine binds to muscarinic receptors on the effector cell surface and stimulates phosphatidylinositol breakdown, alters adenyl cyclase activity and increases the intracellular pool of free calcium release (27). The net result is secretion of pancreatic fluid and enzymes. Muscarinic receptors on the pancreatic acinus can be distinguished by their physical and functional properties from those found in the heart and brain and have been classified as a subclass of $M_2$ receptors, based on their relative affinity for the muscarinic antagonist, pirenzepine; they have been given the tentative designation of $M_3$ (28, 29). Recent data indicate that 85% of muscarinic receptors found in the rat pancreas have a low affinity for pirenzepine (30). The unique character of these receptors may explain the high affinity with which some cholinergic agonists bind to the acinus (29, 31).

**Hormonal control.** The major gastrointestinal hormones controlling pancreatic exocrine secretion are secretin and cholecystokinin (CCK, also known as pancreozymin; Fig. 1) (7, 32). Secretin, produced by the “K” or “S” cells of the duodenum and jejunum, stimulates secretion by the pancreatic ductule cells of a fluid rich in bicarbonate. Release of secretin is stimulated by the entry of unbuffered hydrogen ions into the duodenum (1, 33); secretin, in turn, stimulates the pancreas to secrete bicarbonate, resulting in the rapid neutralization of free acid. The pH of the duodenal contents is thus optimized for the action of pancreatic enzymes. Cholecystokinin is produced by endocrine cells located in the proximal gut, as well as by neurons in the ileum and colon (34). It is released in response to stimulation by $L$-isomers of amino acids, $C_{10}-C_{18}$ fatty acids and divalent cations within the intestinal lumen (Fig. 3) (14, 34). Cholecystokinin release stimulates both the release of digestive enzymes from the pancreatic acinus and gallbladder contraction. In addition, it potentiates and is itself potentiated by secretin. These actions are mediated by cell membrane receptors in the pancreas that have binding characteristics similar to those found in the brain and gallbladder (35–37). Cholecystokinin is one of the most potent pancreatic secretagogues known, eliciting secretory responses that are 166% of those observed with cholinergics such as bethanechol (19, 38). Cholecystokinin also serves a second function as a neurotransmitter in the central and peripheral nervous systems.

Other hormones, including some secreted by endocrine cells within the pancreas, are also known to regulate the flow of pancreatic enzymes and fluids. Pancreatic polypeptide (PP), one member of a family of gastrointestinal hormones and neurotransmitters, inhibits pancreatic exocrine function when administered in vivo (12, 39). It is produced by cells (PP cells) within pancreatic islets, from which it is released by vagal-cholinergic dependent mechanisms (11). There are distinct cephalic and gastric phases mediated by vagal reflexes; in addition, an intestinal phase mediated by hormones such as CCK and neural reflexes plays an important role (Taylor, I. L., unpublished results). Pancreatic polypeptide has been demonstrated to inhibit acetylcholine release from pancreatic nerves, which may explain its inhibitory effects (40). Somatostatin, another product of the pancreatic islets (D cells), has also been shown to decrease pancreatic secretions (41). Finally, insulin may have an effect on the synthesis and release of amylase by the pancreatic acinus (8, 42).

An ever-increasing number of brain-gut peptides, acting as hormones or neurotransmitters, are known
to affect exocrine pancreatic function. Examples include VIP, somatostatin, calcitonin gene-related peptide, neurotensin and peptide YY [24, 41, 43, 44]. Some of these peptides, such as peptide YY and somatostatin, are produced by endocrine cells in the intestinal tract, whereas others, such as calcitonin gene-related peptide and VIP, are localized to nerve cells. In many cases their physiological actions are just beginning to be defined. Peptide YY, for example, has been localized to endocrine cells in the ileal, colonic and rectal mucosa [43], from which it is released in a delayed manner in response to meals. Intraluminal fat and glucose are the most potent stimulators. Peptide YY is known to decrease pancreatic protein secretion, possibly by inhibition of CCK release [45]. The peptide YY response to a meal is characteristically prolonged and it may play a role in curtailing pancreatic secretions.

