Evolving Concepts and Translational Relevance of Enteroendocrine Cell Biology

Daniel J. Drucker

Department of Medicine, Mt. Sinai Hospital, University of Toronto and the Lunenfeld-Tanenbaum Research Institute, Toronto, Canada M5G 1X5

Context: Classical enteroenteroendocrine cell (EEC) biology evolved historically from identification of scattered hormone-producing endocrine cells within the epithelial mucosa of the stomach, small and large intestine. Purification of functional EEC hormones from intestinal extracts, coupled with molecular cloning of cDNAs and genes expressed within EECs has greatly expanded the complexity of EEC endocrinology, with implications for understanding the contribution of EECs to disease pathophysiology.

Evidence Acquisition: Pubmed searches identified manuscripts highlighting new concepts illuminating the molecular biology, classification and functional role(s) of EECs and their hormonal products.

Evidence Synthesis: Molecular interrogation of EECs has been transformed over the past decade, raising multiple new questions that challenge historical concepts of EEC biology. Evidence for evolution of the EEC from a unihormonal cell type with classical endocrine actions, to a complex plurihormonal dynamic cell with pleiotropic interactive functional networks within the gastrointestinal mucosa is critically assessed. We discuss gaps in understanding how EECs sense and respond to nutrients, cytokines, toxins, pathogens, the microbiota, and the microbial metabolome, and highlight the expanding translational relevance of EECs in the pathophysiology and therapy of metabolic and inflammatory disorders.

Conclusions: The EEC system represents the largest specialized endocrine network in human physiology, integrating environmental and nutrient cues, enabling neural and hormonal control of metabolic homeostasis. Updating EEC classification systems will enable more accurate comparative analyses of EEC subpopulations and endocrine networks in multiple regions of the gastrointestinal tract. (*J Clin Endocrinol Metab* **101: 0000–0000, 2015**)

Physiological analyses and careful experimentation enabled Bayliss and Starling to identify secretin through isolation and purification of pancreatic exocrine secretory activity from gut extracts. The discovery of the first "hormone" ushered in the era of modern endocrinology at the turn of the last century. Shortly thereafter, the cellular origin and clinical presentation of carcinoid or gut endocrine tumors was described, further establishing the clinical relevance of sparse, dispersed, hormone-secreting cells within the gut mucosa. Although the gastrointestinal (GI) tract remained an important source for revealing new hormones in the early 20th century, a rapid series of discoveries established the next generation of new hormones outside the gut. The isolation of insulin, followed quickly by

Abbreviations: BA, bile acid; CCK, cholecystokinin; DPP4, dipeptidyl peptidase-4; EEC, enteroenteroendocrine cell; ENS, enteric nervous system; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; HIO, human intestinal organoid; PGDP, proglucagon-derived peptide; PYY, peptide YY; VIP, vasoactive intestinal peptide.

insulin administration for type 1 diabetes, pioneered the lifesaving potential of hormone replacement therapy. Subsequently, multiple adrenal, pituitary, thyroid, islet, and gonadal hormones were identified and characterized, indirectly diverting the attention of most endocrinologists away from the gut. Nevertheless, more than 100 years later, the enteroendocrine cells (EECs) of the GI tract have resurfaced to collectively represent the largest and arguably the most important yet functionally complex "endocrine organ", with EEC-derived hormones exerting pleiotropic actions on local and distant target cells and tissues. EECs are ideally situated to sense and respond to environmental, nutrient, and microbial signals, and constitute an early warning system that coordinates complex physiological responses to changes in the external environment. The importance of EECs and their hormones for maintenance of physiology and energy homeostasis is illustrated in part by loss-of-function mutations in the human neurogenin-3 (*NEUROG3*) gene, a syndrome that presents in infants with failure to thrive, severe feeding-associated malabsorptive diarrhea, and a marked depletion of EEC populations throughout the small and large intestine (1). Herein we summarize evidence establishing key concepts of enteroendocrine biology, and highlight gaps in our understanding and future challenges that merit ongoing exploration and experimental resolution.

Classification, Nomenclature, and Structure of the EEC System and its Hormones

EECs

The original alphabetic nomenclature of EECs was based on a combination of anatomical appearance and localization, histochemical and staining characteristics, and attribution of a predominantly unihormonal phenotype to morphologically distinct EEC populations. More recently, results from analysis of the purified EEC transcriptome and secretome using genetic markers and purified EEC populations from transgenic mice or human intestinal organoids (HIOs) necessitates a major reconsideration or complete abandonment of original nomenclature, which is no longer accurate or useful. Indeed, using the "L cell" as an example, it is difficult if not impossible to identify an EEC population that uniquely expresses the proglucagon (*GCG*) gene, as virtually all "L cells" are plurihormonal and coexpress, depending on their location within the gut mucosa, cholecystokinin (CCK), glucose-dependent insulinotropic peptide (GIP), neurotensin, peptide YY (PYY), and secretin (2, 3). The application of

single-cell transcriptome analyses to EECs isolated from distinct anatomical locations along the GI tract, from normal or injured rodent and human gut epithelium, and from villus vs crypt compartments is exciting. These studies will almost certainly unmask additional complexity and reveal multiple new EEC subtypes based on cell-specific patterns of gene expression (4).

