Microbial endocrinology: host–bacteria communication within the gut microbiome

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Abstract

The human body is home to trillions of micro-organisms, which are increasingly being shown to have significant effects on a variety of disease states. Evidence exists that a bidirectional communication is taking place between us and our microbiome co-habitants, and that this dialogue is capable of influencing our health in a variety of ways. This review considers how host hormonal signals shape the microbiome, and what in return the microbiome residents may be signalling to their hosts.

Introduction

A microbiome may be defined as the collective genomes of the micro-organisms that reside within an environmental niche (Turnbaugh *et al.* 2007). The human microbiome represents an ecological community of commensal, symbiotic and pathogenic micro-organisms (bacteria, fungi, protozoa and viruses) that share the human body space (Turnbaugh *et al.* 2007, Robinson *et al.* 2010). It is estimated that the human microbiome, principally that of the gut, consists of $\sim 10^{13}$–10^{14} micro-organisms, which is more than ten times the number of cells in the human body. The gut microflora benefits their host by protecting against colonisation by pathogens, assisting in intake of nutrients from the diet, metabolising certain drugs to functional forms, and in the absorption and distribution of fat (Sekirov *et al.* 2010). Human microbiome research is leading to an understanding as to how changes in microbiome diversity may be linked to disease states such as diabetes, rheumatoid arthritis, muscular dystrophy, multiple sclerosis, fibromyalgia, and possibly certain cancers (Sekirov *et al.* 2010, Alonso & Guarner 2013).

The obvious question that arises in studying the human microbiome is which host factors shape the resident microflora?

Although it is known that bacterial growth and virulence can be affected by change in host environmental conditions such as temperature, nutrient, and iron availability (Ratledge & Dover 2000), the influence of host hormonal signals on the behaviour of the microflora has now also become apparent. Microbial endocrinology is a microbiology research field that has as its foundation the tenet that, through their long coexistence with animals and plants, micro-organisms have evolved systems for sensing host-associated signals such as hormones. Detecting such signals enables the microbe to recognise that they are within the locality of a suitable host, and that temporally they should initiate expression of genes needed for host colonisation (Lyte 2004, Freestone *et al.* 2008*a, b*, Lyte & Freestone 2009, 2010, Freestone 2013). Owing to the richness of the mammalian gastrointestinal (GI) tract in catecholamine hormones, most microbial

endocrinology studies have focused on interaction of gut bacteria with the fight and flight catecholamines adrenaline, noradrenaline (NE) and dopamine (Freestone 2013; Fig. 1). This focus on the catecholamines and bacteria came about because of the long-held view that stress in humans and animals increases their risk of developing an infection due to stress hormones reducing immune function (Reiche *et al.* 2004, Glaser & Kiecolt-Glaser 2005). Although this review will focus on catecholamine hormone–microbiome interactions, it is important to appreciate that a variety of chemical languages are spoken across the prokaryotic and eukaryotic kingdoms, and that bacteria and fungi can recognise a surprising number of eukaryotic hormones and other signals (reviewed in Freestone (2013)). The dialogue that occurs between microbiome residents and their host, and the relevance to our health of encountering their communication signals will be considered.

Stress and health

Stress is generally described as experiences that are psychologically or physiologically challenging. In animals, stress results in a bi-directional communication between the brain and the peripheral organs and is mediated by a variety of hormones, and neuroactive factors (Goldstein *et al.* 2003, Reiche *et al.* 2004). Perception of stress by the CNS leads to release of stress-associated chemicals, which can directly affect immune function (Goldstein *et al.* 2003, Reiche *et al.* 2004, Glaser & Kiecolt-Glaser 2005). In the infection context, nearly all immune cells possess receptors for catecholamine hormones and neuropeptides, and there is a close connection between nervous and immune systems as sympathetic nerve fibres extensively innervate lymphatic tissue and organ lymph nodes. Exposure to stress hormones has been generally shown to reduce immune effectiveness, particularly protective cell-based immunity (Reiche *et al.* 2004, Glaser & Kiecolt-Glaser 2005).