Integrated regulation of postprandial pancreatic secretion. Although many of the individual elements involved in the control of pancreatic enzyme secretion are understood, the manner in which all of these various neural, hormonal and paracrine mechanisms interact in vivo after food consumption is poorly understood [46]. A number of integrated phases temporally associated with the regulation of pancreatic exocrine function in response to food ingestion have been described, including cephalic-vagal, gastric, intestinal and circulatory or humoral phases [1]. The cephalic phase of secretion is initiated by the sight, smell and taste of food and is mediated directly via efferent impulses of the vagus nerve. The act of swallowing food may also activate vagal reflexes that are in the pharynx and esophagus. Additionally, pancreatic secretion may be indirectly stimulated during the cephalic phase by the vagal-stimulated release of gastrointestinal hormones, such as gastrin [1, 46]. The gastric phase of pancreatic secretion is initiated by the gastric distension and possibly the exposure of the gastric mucosa to nutrients [46]. Although neural reflexes are activated during this phase, another important factor is the stimulation of acid secretion, which in turn stimulates the release of gastrointestinal hormones (such as secretin) from the intestine. During the intestinal phase, acid and nutrients such as amino acids (particularly aromatic amino acids) and fatty acids directly stimulate endocrine cells to release hormones (such as secretin and CCK) that stimulate pancreatic secretion. Absorbed nutrients such as lipids, amino acids and glucose and minerals such as calcium initiate a humoral phase acting directly upon the pancreatic acinar cell to stimulate or inhibit secretions. They may also act indirectly by stimulating the release of hormones such as CCK, insulin or thyroxin [8, 13, 14]. It should be noted, however, that much of the evidence for this phase of pancreatic secretion regulation is equivocal and in need of confirmation.

**REGULATION OF PANCREATIC EXOCRINE FUNCTION IN RUMINANTS**

Although many of the neural, hormonal and humoral mechanisms of regulation of pancreatic exocrine secretion have been identified in nonruminants, their role has not been fully defined in ruminants [42]. The regulation of pancreatic exocrine secretion in ruminants is likely different than that in nonruminants because of the presence of pre-gastric fermentation in the rumen [47]. The continuous absorption of fermentation end products from the rumen as well as the relatively constant flow of digests into the intestinal tract contrasts greatly with the intermittent entry of nutrients into the small bowel coupled with metabolite fluxes that accompany the feeding patterns of nonruminants [47, 48]. Recent information has suggested that a ruminal phase of pancreatic secretion may exist and that short-chain volatile fatty acids (VFA) may mediate this phase of exocrine secretion [49].

Data supporting the existence of various regulators of pancreatic exocrine secretion in ruminants (i.e., neural, hormonal, paracrine, humoral) will be discussed in the context of the recognized phases of postprandial regulation of pancreatic exocrine secretion existing in other species. Information supporting the existence of an additional phase of pancreatic secretory control unique to ruminants will also be discussed.