Gut hormones

Identification of physiological activity emanating from administration of a fractionated gut extract, and later, purified or synthetic hormone, was followed by generation of antibodies that enabled 1) immunocytochemical localization of unique EEC subpopulations, and 2) measurement of tissue and plasma levels of the corresponding immunoreactive hormone. A major advance in revealing the potential for biological and more specifically, hormonal diversity within EECs stemmed from cloning of the cDNAs and genes that encode EEC hormones. These studies illuminated the complexity of prohormone organization as well as the structural and evolutionary relationships of multiple distinct hormones coencoded by the same gene. The contributions of alternative promoter utilization, tissue-specific RNA splicing, together with cell-specific prohormone processing and posttranslational modifications enable tremendous amplification of biological information encoded within a single gene. For example, the relationship of mammalian EEC "L cells" to islet "α" cells, simplistically defined by coexpression of the *GCG* gene, was clarified by the demonstration that in several species (fish, reptiles, and others), alternative RNA splicing yields structurally distinct prohormone precursors and different proglucagon-derived peptide (PGDP) profiles in pancreas vs gut. In contrast, although mammalian alpha and L cells express an identical preproglucagon mRNA transcript in pancreas and gut, cell-specific expression of unique prohormone convertase enyzmes generates considerable diversity of liberated PGDPs. Within the gut "L cell," proprotein convertase subtilisin/kexin type 1 expression produces glicentin, oxyntomodulin, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), and two intervening peptides, IP-1 and IP-2 (5). Surprisingly, transcriptomic analysis of gene expression signatures from single cells has revealed that islet alpha cells are more similar to insulin-producing β-cells than to *GCG*-expressing L cells (2). Hence, it seems reasonable to classify EECs based on 1) their species of origin; 2) their anatomical location, including subdivision by stomach, upper and lower small intestine and colon, and possibly even crypt vs villus localization within the GI tract; and 3) the genes and protein signatures they express (and those they do not) (Figure 1). Such a new classification would
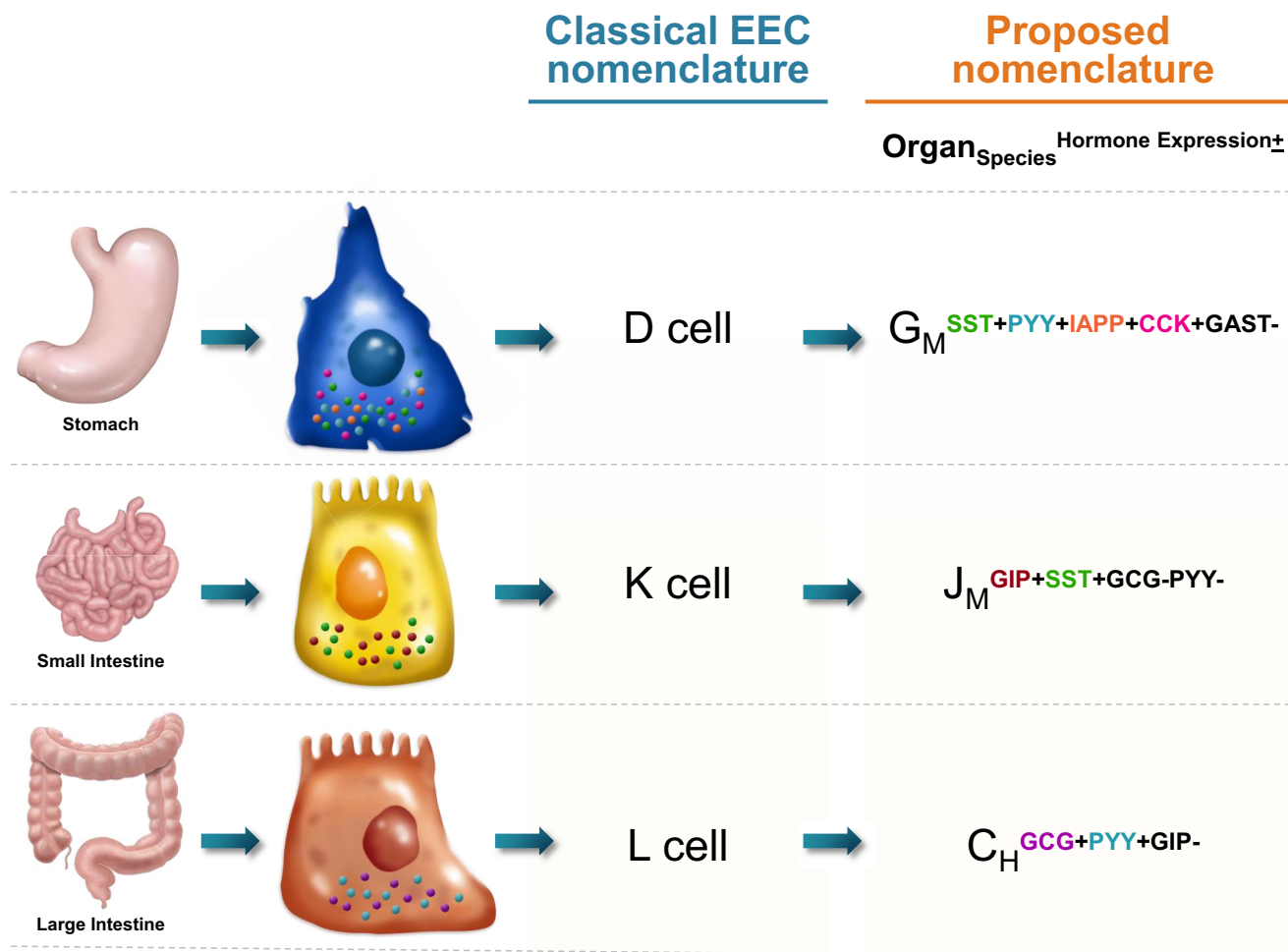
Classical EEC nomenclature   **Proposed nomenclature**

