A novel in vitro model of Brunner’s gland secretion in the guinea pig duodenum

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Moore, Beverley A., Gerald P. Morris, and Stephen Vanner. A novel in vitro model of Brunner’s gland secretion in the guinea pig duodenum. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G477–G485, 2000.—A novel in vitro model that combined functional and morphological techniques was employed to directly examine pathways regulating Brunner’s gland secretion in isolation from epithelium. In vitro submucosal preparations were dissected from guinea pig duodenum. A videomicroscopy technique was used to measure changes in luminal diameter of glandular acini as an index of activation of secretion. Carbachol elicited concentration-dependent dilations of the lumen (EC$_{50}$ = 2 µM) by activating muscarinic receptors on acinar cells. Ultrastructural and histological analyses demonstrated that secretion was accompanied by single and compound exocytosis of mucin-containing granules and the accumulation of mucoid material within the lumen. Inflammatory mediators (histamine, PGE$_1$, PGE$_2$, and intestinal hormones (CCK, gastrin, vasoactive intestinal polypeptide, secretin) also stimulated glandular secretion, whereas activation of submucosal secretomotor neurons by 5-hydroxytryptamine did not. This study directly demonstrates that multiple hormonal, inflammatory, and neurocrine agents activate Brunner’s glands, whereas many have dissimilar effects on the epithelium. This suggests that Brunner’s glands are regulated by pathways that act both in parallel to and in isolation from those controlling epithelial secretion.

THE EMPTYING OF GASTRIC HYDROCHLORIC acid and proteases into the duodenum constitutes a threat to the integrity of the duodenal epithelial barrier. The duodenum responds to this threat by releasing mucin and bicarbonate into the lumen from epithelial cells and Brunner’s glands located beneath the epithelium within the submucosa. Protection occurs when mucin and water combine to form a viscoelastic gel that, when infiltrated with bicarbonate, forms a physicochemical barrier to hydrogen ions and proteolytic enzymes (10). Impairment of this protective response can result in the breakdown of the physicochemical barrier, leading to erosions and ulcers (36).

The factors controlling the epithelial component of duodenal secretion are better understood, largely because the epithelium is more accessible than Brunner’s glands for study. Functional studies have shown that several endocrine, paracrine, and neurocrine mediators can modulate epithelial secretion. These mediators may be released as part of the normal processes of digestion or in response to noxious challenge. For example, hydrochloric acid discharged from the stomach into the duodenal lumen is a powerful stimulant of bicarbonate and mucus secretion (8, 34, 36). Mediation of increased secretion in response to acid involves local release of prostaglandins as well as the activation of neural reflexes. In addition to acid, fatty acids and amino acids liberated by digestive enzymes stimulate the synthesis and release of gastrointestinal hormones, which then enter the systemic circulation, where they can influence secretory responses (8, 9, 19, 38).

In mammalian species, secretions from Brunner’s glands contribute to the flow of mucin by the epithelium (18, 38). Brunner’s glands may also contribute bicarbonate to this mucus secretion (19, 21, 31), but there is some controversy as to the importance of this latter role (1, 30). In addition to this alkaline mucus, immunological and molecular studies indicate that Brunner’s glands contribute a number of other important factors. The presence of immunoglobulins and lysozyme (7) suggests that glandular products may contribute to mucosal defense. In addition, growth factors and trefoil peptides (16, 33), which have been linked to epithelial proliferation and restitution (43), have been identified. Thus Brunner’s gland secretions may also play a role in the regulation of mucosal healing. Pancreatic trypsin inhibitor (4) and a unique serine protease have been identified in acinar and duct cells (44), suggesting that glandular secretions may contribute to the regulation of digestive enzymes. Despite the importance of Brunner’s gland secretions, both in normal digestive function and in mucosal protection and healing, little is known about how secretion is regulated. This is largely due to the fact that much of what is known about duodenal secretion has been derived from studies using duodenal pouches in vivo where glandular and epithelial secretions cannot be separated.

