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Making sense of gut-brain signals

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Introduction

2011 was the centenary of the Gouldstonian Lectures delivered by Arthur Hurst and published as the *Sensibility of the Alimentary Tract*, in which he reviewed what is now called 'gut–brain signalling'. He accurately observed that painful sensations from the gut were predominantly relayed by spinal afferent neurons, whereas sensations of fullness after a meal were mediated by vagal afferent neurons. His analysis remains valid and has provided a foundation for much subsequent work in elucidating mechanisms of signal transduction in the gut, of signalling mechanisms by vagal and spinal afferent neurons, and of central nervous system (CNS) processing of visceral information in health and disease.

Modern views of gut–brain signalling recognise a hierarchical series of interacting networks activated by vagal or spinal afferent neurons following gastrointestinal stimulation.[1] These involve local reflex mechanisms as well as interactions with networks up to, and including, the prefrontal cortex, insula and anterior cingulate cortex. Considerable progress has been made in elucidating the neuronal mechanisms involving enteric and primary afferent neurons, and modern imaging methods are revealing the CNS systems responding to visceral signals. The latter include nutrients, noxious chemicals, inflammation and mechanical stimuli; in addition, there is a rapidly growing body of evidence to indicate that the gut microbiota also influence CNS function. The mechanisms are important in various conditions, including functional bowel disorders, obesity and feeding disorders, and inflammatory bowel disease. Although links between visceral signals and emotional and cognitive states are recognised, the precise nature of these remains largely speculative.

Gut–brain signalling and food intake

Epithelial-derived signalling molecules provide a key link between the luminal contents of the gut and CNS responses. This is well illustrated by the control of food intake mediated by gut hormones released in response to luminal nutrients. The intestinal hormone cholecystokinin (CCK) was first shown to inhibit food intake over 40 years ago and, more recently, glucagon-like peptide (GLP)-1, peptide YY $(PYY)_{3–36}$ and oxyntomodulin have also emerged as putative gastrointestinal satiety hormones.[2] Conversely, in the interdigestive phase, there is release of the gastric hormone ghrelin, which increases food intake. These peptides are produced by epithelial endocrine cells. In addition, there are lipid amide mediators that are probably produced by many different epithelial cells. These include both satiety factors, such as oleoylethanolamide (OEA), which is generated in response to dietary lipid, and the endocannabinoids (the body's own version of the active factor in cannabis), notably anandamide (AEA) and 2-arachidonyl glycerol (2-AG), which stimulate food intake and are produced during fasting.

Enteroendocrine cells secrete gut hormones at their basolateral membrane in response to a variety of luminal stimuli that might be detected by G protein-coupled receptors (GPCRs) located on the apical microvillus membrane; GPCRs that are selective for bitter compounds, long-chain fatty acids, short-chain fatty acids or sweet compounds have all been characterised (Fig 1). There are also other sensing mechanisms based on changes in enteroendocrine cell metabolism that, together with GPCR stimulation, collectively control intracellular calcium to evoke secretion.

The gastrointestinal signals that influence ingestive behaviour work together with signals derived from other organs, notably leptin from adipocytes and insulin from the endocrine pancreas. These act on brain stem and hypothalamic neural networks and collectively constitute the homeostatic mechanisms controlling food intake. In addition, inputs from taste, smell and food texture, together with memory, cognition, reward and emotional states, contribute hedonic control mechanisms.

Both peptide hormones and lipid mediators can act not only on vagal afferent neurons, but also directly on brain stem and hypothalamic neurons via delivery in the circulation. Overlapping populations of vagal afferent neurons express receptors for satiety factors, including CCK, GLP-1, PYY_{3-36}, OEA and leptin, as well as for orexigenic factors including ghrelin, AEA and 2-AG (Fig 2). Vagal afferent neurons produce two neuropeptide transmitters: cocaine- and amphetamine-regulated transcript peptide (CARTp), which inhibits food intake, and melanin-concentrating hormone (MCH), which stimulates food intake.

The homeostatic mechanisms originating at the level of the gut are providing a rich range of potential therapeutic targets for the treatment of obesity and feeding disorders. Bariatric surgery and lipase inhibitors are well established; nutraceuticals, and pre- and probiotics are currently attracting intense interest in view of the emerging role of the microbiota in obesity. In addition, there is interest in the development of agonists at receptors for satiety factors, including GLP-1 and glucagon co-agonists, and antagonists at receptors of orexigenic factors. Although the cannabinoid CB1 receptor antagonist, rimonabant, was withdrawn from clinical use in the treatment of obesity a few years ago because of unacceptable CNS adverse effects, peripherally restricted CB1 antagonists might yet prove to be useful, either alone or in combination therapy.