Organ$_{Species}$$^{Hormone\ Expression\underline{+}}$

Figure 1. Enteroendocrine cell nomenclature. Current (Classical) vs proposed EEC nomenclature is depicted. A classification system that incorporates region of the GI tract (G, gastric; J, jejunum; I, ileum; C, colon), followed by species, then presence or absence of specific hormone gene expression, would greatly enable comparative studies of EEC biology.

resemble in part the nomenclature that has evolved for description of the complexity of individual lymphocyte subpopulations. A more precise detailed informative nomenclature will make it more likely that different scientists are actually studying and describing similar if not identical EEC subpopulations, which, at present, can be challenging or impossible to ascertain. Although recent advances in characterizing EEC populations have largely used the mouse as a model organism, the transcriptomic analysis of human EECs from stem cell-derived HIOs, primary intestinal cultures, or single cells isolated from human gut by laser capture microdissection, should rapidly extend the modern analysis of EEC complexity from rodents to humans.

EEC Hormones and Their Bioactive Products

A simplified traditional view of gut hormone action posits that each EEC-derived peptide or monoamine such as serotonin or histamine exerts its actions through a single, well-defined G protein–coupled receptor. Although this often remains the case, the literature is replete with thousands of reports describing actions of 1) prohormones, or 2) multiple truncated, enzymatically cleaved forms of EEC peptides, through incompletely characterized receptors, ion channels, and signaling pathways. For example, understanding the complexity, biological activities, and cellular mechanisms of action for multiple smaller peptides arising from prohormones encoding CCK and gastrin is a daunting challenge. Similarly, original concepts of enzymatic "inactivation" of EEC hormones, for example via amino-terminal cleavage at a position 2 alanine or proline by dipeptidyl peptidase-4 (DPP4), have been superseded by dozens of studies ascribing biological actions to multiple DPP4-generated peptides (6). Although DPP4 cleavage of NPY and PYY does indeed switch receptor selectivity and preference for GPCR-mediated signaling within the NPY receptor family, the mechanisms of action of GLP-1 (9–36) on hepa-

tocytes, endothelial cells, skeletal muscle myocytes, and cardiomyocytes, remain enigmatic. A critical view of this literature suggests that it contains a predominant bias toward pharmacology, with most experimental studies employing administration of bioactive peptide fragments to achieve exposure levels far in excess of those normally found under physiological conditions. The dominant pathophysiological scenarios associated with increased secretion of EEC hormones potentially unmasking relevance of incompletely processed prohormones, or high levels of cleaved peptides are 1) EEC neoplasia such as gut carcinoids, and 2) following some forms of bariatic surgery. In the former situation, classical prohormone processing is often inefficient, aberrant processing is not uncommon, and tumor-associated production and secretion of incompletely processed prohormones and multiple hormone-derived fragments may result in extremely high levels of peptides with resultant bioactivity. Following gastric bypass surgery, EECs residing within the bypassed proximal gut segment generally exhibit a lower level of basal hormone secretion in the absence of luminal nutrients, whereas more distal EECs exposed to large volumes of incompletely digested nutrients and bile acids produce and secrete higher levels of gut hormones. In contrast, ascertaining the endogenous physiological importance of prohormones (often secreted at higher levels by EEC tumors) and cleaved peptide fragments is challenging. These molecules normally circulate at very low levels; to determine their biological relevance one would need data from informative experiments using selective antagonists, immunoneutralizing antisera, or mouse genetics; studies that may be technically challenging and are often lacking. Hence, it seems useful to separate conceptually the actions of a peptide, prohormone, or peptide fragment described in pharmacological studies, vs essential actions unmasked by interrupting the normal physiological actions of the naturally occurring prohormone or intact and cleaved peptide.

