Epithelial Cells and Their Neighbors.

II. New perspectives on efferent signaling between brain, neuroendocrine cells, and gut epithelial cells

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Flemström, Gunnar, and Markus Sjöblom. Epithelial Cells and Their Neighbors. II. New perspectives on efferent signaling between brain, neuroendocrine cells, and gut epithelial cells. Am J Physiol Gastrointest Liver Physiol 289: G377–G380, 2005; doi:10.1152/ajpgi.00093.2005.—Surface sensory enteroendocrine cells are established mucosal taste cells that monitor luminal contents and provide an important link in transfer of information from gut epithelium to the central nervous system. Recent studies now show that these cells can also mediate efferent signaling from the brain to the gut. Centrally elicited stimulation of vagal and sympathetic pathways induces release of melatonin, which acts at MT2 receptors to increase mucosal electrolyte secretion. Psychological factors as well mucosal endocrine cell hyperplasia are implicated in functional intestinal disorders. Central nervous influence on the release of transmitters from gut endocrine cells offers an exciting area of future gastrointestinal research with a clinical relevance.

enteroendocrine cells; irritable bowel syndrome; melatonin; orexin; uroguanylin

Enterodocrine cells, for example enterochromaffin cells (EC cells), are a complex and specialized population of intestinal epithelial cells that produce a wide range of hormones and transmitters, including 5-HT, CCK, uroguanylin (8, 19), and melatonin (3). Enteroendocrine cells, but not nerve fibers, are in close contact with the intestinal lumen and are considered to act as mucosal taste cells by sensing luminal contents of nutrients as well as changes in luminal osmolarity and luminal acidity (21). The presence of a pH gradient at the surface of the gastroduodenal mucosa is compatible with luminal acidity being sensed as PCO2 rather than pH (2). CO2 would be generated within the surface mucus gel during reaction between luminal H+ and secreted HCO3-. Release of 5-HT from EC cells is also involved in mediating the mucosal secretive response to some bacteriotoxins, e.g., cholera toxin.

Transmitters released from the enteroendocrine cells in response to various stimuli, in addition to their paracrine actions on neighboring enterocytes, activate specific receptors within the enteric nervous system (15). Importantly, enteroendocrine cell products also activate receptors at afferent terminals in vagal and spinal pathways (10). They thus provide an important link in the afferent transfer of information from the gut surface to the nervous system. Particular attention has been paid to the inactivation of intestinal neuroreceptors by 5-HT produced by EC cells and by CCK produced by enteroendocrine C cells. Furthermore, recent studies have shown that two transmitters interact in stimulation of vagal primary afferent neurons (15). The transfer of information from the gut to the central nervous system has been the subject of theme articles in this journal (10, 21). In the present article, the focus is on the role of enteroendocrine cells as mediators of efferent signaling, primarily elicited within the central nervous system and transferred to the gut.

Central Nervous System and Intestinal Function

The importance of the central nervous system in the overall physiological control of the gastrointestinal tract was elegantly illustrated long ago in St. Petersburg by Pavlov. He demonstrated in conscious dogs that sham feeding increases the exocrine secretion from the stomach and pancreas, naming it the cephalic phase of stimulation. Subsequently, a wealth of evidence has shown that gastrointestinal secretions, and motility in general, are influenced by the central nervous system. Actions are mediated not only by vagal and sympathetic pathways but also by peptide hormones, such as β-endorphin, released from the pituitary gland into the systemic circulation. Sham feeding experiments, electrical stimulation of vagal and sympathetic nerves, as well as intracerebral administration of neurotransmitters and drugs are some of the approaches used in elucidating the central nervous influence on intestinal function.

The duodenal epithelium secretes HCO3- at higher rates (per unit surface area) than the more distal small intestine, probably reflecting higher HCO3- selectivity of the apical CFTR channel in this part of the intestine (2). This secretion is currently accepted as the primary duodenal defense against acid discharge from the stomach and is deficient in patients with duodenal ulcer disease. It has thus been the subject of several studies (2). It is now clear that centrally elicited stimulation of the HCO3- secretion occurs on intracerebroventricular infusion of some neuropeptides and catecholamines, including thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone, and phenylephrine (6, 12–14, 26). In contrast, intracerebroventricular infusion of calcitonin gene-related peptide (13) or the adrenoceptor agonist clonidine (12) inhibits the mucosal HCO3- secretion.