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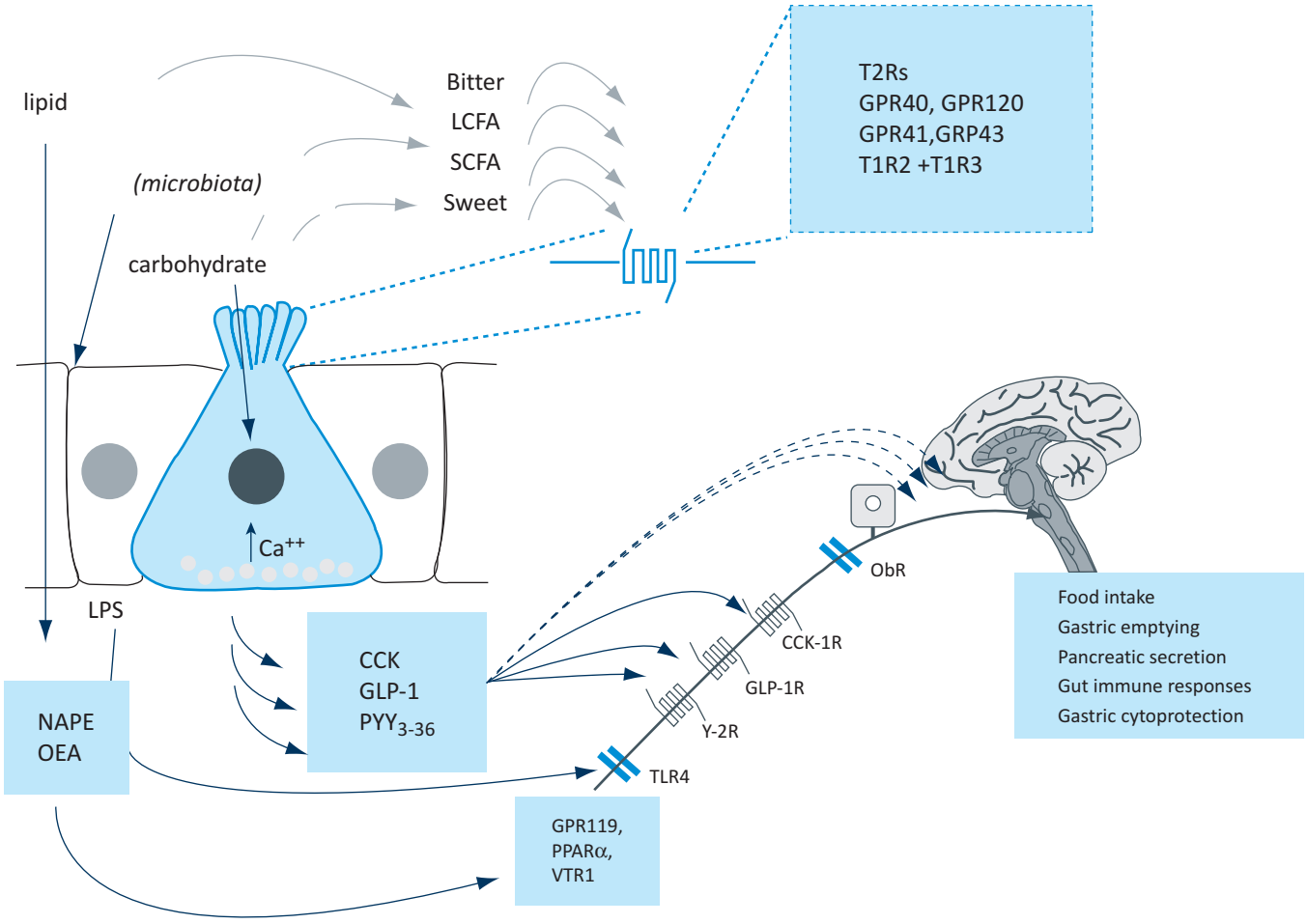

Fig 1. Nutrient sensing and gut–brain signalling. Enteroendocrine cells of the intestine (blue) respond to different dietary components in the lumen via activation of G protein-coupled receptors (GPCRs) including receptors for bitter compounds (T2R), long-chain fatty acids (LCFA; GPR40 and 120), short-chain fatty acid (SCFA; GPR41 and 42) and sweet compounds (T2R2 and T1R3), leading to increased intracellular calcium. Changes in cellular metabolism also increase intracellular calcium and evoke hormone secretion. Cholecystokinin (CCK) released by I-cells, and glucagon-like peptide (GLP)-1 or peptide YY (PYY)$_{3–36}$ released by L-cells, can act at their cognate receptors expressed by vagal afferent neurons; they can also act directly on central nervous system (CNS) neurons via the circulation. Dietary lipid can be converted to N-acyl phosphatidyl ethanolamine (NAPE) or oleoylethanolamide (OEA), which both inhibit food intake. LPS = lipopolysaccharide; ObR = leptin receptor; PPARα = peroxisome proliferator-activated receptor alpha; TLR4 = Toll-like receptor 4; Y2-R = Y2 receptor.