Experimental Models for Studying EEC Biology and Hormones

Although the mammalian digestive tract has evolved substantially in structure and function relative to the gut in organisms such as *Drosophila melanogaster*, understanding the importance of EECs within the context of evolutionary biology may provide valuable insight into conserved and novel functions of the mammalian enteroendocrine system. Loss of EECs in *D. melanogaster* is associated with defective nutrient-stimulated gut growth, impaired stem cell division, defective EEC-muscle com-

munication, and reduced life span, highlighting the importance of EEC-derived tachykinins for muscle insulin-like peptide 3 expression and the control of intestinal growth and energy homeostasis (7, 8). Remarkably, EEC-derived hormones promote intestinal cell division in *D. melanogaster* through recruitment of insulin-like peptides or epidermal growth factor–related signal proteins. Similarly, the EEC-derived PGDP GLP-2 also indirectly promotes growth and regeneration of the mammalian GI tract, stimulating crypt cell proliferation via activation of IGF-I and epidermal growth factor/ErbB ligand signaling (9). Hence, *Drosophila* may be a useful model for genetic dissection of EEC function with potential relevance for understanding human EEC biology (Figure 2). EECs have also been studied through analysis of mixed primary rodent and human intestinal epithelial cell cultures, in perfused intestinal segments, and indirectly through analysis of immortalized rodent and human EEC lines. The development of transgenic mice expressing reporter genes targeted to unique EEC subtypes has also facilitated isolation and analysis of enriched purified EEC subpopulations, enabling studies of hormone secretion and generation of single-cell transcriptomic RNA profiles. Similarly, selective targeting of diphtheria toxin to ablate subpopulations of EECs, or use of homologous recombination to disrupt genes encoding critical EEC transcription factors, hormones, receptors, and growth factors, has provided valuable new insight in the development and function of EECs.