Another interesting example of central nervous influence is the spontaneous circadian rhythmicity of some gastrointestinal secretions. In fasting rats, there are circadian rhythms in the gastric secretion of H+, HCO3-, and mucus (11). The mucus-protective secretion of HCO3- and the potentially damaging secretion of H+ follow rhythms with an 8-h difference in peak.
times. This could, in theory, indicate centrally elicited rhythmicity of mucosal vulnerability to acid injury. The incidence of gastroduodenal ulcer reportedly peaks at certain periods of the year. It should be noted that clock genes are not only timekeepers in the brain but also expressed in cells in peripheral tissues, including the gastrointestinal tract (24). The functional role of their expression at the latter location is interesting but not yet fully understood.

VAGAL AND SYMPATHETIC MEDIATION OF CENTRALLY ELICITED STIMULI

Intracerebroventricular administration of the adrenoceptor agonist phenylephrine acting at $\alpha_1$-adrenoreceptors causes a marked, up to fivefold, increase in duodenal mucosal $\text{HCO}_3^-$ secretion (12, 25, 26). Pretreatment with prazosin, an $\alpha_1$-antagonist, inhibited this rise in secretion when infused into the lateral brain ventricle but not when administered intravenously, confirming that the stimulatory action of phenylephrine is centrally elicited (12). Furthermore, the secretory response was abolished by sublyingeal ligation of all nerves around the carotid arteries but insensitive to truncal vagotomy alone or sympathectomy alone (26). The $\mu$-selective opiate antagonist naloxone was without effect in this system, excluding humoral stimulation by $\beta$-endorphin released from the pituitary gland (12).

In contrast to the findings with phenylephrine, truncal vagotomy alone abolished the rise in duodenal $\text{HCO}_3^-$ secretion induced by intracerebroventricular infusion of TRH (14) or the benzodiazepines diazepam and Ro-15-1788 (22). The maximal rise in secretion after central nervous system administration of TRH and benzodiazepines was also considerably smaller (~2-fold) than that observed with phenylephrine (4- to 5-fold). Secretory responses to intracerebroventricular TRH were completely inhibited by a VIP receptor antagonist (4Cl-d-Phe$^6$-Leu$^7$) and, as found with phenylephrine, by ganglionic blockade (12, 14). Pretreatment with atropine caused a minor decline of the response, whereas bretylium, a compound inhibiting the release of noradrenaline at nerve endings, was without effect. The neurally mediated response to TRH thus seems transferred by the vagal nerves alone; it is dependent on release of VIP and in part involves muscarinic pathways. Noradrenergic pathways appear to be unimportant for transfer of stimuli elicited by intracerebral TRH.

LOCAL MUCOSAL INNERVATION OF INTESTINAL ENTEROENDOCRINE CELLS

Efferent neural stimuli to the intestine may have a direct effect on electrolyte-transporting enterocytes, act at receptors in the enteric nervous system, or induce release of transmitters from enteroendocrine cells. Local mucosal innervation of intestinal EC cells was first demonstrated and studied in guinea pig duodenum nearly 30 years ago (16). Near the basement membrane of all examined EC cells in the crypts, bundles of unmyelinated nerve processes were observed only partially ensheathed in a Schwann cell cover. No typical synaptic arrangements were observed, but the minimal distance between the EC cells and the nerve bundles was 150–250 nm, thus well within the functional limits of the “autonomic gap.” These authors proposed that EC cells are innervated by several types of nervous elements, including catecholaminergic fibers. The same group (1) also studied the effects of long-lasting electrical stimulation of efferent vagal nerves at the cervical level in cats. This stimulation decreased the intracellular concentration of 5-HT in small intestinal EC cells, with very similar effects in duodenum and more distal small intestinal segments. Pretreatment with atropine did not affect the depletion of 5-HT, but it was inhibited by removal of the superior cervical ganglia, as well as $\beta$-adrenoreceptor blockade by propanolol.