Imaging the human CNS responses to nutrient ingestion

Recent work has illustrated the value of magnetic resonance imaging (MRI) for revealing human CNS responses to ingestion of nutrients in physiological loads. Thus, a lipid test meal of dodecanoic acid (C12) produces a robust increase in the blood oxygen level-dependent (BOLD) signal in brain stem and hypothalamus of normal subjects when instilled directly into the stomach in a dose producing a physiological increase in plasma CCK, stimulation of gall bladder contraction and inhibition of gastric emptying, but not nausea.[3] The CCK1 receptor antagonist dexloxiglumide completely inhibits the increase in CNS BOLD signal consistent with the release of CCK by fatty acid and with evidence from animal studies that CCK stimulates brain stem and hypothalamic neurons as a consequence of activation of a vagal satiety pathway.

Interestingly, the gastric orexigenic factor, ghrelin, also increases the BOLD signal in hypothalamus and brain stem in fasted subjects when infused in doses producing physiological changes in plasma concentration. This has been attributed to direct effects on neurons in these regions. However, the CNS BOLD response to ghrelin is crucially dependent on nutrient status because, in the fed state, or after intragastric C12, ghrelin depresses the BOLD signal in hypothalamus and brain stem.[4] The delivery of C12 by the intragastric route avoids confounding effects resulting from possible influences of ghrelin on hedonic states involving the taste, smell or texture of food. It seems, then, that ghrelin suppresses gut-derived satiety signals, which is compatible with evidence of inhibitory effects on vagal afferent neurons. The latter actions are likely to be particularly relevant in the immediate postprandial period as plasma ghrelin concentrations start to fall while those of CCK increase.