The potential for species specificity in EEC biology and actions of EEC-derived hormones complicates direct extension of findings in preclinical species to human subjects, fostering the development of models for studying human EEC biology. Based on rapid advances in human pluripotent stem-cell technology, HIOs have been generated that contain all principal intestinal epithelial cell types (enterocytes, paneth cells, goblet cells, and EECs), providing a valuable model for studying human EEC hormone synthesis and secretion (10). Moreover, EECs within HIOs can be studied ex vivo under well-defined conditions, or following transplantation into immunodeficient murine hosts for physiological studies in vivo. Indeed, the relatively immature cellular and functional phenotypes evident in HIOs propagated in vitro may be overcome, to a limited extent, by the enhanced differentiation and function exhibited by HIOs propagated in vivo (11). Intriguingly, transplanted HIOs exhibit a proliferative response to resection of the endogenous murine ileum and cecum, findings consistent with preservation of growth factor signaling and/or GLP-2 receptor responsivity within the epithelium of transplanted HIOs (11). Furthermore, L cells propagated from HIOs reside in a polarized cell environment, retain a capacity for prolifer-
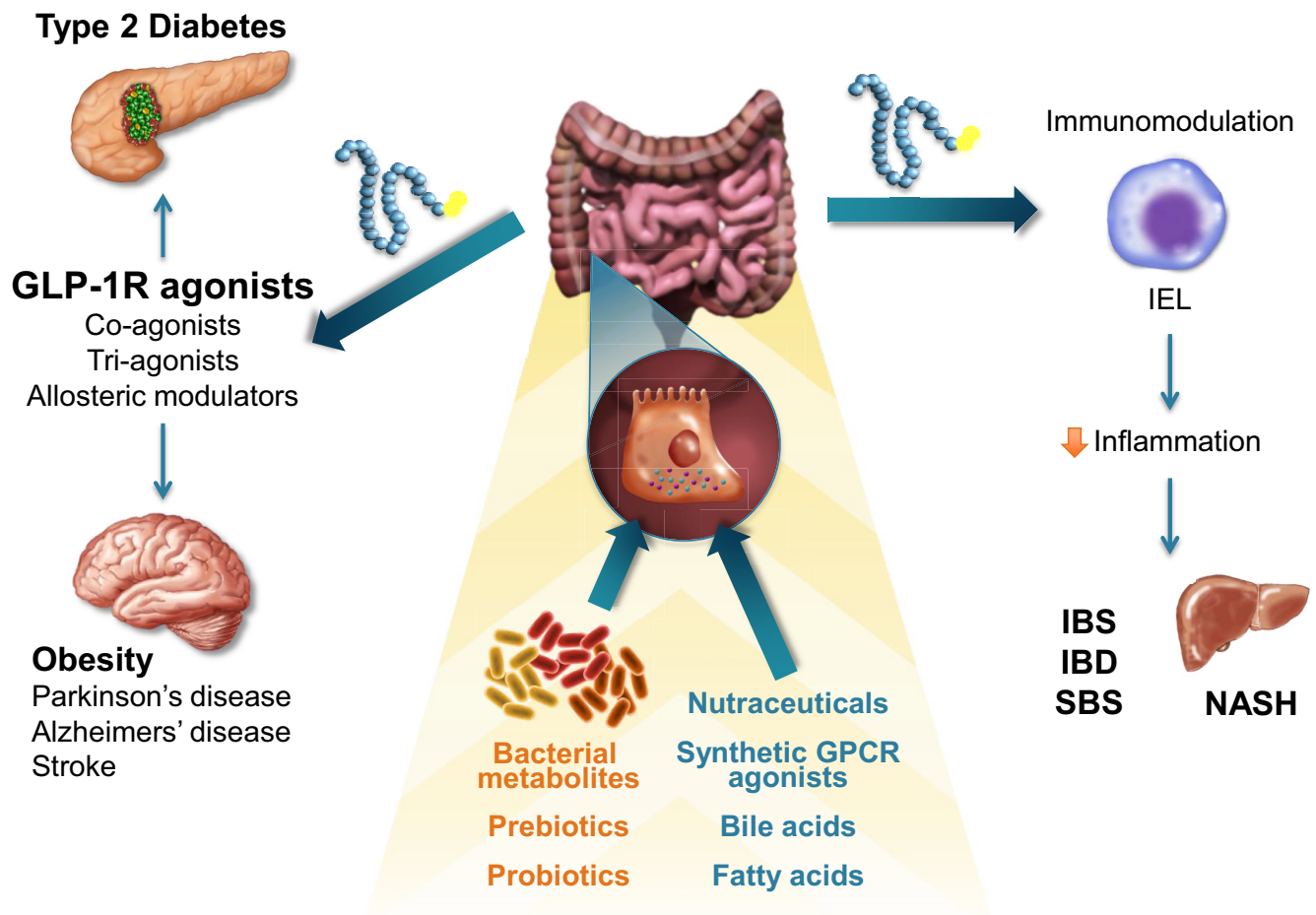
Figure 2. Translational potential of the GLP-1-producing EEC. GLP-1 receptor agonists are approved for the treatment of type 2 diabetes and obesity, and being explored for the treatment of multiple human disorders, as outlined. GLP-1-containing coagonists and triagonists and EEC secretagogues are also under active investigation as therapeutic modalities. IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IEL, intraepithelial lymphocyte; NAFLD, nonalcoholic fatty liver disease; SBS, short bowel syndrome.

ation, and exhibit some preservation of EEC gene expression profiles and well-defined PGDP secretion pathways (12). Nevertheless, current limitations of studying EEC biology in HIOs include incomplete characterization of EEC maturation and hormonal subtypes, and lack of 1) a coexisting normal microbiota, 2) enteric nervous system (ENS), 3) Brunner's glands, 4) fully developed human vasculature, and 5) input from systemic and local immune systems, organs, and cell types with major importance for EEC function (13). Moreover, whether HIOs can be engineered to precisely recapitulate functional EECs that reside within the human jejunum, ileum, and different colonic segments, remains to be established. A detailed molecular profile of individual EECs derived from HIOs, compared with similar profiles generated from single human EECs isolated from different regions of the human gut will improve our understanding of the extent to which HIO-derived EECs recapitulate one or more normal EEC populations.

The EEC Neighborhood and Local Endocrine Networks

The cellular neighborhood within which EECs reside comprises important cell types and organ systems critical for the secretion and action of EEC hormones. The anatomical location of many EECs enables exposure to and sensing of contents within the GI lumen while simultaneously interacting—through hormones released via its basolateral surface—with lymphatics, endothelial cells, glial cells, and enteric neurons. For example, subpopulations of ENS neurons express both neurotransmitters and GPCRs that may transduce local EEC-derived hormonal signals important for control of gut motility, appetite, barrier function, and glucose homeostasis. Conversely, neurotransmitters released by the ENS may alternatively stimulate or inhibit hormone secretion from EECs along the GI tract (14). The anatomical relationship connecting individual EECs and enteric neurons may be more intimate than previously realized, with cytoplasmic projections

from EECs—designated neuropods—that directly impinge upon sensory enteric neurons, enabling EEC-ENS synaptic transmission (15). Whether EEC-ENS transmission is restricted to luminal sensory- and nutrient-derived inputs, or also facilitates ENS infection from enteric viruses, requires additional study. Similarly, the precise roles of mucosal enteric glia in communicating signals from EEC neuropods to regulate gut barrier function, alone, or in part through subsets of neurons within the ENS, are not well defined. Furthermore, given the extent to which the submucosal ENS senses and responds to luminal nutrient cues, whether and to what extent the EEC network, through its anatomical exposure to luminal contents, critically modifies nutrient-regulated ENS activity, is incompletely understood.