**Cephalic phase.** It is questionable whether ruminants exhibit a true cephalic phase of pancreatic secretion, because the sham-feeding of sheep fails to elicit increased pancreatic fluid or enzyme flow unless the sheep have been deprived of food [47]. There are ample data in ruminants suggesting that the nervous system plays a major role in the control of pancreatic exocrine secretion. Reynolds and Heath [50] found that electrical stimulation of the vagal nerves of sheep caused a greater release of trypsin, chymotrypsin and amylase than CCK (Table 1). Pancreatic secretion in response to stimulation of the vagus nerve, which is characterized by increased secretion of both pancreatic fluid and enzymes [47, 50, 51], can be blocked by treatment with atropine [51]. In vivo and in vitro administration of acetylcholine and cholergic agonists (such as carbachol and pilocarpine) causes an atropine-sensitive stimulation of pancreatic fluid and enzymes, including amylase [Fig. 2] [22, 47, 51–53]. Because atropine will block vagally or cholingeric agonist–induced amylase release, one can deduce that muscarinic receptors are involved in the neural regulation of pancreatic function. Indeed, recent studies have shown that intravenous administration of 4-diphenylacetox-y-N-methylpiperidine methiodide to dairy steers reduces basal pancreatic fluid secretion (Harmon, D. G., and Croom, W. J., Jr., unpublished observations).
To date, there have been no attempts to delineate the subtypes of acetylcholine receptors found in the pancreas of ruminants. In one study (54), the in vitro application of acetylcholine to sheep pancreatic acinar cells caused cellular depolarization, demonstrating the presence of a receptor on the acinar cell itself. Slaframine, a muscarinic agonist with a high affinity for the gastrointestinal tract, stimulates pancreatic amylase, protease, lipase and fluid secretion in calves, goats and sheep (31, 55). Slaframine-induced salivation and pancreatic secretion are blocked by atropine in ruminants and chicks (55). The order of potency of various antagonists in inhibiting slaframine-induced mortality in chicks administered slaframine at the LD50 was atropine (blocks all known muscarinic receptor subtypes) > pirenzepine (M1 antagonist) > gallamine (M2 antagonist) [Croom, W. J., Jr., unpublished observations]. This is similar to the order of potency observed for various muscarinic antagonists in blocking carbachol-stimulated amylase release from the splenic lobe of the chick pancreas [atropine = 4-diphenylacetoxy-N-methylpiperidine methiodide (M1 cholinergic receptor antagonist) > pirenzepine > gallamine] (56). The above studies, considered together, suggest that the ruminant pancreas may possess muscarinic receptor subtypes similar to those observed in other species (29). Recent observations by Froetschel et al. (57) that slaframine administration increased postural starch digestion in steers may support this hypothesis. The resolution of this question concerning the subtype of muscarinic receptor present in the ruminant pancreas must await radioligand binding studies.

It would be erroneous to assume that all of the neural stimulation of the pancreas is mediated directly by acetylcholine. Vagal stimulation is known to cause the release of pancreatic and gastrointestinal hormonal secretagogues and other neurotransmitters such as gastrin, CCK and VIP (24, 50). It is possible, even probable, that a component of the secretory response to vagal stimulation in ruminants is secondary to release of hormones or neurotransmitters other than acetylcholine in the gut. In addition, vagal action on pancreatic secretion in sheep is potentiated by the simultaneous release of insulin, which may stimulate pancreatic protein secretion directly (42, 58).

Ruminal phase: supporting evidence! Although a ruminal phase of pancreatic secretion has not been defined in ruminants, VFA stimulate amylase release in vivo and in vitro from the sheep pancreas (49). Indeed, the amylase response to butyrate was similar to that observed with CCK (49). Katoh and Tsuda (59) observed a positive relationship between the length of VFA (2–8 carbon atoms) and pancreatic juice, protein and amylase (data not shown) release, with isovalerate and butyrate increasing amylase release more than propionate and acetate (Fig. 4) (49, 59).

The exact mechanisms by which VFA stimulate pancreatic secretion have not been elucidated. This effect possibly may be mediated through insulin release, because propionate and butyrate are known to enhance insulin release from the ruminant pancreas (59); however, one study indicates VFA act directly on pancreatic acinar cells (49). Short-chain VFA increase calcium efflux and membrane potential of sheep acinar cells in much the same way as acetylcholine (54, 59), although the effects of the VFA are not blocked by atropine. Presumably, the cellular mechanisms involved in the stimulation of pancreatic secretion by VFA are similar to those involved in the
mediation of acetylcholine’s effect and involve intracellular calcium [59]. To date, there is no information to suggest that VFA exert their effects through specific cellular receptors.

One concern in establishing the involvement of ruminal fermentation products, such as butyrate, in the regulation of pancreatic function is the fact that the amount of VFA needed to stimulate acinar cells in vitro is generally larger than the amount found in the peripheral circulation. Harada and Kato [49] found that butyrate stimulated maximal amylase release from isolated sheep pancreatic lobule preparations at a concentration of 1 mmol/L, whereas the minimal effective dose was 10 μmol/L butyrate (Fig. 5). Maximal amylase release from isolated goat pancreatic lobules was observed with 10 mmol/L acetate, propionate and butyrate (Fig. 6) [22]. Arterial concentrations of acetate and butyrate in the range of 1 mmol/L and 20 μmol/L have been observed in concentrate-fed sheep [59], these values are within the lower range of VFA concentrations known to stimulate pancreatic secretion and amylase release in vitro. Additionally, arterial concentrations of acetate, propionate and butyrate (779, 187 and 25 μmol/L, respectively) have been reported in sheep fed alfalfa pellets [60].