In the current study, a novel in vitro model was developed that permitted the examination of Brunner’s gland secretion in tissues that are devoid of epithelial cells. A videomicroscopy technique was adapted to measure changes in the luminal diameter of Brunner’s gland acini as an index of secretion. The presence of mucin in the lumen was used as an indicator of the release of a secretory product. The aim of the study was...
to examine the actions of neurocrine, endocrine, and paracrine mediators to directly determine whether Brunner’s gland secretion is activated by these agents. These findings were compared with previous studies of epithelial secretion to determine whether mediators that stimulate Brunner’s glands do so in parallel with their actions on epithelial secretion or whether some may preferentially activate Brunner’s glands.

METHODS

Guinea pigs (140–225 g) of either sex were obtained from Charles River Laboratories (Montreal, Quebec, Canada). Experiments were performed according to the guidelines of the Canadian Council of Animal Care. Animals were anesthetized with isoflurane and immediately killed by decapitation. The abdomen was opened, and the entire duodenum was removed from a point ~1 cm distal from the pyloric junction to the ligament of Treitz.

Preparation of tissue. Proximal duodenum was opened along the mesenteric border and pinned flat in Sylgard-lined petri dishes with the mucosal surface facing upwards. The tissue was covered with frequent changes of Krebs buffer (in mM: 126 NaCl, 2.5 NaH₂PO₄, 1.2 MgCl₂, 2.5 CaCl₂, 5 KCl, 25 NaHCO₃, and 11 glucose) equilibrated with 95% O₂-5% CO₂. The mucosa was removed from the entire preparation to expose the submucosa. The submucosa containing Brunner’s glands was then dissected free from the underlying muscularis propria and cut into 0.5- to 1-cm² preparations. These preparations were transferred to fresh buffer, where they were maintained under continuous oxygenation at room temperature. All preparations were used for experimental study within 2 h of dissection. Previous studies in our laboratory and many others have demonstrated that the intrinsic submucosal neurons remain functionally intact in this in vitro preparation (i.e., they can be stimulated by agonists, leading to activation of neurally mediated epithelial secretion and dilation of submucosal arterioles [6, 23, 41]) and that nerve terminals from extrinsic nerves are also functionally intact (3, 40).

Videomicroscopy technique. Submucosal preparations were oriented with the mucosal surface upwards and pinned flat in small (1–2 ml), Sylgard-lined Plexiglas organ baths (39, 40). The baths were mounted on an inverted microscope (Zeiss Axiovert 10) equipped with a black and white video camera. Tissues were continuously superfused with oxygenated Krebs buffer (36 ± 0.5°C, pH 7.4) provided by gravity perfusion at 15 ml/min. After an equilibration period of 10 min, Brunner’s glands were visualized using a computer-assisted videomicroscopy system (Diamtrak, T. O. Neilid) to monitor changes in the diameter of the lumen of Brunner’s gland acini as an index of secretion. This videomicroscopy technique was developed for the in vitro measurement of changes in blood vessel diameter (28) and has been used extensively in this laboratory for that purpose (2, 3, 39, 40). The camera captures the image seen through the microscope for analysis by the imaging software (Fig. 1A), which then converts the image to grayscale and displays it on a video monitor. As shown schematically in Fig. 1B, the microscope is focused so that the walls of the acinar cells form two parallel dark lines. Cursors are superimposed on the image and centered across the acinus lumen. On exposure to agonists, the lumen dilates, and the distance between the two dark lines is continuously tracked by the cursors and recorded on a chart recorder. The resolution of this system is ~1 µm, and the sampling rate is 15 Hz.

All drugs, except porcine CCK, were applied by continuous superfusion. One milliliter of a CCK solution (~5 Crick
units), warmed to 37°C, was added directly to the bath immediately after abrupt termination of Krebs superfusion to the bath. The effects were studied for 1 min. After this study period, superfusion was restarted and the solution was washed out from the preparation.