Local vascular beds and lymphatics directly communicate with EECs, and are exposed to high levels of EEC-derived hormones. Several EEC hormones, including ghrelin, GIP, GLP-1, GLP-2, PYY, tachykinin-related peptides, and vasoactive intestinal peptide (VIP), as well as histamine, are all vasoactive and may exert local control of endothelial function and blood flow in the mucosa or within larger vascular beds in the GI tract. Several gut hormones, including PGDPs such as GLP-1, are present at very high concentrations within the lymphatic system of rodents; however, the role and functional importance of EEC-lymphatic communication in primates has not been extensively studied.

The complexity of immune cell subtypes within the gut is emerging as an important modifier of EEC function, and EEC-derived hormones in turn are potent regulators of local and systemic inflammatory responses. Multiple immune cell–derived cytokines and bacterial-derived lipopolysaccharides regulate EEC hormone secretion (Figure 2); some EEC hormones in turn act directly on immune cells to amplify or attenuate inflammatory responses. Alternatively, EEC hormones may indirectly modify local and systemic inflammatory responses through control of gut barrier function, reducing bacterial translocation and indirectly decreasing systemic exposure to microbial toxins. For example, cytokines such as IL-6 enhance EEC PGDP synthesis and secretion (16); GLP-1 and CCK secretion is also rapidly increased by lipopolysaccharides; GLP-1 directly attenuates inflammatory responses by engagement of the cannonical GLP-1 receptor expressed in intestinal intraepithelial lymphocytes (17). Similarly, GLP-2, cosecreted together with GLP-1, also attenuates intestinal inflammation by indirectly improving gut barrier function through poorly understood mechanisms (9). Moreover, EEC and ENS peptides—classically ghrelin, pituitary adenylate cyclase activating peptide, somatostatin, and VIP—also exert local immunomodulatory actions

in the gut. Hence, EEC cells and their hormones function as both sensors and effectors of local inflammation and immune responses, modifying experimental inflammation within the GI tract.

Multiple lines of evidence support the importance of the gut microbiota as a critical determinant of the development and regulated function of EECs. Germ-free mice exhibit increased synthesis and secretion of PGDPs including GLP-1 (18), and the presence or absence of microbiota markedly affects the functional maturation of the digestive tract (including EEC populations) in zebrafish. The molecular signals linking deficiency of the microbiota to alterations in EEC density and function are poorly understood and may encompass changes in energy availability, or a switch in the balance of positive vs negative microbial-derived regulators of EEC growth, survival, and function. The microbiota may directly influence EEC function through nutrient fermentation and subsequent generation of short-chain fatty acids and via related metabolites such as bile acids and indole that directly control EEC hormone secretion (14). Manipulation of microbial populations, through administration of prebiotics or probiotics, may also produce potent salutary effects attenuating mucosal inflammation and improving gut barrier function through enhanced secretion of EEC hormones such as GLP-2 (19). Metabolites produced by spore-forming bacteria induce serotonin synthesis and secretion from colonic EECs, increasing circulating serotonin levels with pleiotropic effects on gut motility and systemic hemostasis (20). Whether the beneficial effects of microbial transplantation experiments to reverse dysbiosis in experimental models of obesity, diabetes, cardiovascular disease, and gut inflammation reflect contributions from one or more microbial metabolites that influence levels of EEC-secreted hormones has not been established and requires further interrogation.

Therapeutic Potential and Relevance of the EEC Network

EEC hormones as therapeutic targets

Returning to Bayliss and Starling and the discovery of secretin, the clinical approval of secretin for use as an investigative agent to assess pancreatic exocrine dysfunction, or as an adjunctive agent to facilitate endoscopic retrograde cholangiopancreatography, clearly established the clinical relevance of EEC hormones. More recently, multiple GLP-1 receptor agonists have been approved for the treatment of both type 2 diabetes and obesity (5), oxyntomodulin analogs are being assessed in clinical trials for diabetes and obesity, and a GLP-2 analog has been