Structurally, the catecholamine stress hormone family are a group of widely acting effector compounds derived from tyrosine and other dietary amino acid sources. They chemically comprise a benzene ring with two adjacent hydroxyl groups and an opposing amine side chain, which contributes to receptor specificity (Goldstein *et al.* 2003). The synthesis pathway for catecholamines begins with dietary L-dopa, which is enzymatically converted into dopamine, NE and finally adrenaline (Fig. 2). Besides playing endocrinological roles such as controlling cognitive abilities, mood and gut motility, dopamine, NE and
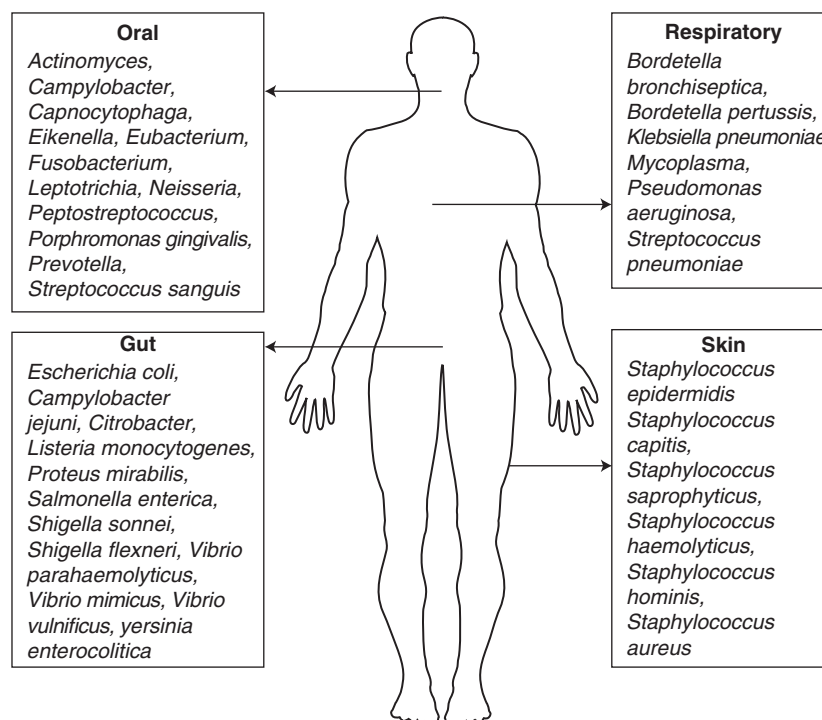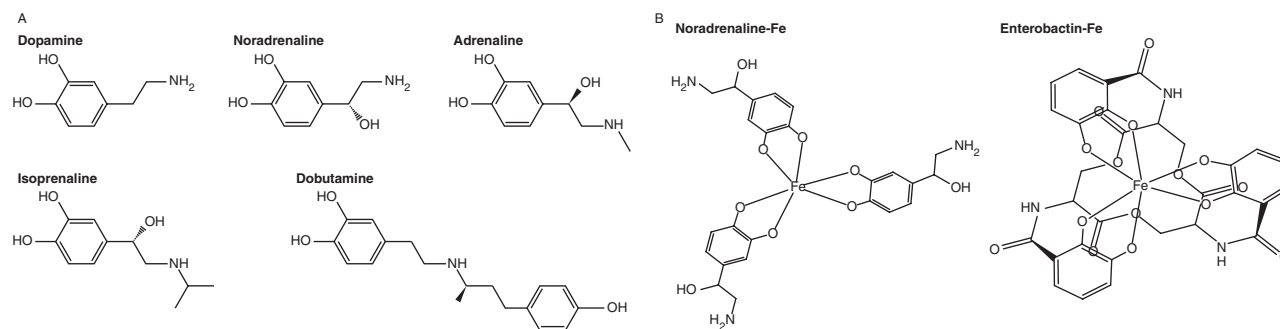

Oral
Actinomyces, Campylobacter, Capnocytophaga, Eikenella, Eubacterium, Fusobacterium, Leptotrichia, Neisseria, Peptostreptococcus, Porphromonas gingivalis, Prevotella, Streptococcus sanguis

Respiratory
Bordetella bronchiseptica, Bordetella pertussis, Klebsiella pneumoniae, Mycoplasma, Pseudomonas aeruginosa, Streptococcus pneumoniae

Gut
Escherichia coli, Campylobacter jejuni, Citrobacter, Listeria monocytogenes, Proteus mirabilis, Salmonella enterica, Shigella sonnei, Shigella flexneri, Vibrio parahaemolyticus, Vibrio mimicus, Vibrio vulnificus, yersinia enterocolitica

Skin
Staphylococcus epidermidis Staphylococcus capitis, Staphylococcus saprophyticus, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus aureus

Figure 1
Microbiome locations of stress hormone-responsive bacteria.

Figure 2

Catecholamine structures and Fe complexes. (A) Chemical structures of the most studied endogenous and therapeutic catecholamines. (B) Shows the chemical structure of the noradrenaline–ferric Fe complex and its similarity to the ferric Fe-chelating siderophore enterobactin.

adrenaline also directly function as neurotransmitters and are utilised in both the CNS and peripheral nervous systems. Noradrenergic and dopaminergic receptors containing nerve terminals are widely distributed within the mammalian body, including the GI tract where they are components of the enteric nervous system (ENS) (Costa *et al.* 2000, Goldstein *et al.* 2003, Furness 2006).