Histology. Duodenal submucosal preparations were mounted on the experimental apparatus as described above and superfused for 1 min with either Krebs buffer alone or buffer containing 3 or 10 µM carbachol. The superfusate was then drawn off, and the preparations were immediately covered with fixative (10% Formalin in 0.1 M sodium phosphate buffer, 5°C, pH 7.4). Tissues were fixed overnight, washed several times with 0.1 M phosphate-buffered saline (PBS, pH 7.4), transferred to fresh PBS, and stored for a maximum duration of 3 days before processing for histology.

The fixed tissues were dehydrated to 100% ethanol and sequentially equilibrated in 50, 75, 90, and 100% LR White acrylic resin (JBS Supplies) in ethanol. Tissues were embedded in 100% LR White in gelatin capsules and polymerized at 20°C overnight. Sections were cut at ~5 µm on a Sorvall MT2-B ultra-microtome using glass knives, and then stained with toluidine blue. A second group of sections was processed using the periodic acid-Schiff (PAS) reaction for glycoproteins and counterstained with hematoxylin. In some experiments, whole mounts of duodenal submucosa containing unstimulated or stimulated Brunner’s glands were processed for PAS reactivity. Slides were coded to avoid observer bias.

Ultrastructural analyses. Submucosal preparations were superfused for 1 min with either Krebs buffer or buffer containing 3 µM or 10 µM carbachol (n = 3 for each) (as described in Histology). Preparations were fixed for 2 h at room temperature in 2% glutaraldehyde buffered with sodium phosphate to pH 7.0 and washed in sodium phosphate buffer. The preparations were then cut into segments of ~2 mm² and postfixed for 1 h in phosphate-buffered 1% osmium tetroxide. The preparations were <0.1 mm thick. After routine processing, tissue blocks were embedded in Eponaldrite. Semithin sections (0.5–1.5 µm thick) were cut perpendicular to the surface of the submucosal preparations and placed on glass microscope slides. Brunner’s glands were identified in sections stained with toluidine blue, and the plastic blocks were trimmed to surface areas of ~0.5 mm². Ultrathin sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate by conventional methods. Sections were viewed using a Zeiss EM 10 CR transmission electron microscope. All grids were coded to avoid observer bias.

Statistical analysis. Results are expressed as means ± SE. Data were compared using the two-tailed Student’s t-test for unpaired values where appropriate. P values of <0.05 were considered significant.

Materials. Carbachol was purchased from Aldrich (Oakville, ON, Canada); TTX, 5-hydroxytryptamine (5-HT), PGE₁ and PGE₂, histamine, synthetic human [Leu₁₅]gastrin, vasoactive intestinal polypeptide (VIP), and CCK-8 were from Sigma (Oakville, ON, Canada); porcine pancreozymin (CCK) was from ICN Biochemicals (Montreal, QC, Canada); purified human secretin was from Bachem (Torrance, CA); and 4-diphenylacetoxy-N-(2-chloroethyl)-piperidine hydrochloride (4-DAMP) and 1,1-dimethyl-4-phenyl-piperazinium were from Research Biochemicals (Oakville, ON, Canada). All other materials were of reagent grade.

RESULTS

Characterization of the in vitro model. The ACh analog carbachol was employed in initial experiments because cholinomimetics have been found to stimulate secretion from secretory cells (6, 37, 46). Carbachol (3 µM) reproducibly evoked large dilations of Brunner’s gland acinar lumen (see Fig. 1) in all preparations studied (n = 22). The onset of dilation typically occurred within 10–20 s after addition of carbachol and reached maximum within 1 min. Prolonged exposure to carbachol (5 min) did not result in further dilation. On washout of the drug, luminal diameter gradually returned to near resting levels, stabilizing within 10–15 min. However, recovery decreased with repeated application of carbachol. Therefore, in subsequent studies, only one application of a secretagogue was applied to each preparation.