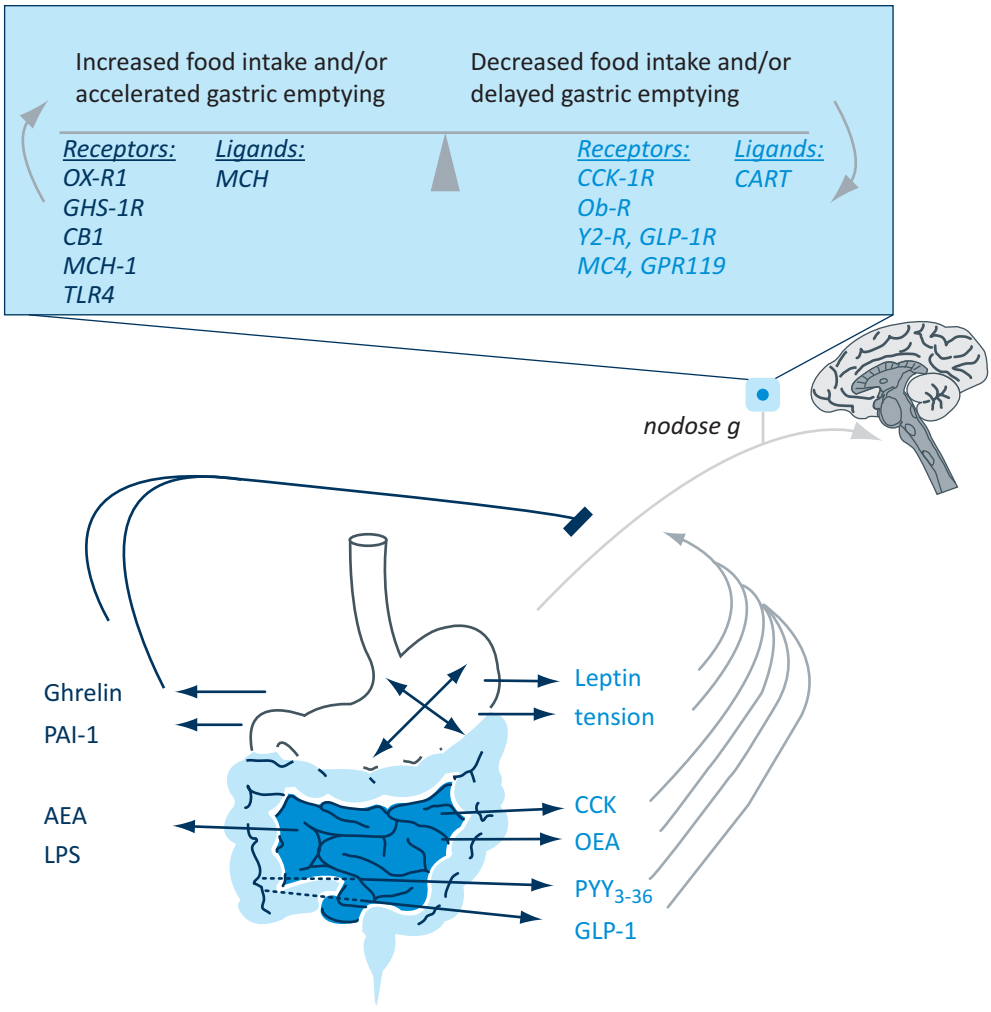

Fig 2. The neurochemical phenotype of vagal afferent neurons. Vagal afferent neurons serving the gastrointestinal tract provide a common afferent pathway for gut-derived signalling molecules associated with inhibition of food intake and/or gastric emptying, such as cholecystokinin (CCK), glucagon-like peptide (GLP)-1, oleoylethanolamide (OEA) or with stimulation of food intake and/or gastric emptying, such as ghrelin and anandamide (AEA). Gut-derived satiety factors stimulate vagal afferent neuron discharge, whereas orexigenic factors inhibit these neurons. The receptors expressed by vagal afferent neurons are shown in the box; some (eg CB1, MCH1 and Y2) exhibit changes in expression with fasting for 6 h or longer, and endogenous or exogenous CCK reverses these changes. The sensitivity of vagal afferent neurons to CCK is increased by leptin and decreased by plasminogen activator inhibitor (PAI)-1 and lipopolysaccharide (LPS). CARTp = cocaine- and amphetamine-regulated transcript peptide; CB1 = cannabinoid receptor type 1; GHS-1R = growth hormone secretagogue receptor 1; GPR119 = G protein-coupled receptor; MC4 = melanocortin 4; MCH = melanin-concentrating hormone; ObR = leptin receptor; OX-R1 = orexin receptor 1; $PYY_{3–36}$ = peptide YY; TLR4 = toll-like receptor 4; Y2-R = Y2 receptor.

Recent work indicates that C12-stimulated CNS responses might also be linked to emotional states. Thus, the change in BOLD signal in response to sad emotion (following musical and visual cues) was attenuated by intragastric C12.[5] Interactions such as this provide a basis for understanding how 'comfort foods' might act. Studies in mice show stress (in the form of social defeat) increases plasma concentrations of active ghrelin, and promotes ghrelin-dependent preferences for dietary fat and stress-induced food-reward behaviour.[6] Thus, anorexigenic and orexigenic signals from the gut together provide links between food and emotional states that are relevant to understanding how homeostatic and hedonic control mechanisms might interact in obesity and feeding disorders.

Plasticity of vagal afferent neurons

Plasticity is a feature of many aspects of gut–brain signalling. These include increased sensitivity (hyperalgesia and allodynia) of spinal afferent neurons in inflammation, and lowered thresholds for painful perceptions of mechanical stimuli in functional bowel disorders in the absence of evident pathology in the gut. Recent work has highlighted examples of nutrient-dependent plasticity in vagal afferent neurons. Specifically, these neurons exhibit changes in receptor and neuropeptide expression depending on food intake over the previous 6–48 h. Whereas some receptors seem to be more or less constitutively expressed over these periods (eg CCK1, leptin receptor (ObR) and growth hormone secretagogue 1 (GHS1)), others exhibit marked changes in expression. Thus, in animal models, fasting for 6 h or longer depresses expression of the Y2 receptor, which responds to the intestinal satiety peptide $PYY_{3–36}$, and increases expression of the CB1 receptor, which responds to the endocannabinoids.[7] Changes in expression of the two receptors exhibit reciprocal time courses. This switch in neurochemistry seems to be regulated by CCK, which is capable of rapidly reversing both the increase in CB1 and the decrease in Y2 receptors (Y2R). Moreover, CCK also regulates expression of the vagal afferent neuropeptide transmitters: in fasting, there is increased expression of the orexigenic peptide MCH, and depressed expression of the satiety factor CARTp. These changes are reversed by refeeding through a CCK-dependent mechanism. In addition, it is now becoming clear that changes in the neurochemical phenotype of these neurons can also be induced over the longer term by high-fat diets possibly involving alterations in the microbiota (see below). These differences in neurochemical

phenotype clearly have implications for the design of physiological and pharmacological studies, and they will also need to be taken into account in the development of therapeutic strategies targeted at this pathway.