Stress results in release of a variety of potent biological effectors and has led to the view that the increased infections that occur following stress are due to stress hormone reduction in immune effectiveness (Glaser & Kiecolt-Glaser 2005). It was not until the work of Lyte & Ernst (1992) that a direct stress hormone effect on infectious bacteria was demonstrated. A later study (Freestone *et al.* 1999) revealed that recognition of eukaryotic stress hormones was widespread across the prokaryote kingdom. Published reports of bacteria–catecholamine interactions are now many in number (reviewed in Freestone (2013)), and the microbiome locations of these stress hormone-responsive bacteria are shown in Fig. 1.

Stress and the skin microbiome

An average adult human is covered by $\sim 2\,m^2$ of skin (Kong 2011). Bacteria residing on the outer dermal layers can either be transient or resident, living on or within skin folds and crevices. The resident skin microflora is usually non-pathogenic and comprises microbes that are either commensals or mutualistic. Major species include the coagulase-negative staphylococci such as *Staphylococcus epidermidis*, *Streptococcus* spp., *Staphylococcus aureus*, *Bacillus* spp., *Malassezia furfur*, corynebacteria, *Propionibacterium acnes*, *Candida* spp. and occasionally *Mycobacterium* spp. (Grice *et al.* 2009, Kong 2011, Nakatsuji *et al.* 2013). The skin microbiome is characteristically diverse and

microflora niches form because of variability in humidity, temperature or the presence of antimicrobial factors. Although the environmental conditions to which skin is exposed can vary considerably, the composition of the skin microbiomes tends to be relatively stable (Nakatsuji *et al.* 2013), which suggests that the skin through some mechanism actively regulates the microflora that populate it. The human skin is also an important endocrinological organ and produces a variety of systemically acting hormones (Zouboulis 2004, 2009). Cells of the skin also express an array of receptors for a variety of hormones and neurotransmitters (Zouboulis 2009) including the catecholamines. Stress has been known for some time to exacerbate certain skin conditions. One of the most common is acne, which occurs when dead skin cells and sebum released from sebaceous oil glands block hair follicles, leading to bacterial overgrowth and inflammation. *P. acnes*, while part of the normal skin microflora, is thought to be a causative agent of acne, though its exact role is unclear (Zouboulis & Böhm 2004). *P. acnes* utilises sebum as a nutrient source, and sebaceous oil gland cells express receptors for catecholamines (Zouboulis 2009), so stress hormone-induced elevations in sebum levels could increase bacterial numbers and explain the worsening of acne symptoms in stressed acne patients (Zoubouilis & Böhm 2004). Metagenomic profiling of acne lesions shows that several bacterial species are also present along with *P. acne*, with *S. epidermidis* being one of the most prevalent bacterial species (Kong 2011). Interestingly, several studies have demonstrated the staphylococci to be highly catecholamine responsive (Freestone *et al.* 1999, 2008*a*,*b*, Neal *et al.* 2001, Lyte *et al.* 2003), suggesting that hormones released during stress could be directly acting upon acne-associated bacteria.

Although commensal in nature, the skin microflora may on occasion cause severe disease, especially if they stray into normally sterile tissues such as blood. The coagulase-negative staphylococci pose a particular infection risk for intensive care patients because of their ability to form biofilms, particularly within i.v. lines (Lyte *et al.* 2003). Skin staphylococci are highly sensitive to the neuroendocrinological status of their host and to certain of the drugs given to critically ill patients. The stress hormones NE, adrenaline, dopamine and synthetic catecholamine inotropes dobutamine and isoprenaline all increased staphylococcal growth in blood-based media by up to 100 000-fold and catalysed recovery to active growth bacteria that had been severely damaged by antibiotics (Freestone *et al.* 1999, 2008*a,b*, Lyte *et al.* 2003). Formation of a biofilm by bacteria is a highly important aspect of virulence as it confers resistance to attack from antibiotics and immune defences. Catecholamines at the concentrations routinely infused down i.v. lines were all found to massively enhance skin staphylococci biofilm formation when bacteria were seeded onto a catheter plastic polymer. This unexpected inotrope side effect may explain why some normally non-harmful skin bacteria can pose a major clinical problem (Lyte *et al.* 2003).

Host stress is sensed by the oral microflora

The oral cavity is of a more uniform temperature than the skin and is permanently moist, periodically rich in nutrients, and so the microbiome is occupied by a wider variety of more than 700 different species of microbes (Paster *et al.* 2001, Dewhirst *et al.* 2010). Oral bacteria occupy niches on both hard and soft oral tissues, and dental plaque on the teeth margins is especially rich in biofilm-forming bacteria. Species present within the mouth can be both transient (entering from the skin or food) and/or resident. Typical resident oral cavity species include: viridans streptococci, coagulase-negative staphylococci such as *S. epidermidis*, *Streptococcus* spp. e.g. *Streptococcus pneumoniae*, *S. aureus*, *Veillonella* spp., *Fusobacterium* spp., *Treponema* spp., *Porphyromonas* spp., *Prevotella* spp., *Candida* spp., *Haemophilus* spp., *Actinomyces* spp. and *Eikenella corrodens* (Paster *et al.* 2001, Dewhirst *et al.* 2010). It can be observed from Fig. 1 that many of these species are stress hormone responsive.