In a new and elegant approach (23), EC cells from the rat ileum were purified by a combination of elutriation and density gradient centrifugation. Immunostaining showed that 84% were 5-HT positive cells in the enriched EC fraction compared with 12% in unfractioned cells and cytochemistry. The presence of $\alpha_1$- and $\beta$-adrenoreceptor isoforms, muscarinic M3, and GABA-A receptors was demonstrated by RT-PCR and cytochemistry. Release of 5-HT was stimulated by noradrenaline, and to some extent by carbachol, whereas GABA was without effect. The stimulation of EC cells by noradrenaline is in line with earlier studies of intact mucosa (1). However, more studies and new methodologies are required to elucidate the apparently complex mucosal innervation of EC cells and, in particular, the role of vagally mediated stimuli and muscarinic receptors. Present methodologies are associated with some difficulties. In experiments in vivo and with sheets of intact mucosa in vitro, a problem is that EC cell-mediated responses may not be primary but are induced by transmitters released from other populations of enteroendocrine cells or from other parts of the enteric nervous system. Isolated intestinal epithelial cells are more difficult to maintain viable than, for instance, gastric parietal cells or pancreatic beta cells (27). The life span of small intestinal enterocytes in situ is very restricted (4–6 days for human enterocytes). In addition, enterocytes in situ rapidly respond to irritating compounds by apoptosis and expulsion. The vulnerability of isolated EC cells (23) seems similar to that of isolated enterocytes (27) and may affect the expression of receptors in some preparations.

EC CELLS IN MEDIATION OF CENTRAL NEURAL STIMULI

The neurohormone melatonin is produced by EC cells in the intestinal epithelium as well as the pineal gland in the central nervous system. The total amount of melatonin in the intestine is ~400 times greater than that in the brain, but effects of melatonin on intestinal secretion and protection are not well understood (3). Melatonin receptors are reported to be distributed throughout the gastrointestinal tract, and in rat, the highest concentration of 2-iodomelatonin binding sites is located in the villi of the small intestine. Recent studies (26) have demonstrated that close intra-arterial infusion of melatonin or melatonin receptor agonists to the duodenal segment in rats increased mucosal $\text{HCO}_3^-$ secretion, with a low dose of melatonin (20 nmol·kg$^{-1}$·h$^{-1}$), causing maximal stimulation. A similar stimulatory action was observed with luminally administered melatonin. Furthermore, the melatonin MT$_2$-selective antagonist luzindole almost abolished the marked rise in secretion induced by intracerebroventricular infusion of the adrenoreceptor agonist phenylephrine but did not affect the rise in release of melatonin to the duodenal lumen (25). These findings strongly suggest that a centrally elicited neural stimulus releases melatonin from EC cells in the duodenal mucosa and...
that released melatonin acting at MT₂ receptors increases enterocyte electrolyte export.

The action of melatonin on enterocyte intracellular Ca^{2+} concentration ([Ca^{2+}]_{i}) signaling has been studied in clusters of human and rat duodenal enterocytes that provide more viable preparations than isolated enterocytes (27). Melatonin and melatonin receptor agonists increased enterocyte [Ca^{2+}]_{i} with the EC_{50} of melatonin as low as 17.0 ± 2.6 nM. As found with duodenal secretion in vivo, MT₂ receptor antagonists abolished the enterocyte [Ca^{2+}]_{i} response. These combined results provide experimental support that EC cells do provide an important link in efferent transfer of information between the central nervous system and the intestine (Fig. 1). It is also clear that, as illustrated by comparison with the centrally elicited action of TRH (6, 14), that pathways for transfer of neural signals from brain to intestine depend on the nature of an eliciting (intracerebroventricular) stimulus. The differences in contribution by sympathetic and noradrenergic pathways in these systems would be of particular interest.