The case for the existence of a ruminal phase of pancreatic stimulation is supported by a number of other observations. First, pancreatic responses to VFA seem limited to ruminants and animals with gastrointestinal fermentations such as Japanese field voles [22]. The pancreas of mice is not stimulated by VFA. Second, most of the ruminal VFA are absorbed directly across the rumen wall into the portal venous system [61]. Because VFA represent the major energy substrates in ruminants and exhibit large postprandial changes, it is logical to assume they would play a role

**FIGURE 4** Effect of various doses of acetate, propionate and butyrate on sheep pancreatic juice flow (A) and protein output (B). Fatty acids were injected via the jugular vein. Individual values represent response over 20 min. Reproduced with permission from Harada and Kato [49].
in the regulation of digestive processes. Unfortunately, information available concerning pancreatic secretion in relation to feeding indicates there is little postprandial effect [47]. Clearly, more research is needed to further define the role of ruminal fermentation in pancreatic secretion regulation.

Gastric and intestinal phase. A number of gastrointestinal hormones and peptides have been identified in the ruminant digestive tract in the abomasum, pancreas and intestines. These include gastrin, secretin, CCK, VIP and PP [62, 63]. Efforts to elucidate their roles in regulating digestive function have been largely confined to effects on gastric juice secretion, gut motility and digesta transit time. There has been little effort to describe their actions on pancreatic exocrine tissue.

The importance of a gastric phase in the regulation of ruminant pancreatic function is believed to be minimal [47]. In humans, neural reflexes mediate this phase. In dogs, gastrin has been implicated as a mediator of the gastric phase of secretion [1]. Although Reynolds and Heath [50] reported enhanced secretion of fluid and enzymes from the pancreas of sheep in response to pentagastrin, Harada et al. [62] found exogenous pentagastrin was a weak stimulator of pancreatic fluid flow and increased pancreatic protein secretion only when administered at a non-physiological dosage range. In contrast, the intestinal phase is believed to play a dominant role in the regulation of pancreatic fluid and enzyme secretion, by altering the flow rate and pH of digesta entering the duodenum [47, 51, 52, 64]. Secretin is the major mediator of pancreatic fluid and bicarbonate secretion; its release is stimulated by free hydrogen ions in the duodenal lumen (Fig. 7) [64]. Cholecystokinin and CCK-like peptides are the major hormonal stimulants of pancreatic protein and enzyme release (Fig. 8) [5, 50, 51, 53, 64]. These two hormones potentiate each other's biological effects.

The relative importance of CCK in regulating ruminant pancreatic exocrine function has been questioned [65]. Thus, it is likely that CCK's importance as a ruminant pancreatic secretagogue may not be as great as in nonruminants. A number of observations support this possibility. First, the ruminant pancreas may not be as responsive to CCK as the pancreata of rats and pigs [19]. Secondly, the pancreatic protein and fluid response to intraportal injection of secretin was greater than that of CCK-8 [62]. If such species differences in pancreatic regulation exist, they undoubtedly are related to the continuous nature of acidic digesta flow in ruminants. The cellular basis for this species difference is unclear. In sheep, as in nonruminants, duodenal acidification has been shown.
Pancreatic function and ruminant starch digestion. The role of pancreatic amylase in the utilization of dietary starch by ruminants has been rigorously debated [3, 4]. Owens et al. [4] argued that the form of dietary starch reaching the small intestine was the limiting factor determining starch utilization. Implicit in this argument is the assumption that amylase secretion is adequate, however, they did suggest that the residence time of starch in the intestinal tract may be limited during the feeding of certain diets. Other authors have implicated pancreatic amylase secretion or intestinal maltase as rate-limiting factors in the digestion of starch [3, 67]. None of these arguments have been satisfactorily proven to the exclusion of the others.