Carbachol-evoked dilations were concentration dependent (0.1–10 µM; EC₅₀ = 2 µM; Fig. 1D). Concentrations >10 µM caused massive dilation of the acinus to the extent that the architecture of the lumen became distorted and its boundaries could no longer be distinguished by the imaging system. Thus 10 µM was accepted as the concentration that produced the maximum measurable dilation. In this and all subsequent experiments, dilations were standardized by comparing them to the maximum dilation that could be elicited in each preparation. After washout of the secretagogue under study and after the resting diameter had stabilized (within 10–15 min), the preparation was superfused with 10 µM carbachol. Luminal dilations obtained in response to the test secretagogue were then expressed as percentage of the maximum dilation.

Preparations responded consistently to agonists for at least 2 h after mounting the tissue in the bath. In control experiments, the diameter of the resting lumen remained unchanged during continuous monitoring for 2 h (5.7 ± 0.56 µm at t = 0 min vs. 4.6 ± 0.48 µm at t = 2 h; n = 3) and responses to carbachol (3 µM) at 2 h (53 ± 3% n = 3) were not significantly different from those obtained in separate preparations at 30 min (47 ± 12%; n = 3).

Representative photomicrographs comparing toluidine blue and PAS-stained sections of acini from unstimulated tissue and from tissue exposed to carbachol (3 µM; n = 3) are shown in Fig. 2. In unstimulated tissues, the apical portion of the acinar cells took up little toluidine blue stain but were intensely reactive for PAS. The acinar lumen was formed by the juxtaposition of the apical surfaces of the acinar cells. After exposure to carbachol, the lumen was greatly expanded. In PAS-stained sections, this increase in diameter was accompanied by a reduction in staining within the acinar cells and the accumulation of PAS-positive material within the lumen (Fig. 2B).

The ultrastructure of unstimulated and stimulated glands was studied in greater detail using transmission electron microscopy (Fig. 3; n = 3). Brunner’s gland acinar cells exhibited basal nuclei, and the apical cytoplasm was filled with membrane-bound, electronlucent granules similar to those described for mucin secreting cells (11). In the unstimulated tissue, individual mucin granules could be clearly identified. The membranes of some adjacent granules were fused.
After exposure to 3 µM carbachol, granular fusion was more extensive, with chains of compound granules evident. Both individual and compound granules had migrated to the apical membrane, and their contents were extruded into the acinar lumen. Dilation of the acinar lumen was apparent. Exfoliation of single acinar cells was occasionally observed (Fig. 3B). This holocrine secretion was accompanied by ingrowth of cytoplasmic extensions (Fig. 3, C and D) from adjacent acinar cells.

After exposure to 10 µM carbachol, few apical granules remained and the acinus lumen was greatly expanded. Indications of accelerated synthetic activity were evidenced by dilated endoplasmic reticulum in the acinar epithelial cells (Fig. 3E).

Mechanism of action of carbachol. To determine whether carbachol was acting directly on the Brunner’s gland acinar cell and/or indirectly by activating neural reflexes within ganglia of the submucosal plexus, preparations were pretreated for 3 min with either 4-DAMP, the muscarinic antagonist, or TTX, a blocker of sodium-dependent neuronal transmission (Fig. 4). Carbachol (3 µM; n = 6) had no effect on luminal diameter in the presence of 4-DAMP (1 µM). In the presence of TTX (1 µM), carbachol diluted Brunner’s glands (n = 4) to a similar degree to that seen in preparations not treated with TTX (Fig. 4). Previous electrophysiological studies (39) demonstrated that 5-HT (10–30 µM) activates virtually all secretomotor neurons in similar in vitro submucosal preparations from the small intestine and that these neurons can evoke dilation of submucosal arterioles (41) and activate secretion from epithelium in Ussing chamber studies when the epithelium is intact (6). In the current study, 5-HT (30 µM; n = 4) had no effect on Brunner’s gland luminal diameter.