POSSIBLE ROLE IN DISEASE

Central nervous influence on the release of transmitters from gut endocrine cells offers an exciting area of future gastrointestinal research with a clinical relevance. Irritable bowel syndrome (IBS) and other gastrointestinal secretory and motility disorders are major prevalent disabling gastrointestinal diseases, and although affecting up to 10–20% of the population in several countries, their pathophysiology is not understood. There is, however, general agreement that there is EC-cell hyperplasia of the intestinal mucosa in IBS patients and in particular after intestinal infections (5). Studies of the plasma concentration of 5-HT in humans after a meal suggest that increased 5-HT release might relate to symptoms of IBS, and actions of 5-HT have been the focus of extensive research in relation to the development of treatment of intestinal disorders (4). Considerably less attention has been paid to other transmitters and neuropeptides produced by the intestinal enteroendocrine cells. However, with the clear role of psychological factors in the development of IBS (5), the central nervous influence on intestinal EC-cell function seems of considerable interest.

As described recent results (25–27) strongly suggest that EC cell melatonin is involved in mediating central nervous influence, via both vagal and sympathetic nerves on anion secretion by the duodenal mucosa. Furthermore, it has been reported (18) that intraperitoneal administration of a relatively high dose of melatonin (10 mg/kg) protects against sepsis-induced decreases in contractility in rat ileum and urinary bladder. There is also a strong disturbance of melatonin secretion in the exacerbation, as well as in the remission stage, in patients with duodenal ulcer disease (17).

Uroguanylin and the structurally related peptide guanylin like melatonin are candidate mediators of mucosal protection in the intestine. Both peptides are released into the intestinal lumen and both are endogenous ligands for the apical transmembrane guanylate C receptor (8). Their site of action is thus different from the basolateral sites observed with other transmitters released by intestinal enteroendocrine cells. Uroguanylin is produced by a subpopulation of EC cells in the mucosa of the proximal parts of the small intestine (19). In contrast, guanylin is produced mainly in the distal parts of the intestine and the specific cellular site of guanylin mRNA, originally proposed to be EC cells, is under debate. Furthermore, uroguanylin but not guanylin is resistant to digestion by the pancreatic enzyme chymotrypsin (8). Interestingly, uroguanylin is released when gastric acid enters the duodenal lumen and is a stimulator of the HCO₃⁻ secretion by the duodenal mucosa (9). These findings are further supported by the recent observation (20) that the rise in duodenal alkaline secretion in response to luminal acid is markedly smaller in guanylate C receptor knockout mice than in wild-type animals. In the more distal small intestine, guanylin as well as exogenous uroguanylin cause marked increases in the secretion of electrolytes and fluid and in doing so provide protection by diluting luminal contents. Antagonists to uroguanylin and guanylin may offer a means for treating IBS and colilaxin-induced diarrheas (8) although whether central or local neural pathways influence their release is presently not known.

Finally, most interesting, are recent studies showing that feeding status can rapidly alter the intestinal response to certain hormones. Consequently, it is important in studies of actions of neuroendocrine cell products on intestinal function that feeding status is considered. Orexins, which are involved in the central nervous control of appetite and behavior, are also present in enteroendocrine cells in the intestine. Duodenal close intrarrenal infusion of low doses of orexin-A markedly increased...
the duodenal alkaline secretion in rats (7). However, stimulation occurred only in animals that had continuous access to their regular food. Thus short (overnight) deprivation of food, which is a standard procedure in studies of gastrointestinal function and pathophysiology, abolished this stimulation of the duodenal secretion. Short food deprivation also caused a 100-fold increase in the amount of the muscarinic agonist bethanechol required for stimulation but, in contrast, did not affect responses to VIP or melatonin. An attractive explanation for the changes in sensitivity to orexin-A and bethanechol would be that the procedure of food intake or the presence of food constituents in the intestinal lumen via the central or enteric neural pathways stimulates the activity or expression of receptors in the intestinal mucosa.

In conclusion, recent studies show that enteroendocrine cells can provide an important link in transfer of information from brain to gut. The central nervous influence on enteroendocrine cell release of transmitters as well as effects of feeding on brain to gut. The central nervous influence on enteroendocrine can provide an important link in transfer of information from tors in the intestinal mucosa.

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In conclusion, recent studies show that enteroendocrine cells can provide an important link in transfer of information from brain to gut. The central nervous influence on enteroendocrine cell release of transmitters as well as effects of feeding on receptor expression is a particularly interesting area for future research and, quite likely, for therapeutic developments.

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REFERENCES