approved for the treatment of human subjects with short bowel syndrome (9). Moreover, DPP4 inhibitors, which potentiate the glucoregulatory actions of GIP and GLP-1, are now widely used for the treatment of type 2 diabetes. These therapeutic advances, based on harnessing the pharmacological properties of EEC-derived hormones, have sparked a resurgence of interest in the properties and therapeutic potential of EECs (Figure 2). The recognition that EECs control metabolism through secretion of multiple hormones with complementary mechanisms of action has fostered investigation of the feasibility of combination hormone therapy for the treatment of diabetes and/or obesity. Similarly, clinical observations demonstrate that bariatric surgery is associated with increased circulating levels of multiple PGDPs, PYY, and other satiogenic and glucoregulatory hormones, including bile acids. Hence, there is considerable interest in the development and characterization of gut peptide–based coagonists and triagonists for the therapy of diabetes and obesity (21–23). The complexity and number of potential combinations is considerable, and for the time being it remains difficult to predict precisely which ratios and combinations of peptide epitopes will prove effective and safe in the clinic. The encouraging results obtained with some of these new agents in preclinical studies is tempered by the realization that some gut hormones, including novel molecular entities enhancing GLP-1 action, activate the sympathetic nervous system in rodents, which in turn, indirectly enhances energy expenditure and increases heart rate.

Sympathetic nervous system activation is undesirable for a chronic therapy to be used in humans with obesity, diabetes, or cardiovascular disease; hence, careful scrutiny of the mechanisms underlying preclinical efficacy, coupled with rigorous assessment of the cardiovascular profiles of these agents in humans, is mandatory.

Coincident with elucidation of plurihormonal EEC profiles, and potent metabolic activities embodied within EEC hormones, there is increasing interest in identification of novel EEC secretagogues that might mimic, in part, elevations in circulating levels of multiple gut hormones detected following bariatric surgery. Although the extent to which one or more gut hormones mediate the anorectic and antidiabetic efficacy of bariatric surgery remains controversial, it seems clear that gut hormone therapy exhibits considerable potential for achieving weight loss and glucose control in humans (23). Indeed, the right combination of secretagogues may theoretically be capable of stimulating subsets of EECs to release multiple hormones with therapeutic activity enabling control of glycemia, inflammation, and appetite (Figure 2). Immortalized EEC cell lines and purified populations of murine EECs, as well as HIOs, all represent useful models for screening of chem-ical libraries to identify EEC secretagogue activity. To date, fatty acids including butyrate and propionate, bile acids (BAs), and indole have been shown to enhance PGDP secretion from EECs (14). Nevertheless, the secretory action of some of these agents is complex, given that both indole and bile acids exert both stimulatory and inhibitory effects on EEC-derived GLP-1 secretion, depending on the concentration and exposure (indole), and the selective targeting of specific BA receptor-signaling pathways (TGR5 vs FXR) is challenging. Similarly, the regulation of EEC hormone secretion by nutrients is equally complex, given that food ingestion triggers PGDP secretion yet inhibits the release of insulin-like peptide 5 from colonic L cells. There is also considerable variability in the extent to which ligands for taste receptors stimulate, or fail to regulate GLP-1 secretion, in rodents vs humans, respectively. Moreover, BAs robustly increase GLP-1 secretion via TGR5, yet activate farnesoid X receptor–dependent signaling pathways to inhibit GLP-1 synthesis and secretion from the same cell types. How nutrients and ligands for nutrient sensing GPCRs, BAs, and energy availability alternately stimulate and inhibit hormone secretion from similar EEC populations is not currently understood.

The recognition that the microbial metabolome is an important source of positive and negative regulators of EEC secretory activity provides yet another source of complex compounds for testing. Indeed, it should be feasible to screen microbial metabolomes generated from the gut microbiomes procured from individuals characterized as "robust GLP-1 secretors" or from subjects with the highest levels of GLP-1 after bariatric surgery. The recognition that EECs with overlapping hormonal phenotypes may exhibit different molecular profiles along the stomach and small and large intestine raises important questions about the feasibility of directly translating findings from reductionist EEC screening models to complex human populations with inherent variation in genetic backgrounds. These efforts are likely to be further complicated by the presence of diseases that may modify EEC biology and responsivity. Furthermore, EEC hormone secretion is also regulated by circadian rhythms, providing additional complexity for assessing the efficacy and therapeutic potential of novel secretagogues. Nevertheless, none of these challenges should diminish enthusiasm for scientific approaches to identify EEC secretagogues for the treatment of human disease.

Although considerable effort has been expended in understanding the plasticity of pancreatic endocrine cells, and the mechanisms through which interconversion of islet α- and β-cells occurs, there has been much less enthusiasm in studying the plasticity of the EEC system. Nevertheless, EECs can be coaxed into production and

secretion of nonautonomous hormones such as insulin through genetic manipulation of mice or HIOs, establishing the feasibility of targeting EECs for the production of therapeutic proteins. Recent findings in pancreatectomized patients suggest that EECs may also be capable, through poorly understood mechanisms, of switching on prohormone convertase 2 in a subset of GCG$^+$ EECs, resulting in production of authentic 29 amino acid glucagon. Furthermore, expression of transcription factors through genetic manipulation or viral transduction converts a subset of enterocytes to EECs with the capacity for glucose-regulated insulin secretion. Hence, understanding the mechanisms controlling the generation, proliferation, and plasticity of EEC populations holds therapeutic promise.