Paracrine and hormonal mediators. The paracrine inflammatory mediator histamine evoked concentration-dependent dilation of Brunner’s gland acinar lumen (0.3–30 µM; EC₅₀ = 2 µM; n = 4; see Fig. 5). When histamine (10 µM) was superfused after superfusion of the H₁ receptor antagonist pyrilamine (200 nM) for 3 min, dilations were completely blocked (n = 4). The nonmethylated synthetic prostaglandins PGE₁ (10 µM; n = 5) and PGE₂ (10 µM; n = 4) evoked a similar magnitude of glandular secretion to that seen with histamine (Fig. 5).

The intestinal hormones CCK, gastrin, VIP, and secretin were also found to activate secretion (Fig. 6). Porcine CCK (5 Crick units; n = 4) evoked large dilations (Fig. 6). The selectivity of this action for CCK receptors was supported by the actions of synthetic CCK-8 (600 nM; n = 3), which also diluted Brunner’s glands (38 ± 4% of maximal carbachol secretion). Gastrin (600 nM; n = 6), VIP (300 nM; n = 3), and secretin (300 nM; n = 3) also evoked large Brunner’s gland dilations, similar in magnitude to those obtained with histamine and prostaglandins.

The presence of PAS-stained material was examined in whole-mount submucosal preparations to determine if luminal dilation was associated with the presence of mucin. The dilated lumens evoked by each of the paracrine and hormonal mediators were filled with PAS-positive material (n = 2).

DISCUSSION

The study of proximal duodenal secretion in vivo has been complicated by the inability to separate contributions from the duodenal epithelium and from Brunner’s
Fig. 3. Transmission electron micrographs comparing ultrastructure of Brunner’s glands in control and carbachol-stimulated tissue. A: unstimulated Brunner’s acinus demonstrating typical morphology of polarized secretory cells that release materials from storage granules. Note the basal nucleus (N), and densely packed electron-lucent mucin granules (GR) within apical cytoplasm. Apical surfaces of acinar cell form boundary of acinus lumen (L). Magnification = ×3,250. B: stimulated Brunner’s gland (carbachol, 3 µM). Apical mucin is reduced. Many adjacent secretory granules have fused to form chains of compound granules (*). Mucin granules have migrated to apical surface of acinar cell where their contents are extruded into acinus lumen (L) by single and compound exocytosis. Occasionally, an entire acinar cell is released into lumen (arrow). Magnification = ×6,000. C: site of acinar cell exfoliation. Epithelial integrity is maintained by ingrowth of processes from adjacent acinar cells (AC). L, acinus lumen. Magnification = ×20,000. D: enlarged view of C, showing point of contact of ingrowing processes (arrow). Magnification = ×20,000. E: stimulated Brunner’s gland (carbachol, 10 µM) showing dilated endoplasmic reticulum (ER). Apical cytoplasm is largely depleted of mucin granules. Magnification = ×5,100.
glands. One approach has been to focus on segments of the mammalian duodenum that are devoid of Brunner’s glands (8, 14) or on species, such as the bullfrog, in which these glands are absent (9). In addition, because the epithelium is more accessible, models of isolated epithelium have enabled the direct study of epithelial secretion (9). Consequently, there continues to be a relative paucity of information concerning the regulation of Brunner’s gland secretion. This study describes a novel in vitro model of Brunner’s gland secretion that allows combined functional and morphological study of the secretion by these glands in preparations that are devoid of epithelial cells. Our findings provide direct evidence that glandular secretion is recruited through the activation of multiple regulatory pathways, including cholinergic mechanisms, intestinal hormones, and inflammatory mediators.