Vagal afferent neurons provide a locus for integration of different peripheral satiety signals. It has been known for some time that there is potentiation between leptin and CCK for stimulation of vagal afferent neuron discharge and for inhibition of food intake. In addition, leptin strongly increases the activity of CCK in stimulating expression of CARTp, but has little effect on its own. The action of CCK is dependent on the immediate early gene, early growth response 1 (*EGR1*), which relocates from the cytoplasm to the nucleus in response to CCK.[8] Leptin potentiates this effect of CCK while having little or no effect on nuclear translocation on its own; however, importantly, leptin regulates the abundance of EGR1, whereas CCK has no effect of EGR1 expression *per se*. Thus, leptin determines the capacity of vagal afferent neurons to synthesise CARTp in response to CCK, but alone has little effect; CCK is the primary regulator, but depends on leptin to set the gain in the system. Ghrelin inhibits the effect of both CCK and leptin by excluding transcription factors from the nucleus. Insensitivity to leptin is well known in obesity, and is generally considered to be a CNS phenomenon. However, resistance to leptin is exhibited by vagal afferent neurons before the development of insensitivity in hypothalamic neurons and recent work suggests that the change in vagal function is related to alterations in the gut microbiome.[9]

Emerging roles of the microbiota in gut–brain signalling

The role of the microbiota in gastrointestinal function in health and disease is attracting increasing attention, at least in part as a consequence of the application of metagenomic methods that greatly facilitate characterisation of the microbiome. Interactions between host, diet and the microbiota have been defined that influence many different host systems. In the present context, it should be noted that the microbiota function in both brain to gut, and gut to brain signalling networks. Roles for the microbiota in modulating pain perception that are relevant to inflammatory states, and to hypersensitivity disorders, including post-infectious irritable bowel syndrome (IBS), are well established. In recent years, roles in energy harvesting have been defined, together with evidence that obesity is characterised by functionally important changes in the microbiota. The field is expanding rapidly and, recently, emerging roles in brain development, stress responses and behaviour have also been described.

There are several different ways in which the gut microbiota influences gut–brain signalling that are relevant to the control of appetite and food intake. It is well known that intestinal infection leads to loss of appetite and sensations of distension and bloating. Infection of the small intestine, for example with *Giardia* in humans or with *Trichinella spiralis* infection in mice, leads to elevated plasma CCK concentrations; in the mouse model, there is CCK-cell hyperplasia, loss of body weight and inhibition of food intake, which is reversed by a CCK1-receptor antagonist.[10] Moreover, proinflammatory cytokines, including interleukin (IL)-1β, that are generated during inflammation potentiate CCK effects of vagal afferent neurons.

However, in other conditions, the microbiota can be linked to weight gain. Thus, aside from increased energy harvesting, the production of lipopolysaccharide (LPS) appears to activate pathways leading to leptin resistance in vagal afferent neurons. Raybould has suggested that vagal afferent insensitivity is attributable to LPS acting at Toll-like receptor (TLR)-4 expressed by these neurons to induce suppressor of cytokine signaling 3 (SOCS3) and inhibit the action of leptin in potentiating CCK stimulation.[11] However, there might also be endogenous factors induced during inflammation, including plasminogen activator inhibitor (PAI)-1, that desensitise vagal afferent neurons to CCK and, at least in animal models, lead to hyperphagia and obesity.

Summary

The gastrointestinal tract and brain engage each other in two-way communication that is relevant to a wide range of gastrointestinal disorders, including functional bowel diseases, inflammatory bowel disease, obesity and feeding disorders. The CNS response to ingestion of a physiological load of fatty acid can be studied by MRI. Increased signals in the hypothalamus and brain stem are mediated by CCK and are consistent with animal studies showing that CCK acts on vagal afferent neurons. The latter exhibit nutrient-dependent changes in neurochemical phenotype that are mediated by CCK and that need to be taken into account when manipulating this pathway either experimentally or therapeutically. Leptin potentiates and ghrelin inhibits the action of CCK. Vagal afferent neuron insensitivity to leptin occurs in diet-induced obesity and has been attributable to changes in the microbiota. Progress at present is rapid, and there are multiple emerging targets with therapeutic potential.

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