Although endocrinologists may logically view obesity, diabetes, and postprandial dyslipidemia as logical disease targets for EEC therapies, it seems reasonable to consider whether EEC hormones may exhibit therapeutic potential elsewhere. The finding that GLP-2 rapidly improves gut barrier function and reduces intestinal permeability has potential therapeutic applicability for reducing local and systemic inflammation (9). Indeed, the leaky gut has been viewed as a culprit in perpetuating inflammation in the setting of food allergy, inflammatory bowel disease, irritable bowel syndrome, cardiovascular disorders, insulin resistance, and nonalcoholic fatty liver disease. Whether GLP-2- or EEC-derived therapies may exhibit therapeutic potential in the context of improving intestinal barrier function has not been adequately explored. Similarly, manipulation of the microbiome and correction of microbial dysbiosis yields exciting therapeutic benefit in a wide range of diseases in preclinical studies. The extent to which microbial transplantation, prebiotics, or probiotics exert some of their beneficial actions through modulation of EEC hormone secretion has not been carefully explored. The exciting findings that some of the metabolic benefits ensuing from bariatric surgery can be recapitulated by transfer of bacteria to germ-free hosts requires further characterization of mechanisms, with attention to putative changes in profiles of EEC hormones that accompany such manipulations.

EEC Neoplasms: A Window of Opportunity

Many endocrinologists continue to first encounter the EEC system and its hormonal complexity through interaction with a patient presenting with watery diarrhea, hypokalemia, increased levels of VIP, and Verner Morrison syndrome; peptic ulcer disease, hypergastrinemia, and Zollinger-Ellinson syndrome; or flushing, diarrhea, excess serotonin, and carcinoid syndrome. Remarkably, although these clinical syndromes and their corresponding paraneoplastic features have historically provided a rich opportunity for hormone discovery, there has been little systemic effort to interrogate the transcriptome of different human gut endocrine tumors, occasionally referred to as "GI-NETs" (gastrointestinal neuroendocrine tumors). Indeed, the World Health Organization classification of EEC tumors seems outdated, incorporating the number of mitotic and Ki67$^+$ cells as dominant features for tumor classification, which has been further modified by the American Joint Committee on Cancer to include a tumor size, lymph node involvement, or distal metastasis subclassification (24). Just as we have encouraged a reconsideration of historical nomenclature for non-neoplastic EECs (Figure 1), the current classification of EEC tumors does not incorporate site and cell type of origin, or modern concepts of molecular pathogenesis. Although efforts to determine the molecular underpinning of pancreatic neuroendocrine tumors have been initiated, to date there is a paucity of meaningful progress in our molecular understanding of EEC tumor pathogenesis that might better inform treatment. Current trials have shown some success using agents that target the somatostatin receptors, vascular endothelial growth factor, and mTOR (everolimus); and additional trials using combinations of agents are underway (24). Whole-exome sequencing of well-differentiated EEC tumors from the small intestine revealed somatic single-nucleotide variants within the *FGFR2, MEN1, HOOK3, EZH2, MLF1, CARD11, VHL, NONO*, and *SMADEEC* genes, and mutations and pathways amenable to therapeutic intervention included SRC, SMAD family genes *AURKA, EGFR, HSP90*, and *PDGFR* (25). Ideally, this type of molecular profiling will become routine, greatly enriching our understanding of the EEC genomic landscape. As EEC cancers have historically been difficult to treat, frequently resistant to chemotherapy regimens, they represent ideal targets for personalized medicine. Rapid advances in whole-exome sequencing have spurred the molecular interrogation of aberrant gene expression and signal transduction pathways in tumor subtypes. Hence, it seems predictable that molecular dissection of EEC tumors arising from different regions of the GI tract at the genomic and proteomic level will enable new opportunities for development of innovative targeted therapeutics, as well as for discovery of new hormones, growth factors, and signaling pathways critical for EEC differentiation, proliferation, and secretion.

Summary

The skin, lungs, and GI tract represent important organs with differentiated functions that also serve as "first

responders" sensing and responding to environmental cues and pathogens. Unique among these organs the gut controls energy intake, digestion, absorption, and assimilation, functions critically regulated at multiple levels by EEC-derived hormones. Furthermore, the GI tract and its EECs sense and respond to pleiotropic microbial and viral signals, activating mechanisms that minimize local and system inflammatory injury. The emerging complexity of EEC subtypes, coupled with the tremendous therapeutic potential of EEC-derived hormones, heralds a golden era in harnessing the secrets of EEC biology for the treatment of human disease. Unlocking the scientific complexity and richness of the EEC and its hormonal repertoire should yield tremendous future dividends for understanding disease pathophysiology and enable the development of new therapies for GI and metabolic disorders.

Acknowledgments

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