The guinea pig duodenal submucosal preparation described in this study offers several advantages over other models of Brunner’s gland secretion. Although we employ an in vitro preparation in this study, videomicroscopy monitoring allows functional measurement of secretion that is uncontaminated by epithelial secretions. Furthermore, previous electrophysiological studies in vitro have demonstrated that submucosal neurons (39) and the nerve terminals of extrinsic nerves (3) can be activated electrically and that numerous agonists stimulate submucosal neurons in this preparation (23). Studies in the same preparation have also shown that these nerves, as well as inflammatory cells, are functionally intact. For example, activation of submucosal neurons or mast cells can dilate submucosal arterioles (2, 3), and stimulation of sympathetic nerve terminals hyperpolarizes neurons and constricts arterioles (3). The guinea pig submucosal preparation is also ideal for pharmacological studies because the preparation is only 60–80 µm thick, affording a minimal diffusion barrier to pharmacological agents. In addition, this preparation is well suited to the application of histological techniques, enabling combined functional and histological study in the same preparation. Guinea pig Brunner’s glands are of the mucoid type and are largely confined to the proximal duodenum. They closely re-
semble human Brunner's glands (12), but there are important differences in distribution and type in other species.

The presence of mucoid material in the lumen was demonstrated in this study to establish that secretory products moved from the acinar cell to the lumen as the lumen dilated. This material was PAS positive, indicating that the secreted product contained the glycol moieties characteristic of epithelial mucin glycoproteins. Ultrastructural analysis indicated that the mucoid material is confined to membrane-bound secretory granules similar to those described for other mucin-secreting cells, such as acinar cells of submaxillary glands (17) and intestinal goblet cells (37). Exposure to carbachol evoked fusion of many granules followed by the release of their contents into the glandular lumen by single-granule and compound-granule exocytosis (17, 37). In the current study, this process was observed at concentrations that had caused submaximal dilations, and, at concentrations that evoked maximal luminal dilation, almost all mucin granules were released from the acinar cell (see Fig. 3). Occasionally, holocrine secretion was observed in which entire acinar cells were exfoliated into the glandular lumen. This occurred without disruption of epithelial continuity within the acinus, as evidenced by the extension of processes from adjacent acinar cells beneath the exfoliating cell. This mechanism is consistent with that reported for other epithelial cells (24), including those that release mucin from storage granules (13). Holocrine secretion provides a mechanism for the shedding of senescent cells while maintaining epithelial integrity (22). In addition, it can result from accelerated epithelial cell turnover elicited by physiological stimuli (15).

The secretory action of cholinergic agonists has been observed in a wide variety of secretory cells, including columnar and goblet cells (6, 37) and gastric surface mucus cells (46), and a number of cholinergic neural pathways mediating this action have been implicated. Within the gastrointestinal tract, the cholinergic regulation of secretory responses can involve intrinsic and/or extrinsic pathways (6). Thus the activation of cholinergic submucosal motoneurons might stimulate Brunner's gland secretion in addition to their presecretory effects on intestinal enterocytes. Alternatively, or in addition, glandular secretion may be under vagal regulation. Electrical stimulation of vagal efferents was shown previously to stimulate Brunner's gland secretion (42), although it was not known whether this was due solely to a direct effect on the acinar cells or whether it may have involved intrinsic submucosal neural pathways. In the current study, carbachol-evoked Brunner's gland secretion was abolished by the muscarinic receptor antagonist 4-DAMP but was unaffected by blockade of sodium-dependent neural transmission (Fig. 4). These findings suggest that the stimulatory action of carbachol occurs via the activation of muscarinic receptors present on the acinar cell rather than through the excitation of submucosal secretomotor neurons. The muscarinic receptor antagonist 4-DAMP has selectivity for the M3 receptor, the predominant subtype mediating muscarinic responses in exocrine gland secretion (27). The possible role for submucosal neurons was explored further by activating them directly using 5-HT. Previous studies employing intracellular recording techniques showed that 5-HT reproducibly activates submucosal neurons, causing action potential discharge (39), and that activation of these neurons by 5-HT elicits chloride-dependent fluid secretion (6) and mucin secretion (26) from the intestinal epithelium. 5-HT, however, had no effect on Brunner's gland secretion. When these findings are taken together with previous studies, it seems most likely that cholinergic inputs to Brunner's glands originate from nerve fibers whose cell bodies are extrinsic to the submucosal plexus.