Ruminant nutritionists have failed to adequately examine several issues in considering the role of pancreatic amylase secretion in intestinal starch utilization. First, it seems that pancreatic fluid and amylase secretion do not exhibit a marked post-feeding increase in mature sheep and in calves [47, 68]. This suggests that, during the feeding of high grain diets, there is an asynchrony between the flow of dietary starch into the intestines and the secretion of pancreatic amylase; this is especially so when one considers that amylase secretion accounts for only 2% of pancreatic fluid protein (Table 2) [69]. This pancreatic regulation needs further and more detailed investigation.

**Humoral phase.** As with nonruminants, the experimental evidence supporting a post-absorptive or humoral phase of pancreatic regulation in ruminants is tenuous. Short-term stimulation of pancreatic amylase secretion by glucose infusion and insulin injection has been documented in sheep [15]. Increased glucose absorption, such as occurs during intestinal infusion of glucose, may enhance pancreatic enzyme secretion via a paracrine effect of insulin [15]. Insulin is known to stimulate protein synthesis in the pancreas, and this may include enhanced synthesis of pancreatic enzymes [42]. However, it is doubtful this could represent a regulatory mechanism, because ruminants evolved to consume diets that result in the absorption of limited amounts of glucose. It is arguable that the proposed stimulation of pancreatic secretions by VFA could be classified as a humoral effect, because VFA absorbed from the rumen enter the portal vein in a way similar to that of intestinally absorbed nutrients (see section on “Ruminal phase: supporting evidence?”). Clearly, the humoral phase of pancreatic regulation needs further and more detailed investigation.
could contribute to incomplete intestinal starch digestion. There is even a report (15) of an actual decrease in pancreatic amylase secretion during duodenal starch infusion in wethers. On the other hand, Van Hellen (70) reported increased postprandial pancreatic secretion in steers fed a high grain diet. Scrutiny of this data, however, suggests large variation, independent of feeding time, in release of pancreatic secretion. There is no statistical analysis to support their interpretation of increased postprandial pancreatic secretory pulses.

There is a debate as to whether all pancreatic enzymes are synthesized, transported in the cell and secreted in a parallel manner under different secretory stimuli. Some have argued that different stimuli result in the release of different ratios of pancreatic enzymes. In sheep, vagal stimulation results in increases in the relative amounts of chymotrypsin and amylase secreted compared with trypsin (Fig. 10) (50). No consistent changes in these enzyme ratios were observed with introduction of acid into the duodenum or with gastrin infusion (50). Additionally, it is possible that different regions of the pancreas may secrete variable proportions of enzymes, as has been demonstrated in rats (71). Under different feeding conditions and diets, these types of mechanisms possibly may affect the ability of the ruminant pancreas to secrete amylase. To date, there have been no detailed studies in ruminants addressing these issues.

A number of post-secretory events may affect the efficiency with which pancreatic amylase degrades starch. Although the pancreatic amylase of some species is highly resistant to intraluminal proteases,
the presence of adequate quantities of calcium and other divalent cations in the intestinal lumen may enhance its stability [72, 73]. Conversely, the presence of pancreatic secretory products such as trypsin in the intestinal lumen may decrease amylase output [74].

In conclusion, the factors regulating pancreatic function in ruminants are poorly elucidated compared with nonruminants. The processes discussed in this paper are presented in the light of our present knowledge, with the acknowledgment that regulation of the digestive tract is one of the most complex phenomena in the body. New brain-gut peptides are constantly being discovered within the gastrointestinal tract. More dynamic theories integrating the role these substances play in regulating digestion as they involve the pancreas are being proposed.

Because of the central role the pancreas plays in digestion of nutrients within the gastrointestinal tract, a more complete understanding of its regulation in ruminants may allow modulation of its function to maximize food utilization. Indeed, this concept has been championed by a number of laboratories. For example, the administration of such exogenous secretagogues as caerulein has been proposed "as a possible new tool to obtain better digestibility of nutrients in ruminants" [5]. Additionally, the ability to stimulate the flow of digestive enzymes may be used to attenuate the decreased intestinal nutrient digestibility noted with certain fiber-rich foodstuffs [6].

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LITERATURE CITED