The actions of inflammatory mediators on duodenal secretion are of particular importance in view of the role played by these secretions in duodenal mucosal defense. Large numbers of mast cells are found within the duodenum of human and other species (29) that can release a variety of mediators, including histamine. It appears, however, that histamine has little if any effect on secretion from the epithelium. One study of intestinal anaphylaxis in vivo did suggest that mucin secretion from epithelial cells paralleled increases in tissue histamine after mast cell degranulation (20). However, many other mediators could have been implicated. In addition, a more recent study has contradicted these findings, demonstrating that epithelial mucin secretion did not occur during anaphylaxis (32). This latter finding is supported by in vitro studies, using T84 mucin-secreting epithelial cells, which demonstrated that histamine had no direct effect on epithelial mucin secretion from these cells (25). In contrast, the current study demonstrates that histamine is a potent stimulator of Brunner's gland mucin secretion. This action took place at concentrations of histamine in the same range found in the submucosa of guinea pig ileum when mast cell degranulation occurred (2, 3) and involves activation of H3 receptors, whereas H2 receptors mediate histamine actions on intrinsic neurons (6). Prostaglandins of the E series were also found to be potent secretagogues of Brunner's gland mucin secretion. In addition, however, these inflammatory mediators also have potent actions on epithelial secretion when applied topically or released locally in response to luminal acid (8, 9, 14, 25, 34–36). The current findings provide direct evidence that Brunner's gland secretion and epithelial secretion may act independently or in parallel depending on the inflammatory conditions and that activation of Brunner's gland secretion is an important response during inflammation.

Gastrointestinal hormones also play a major role in stimulating the release of duodenal mucus and bicarbonate. Classical studies in vivo employing proximal duodenal pouches demonstrated that mucus and bicarbonate were released in response to secretin, gastrin, and CCK or in response to the emptying of gastric contents into the duodenum (18, 38). It was unclear from these studies, however, whether the secretions originated from the epithelium or from Brunner's glands. The
current study demonstrates that gastrin, secretin, CCK, and VIP are potent secretagogues stimulating Brunner’s gland secretion, suggesting that they play a role in hormone-evoked secretion. These findings are in keeping with results from in vivo studies, which showed that secretin and gastrin elicited a mucin secretory response only in regions of the rabbit intestine that contain Brunner’s glands (21) and with histological evidence for mucin depletion from Brunner’s glands after in vivo secretin stimulation in the rat (18). Not all hormonal actions are confined to Brunner’s glands, however, since some have also been shown to stimulate epithelial cells. For example, although CCK stimulates Brunner’s gland secretion (19), it has also been shown to activate secretory pathways in epithelial cells (5). In addition, VIP, which has potent actions on Brunner’s glands, has also been shown to stimulate secretion of mucus and bicarbonate from epithelial cells (25, 45). These data support the conclusion that some gastrointestinal hormones, such as gastrin and secretin, preferentially stimulate Brunner’s gland secretion in isolation from epithelial secretions. These actions may be necessary to ensure that during specific conditions, such as during the added duodenal acid challenge with a meal, additional secretions from Brunner’s glands are provided to ensure that the mucosal integrity is maintained. In addition, these selective hormonal actions are intriguing given recent descriptions of potentially important digestive enzymes found within Brunner’s glands, such as duodenase (44) and pancreatic trypsin inhibitor (4), which may be released in addition to mucin and bicarbonate.

In summary, this study describes a novel in vitro model that enables the multidisciplinary study of the cellular mechanisms underlying Brunner’s gland secretion. On the basis of the findings of this study, these glands appear to be under complex regulation by paracrine, neurocrine, and endocrine pathways. Together with the results of previous studies, these findings support the hypothesis that Brunner’s gland secretion may be activated both in concert with and in isolation from the regulation of mucosal epithelial secretion, depending on the physiological and pathophysiological settings. This model will enable a detailed analysis of the constituents of Brunner’s gland secretions and the cellular mechanisms underlying the secretagogues that release them.

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