Dentists recognise psychological stress as a major risk factor for development of oral health problems such as periodontitis, a sub-gingival inflammatory gum condition which accounts for more human tooth loss than dental caries (Iacopino 2009, Akcali *et al.* 2013). In terms of development of periodontitis, normal members of the oral microbiota are implicated to which there has been an inappropriate inflammatory immune reaction, possibly exacerbated by the co-presence of disease-associated bacterial species (Akcali *et al.* 2013). The mechanism(s) whereby stress affects the pathogenesis of periodontal disease is unclear, but catecholamine stress hormones have been detected in saliva and are known to increase during stress (Schachman *et al.* 1995, Mitome *et al.* 1997). A study by Roberts *et al.* (2002, 2005) was the first to investigate stress hormone responsiveness in oral bacteria, which are implicated as being causative or contributory agents of periodontal disease. Of the bacteria tested, around half of the species exhibited significant catecholamine-induced growth enhancement or inhibition, indicating that stress hormones released into the oral cavity might directly and differentially modulate the growth and composition of the sub-gingival microbiome. A later investigation by Graziano *et al.* (2014) found that stress hormone exposure had no effect on the growth of the periodontal pathogen *Porphyromonas gingivalis,* but did increase its virulence by enhancing expression of genes related to haemolytic activity, oxidative stress and iron acquisition. Collectively, the Roberts and Graziano studies suggest that enhancement of oral microbe growth and virulence by stress-released catecholamines may be a contributory factor in development of periodontal disease.

Host stress changes the behaviour of the gut microbiome

The GI tract hosts the most highly diverse microbial community of the human body. It is estimated that the gut microbiome comprises several thousand species of bacteria, archaea, eukarya, and viruses estimated numerically to be ∼100 trillion cells (Ley *et al.* 2006, Turnbaugh *et al.* 2007, Robinson *et al.* 2010, Sekirov *et al.* 2010). The gut microbiome is a highly complex ecosystem in which many different species of microbes compete and cooperate with one another and with also the cells of their host in order for all to survive. Environmental factors such as diet, surgery and antibiotics can all affect the diversity of microbes present, and changes in the composition of the gut microflora have been implicated in a wide variety of human disease states from diabetes to depression (Alonso & Guarner 2013). In fact, the gut microbiome is now considered by some as a virtual organ in its own right (Evans *et al.* 2013), because the gut microflora produce an array of bioactive molecules that directly interact with the

endocrine, nervous and immune systems of their host, though the functional significance of this activity is not as yet fully understood.

In terms of the relevance of microbial endocrinology in understanding the role of host hormones in gut health and disease, it should be appreciated that the GI tract is highly enervated by the ENS that has close connections to the CNS (Furness 2006). Within the ENS, NE is released from storage within sympathetic nerve fibres within the pre-vertebral ganglia innervating the gut mucosa (approximately half of the NE made within the mammalian body is produced within the gut). Dopamine is synthesised in non-sympathetic enteric neurons located within the intestinal wall (Eisenhofer *et al.* 1997, Costa *et al.* 2000, Goldstein *et al.* 2003, Furness 2006). However, neurons containing phenylethanolamine *N*-methyltransferase, the enzyme required for the synthesis of adrenaline from NE, are not expressed in the intestinal mucosa (Furness 2006), making it unlikely that adrenaline would normally be present at any significant level. In addition to the ENS contributions to the presence of catecholamines within the gut, evidence is increasing that the endogenous microflora has the ability to also add to the levels of catecholamines. Butyrate is synthesised by colonic bacteria and has been shown to enhance transcription of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (Patel *et al.* 2005). *Bacillus* spp., *Proteus vulgaris*, *Serratia marcescens*, and *S. aureus* can directly synthesise catecholamines that are exact analogues of the mammalian hormones (Tsavkelova *et al.* 2006). Asano *et al.* (2012) found that commensal gut bacteria express β-glucuronidase enzymes, which are able to generate free NE and dopamine via the cleavage of their pharmacologically inactive conjugated forms. These workers also showed that GI tract catecholamines can be isolated from the gut lumen, and that levels of NE and dopamine increased when there was a bacterial presence. Interestingly, compared with the colonic levels of NE and dopamine (48 ± 7 and 132 ± 19 ng/g luminal contents respectively), barely detectable levels of adrenaline were found within the gut. It is not currently possible to state the magnitude of the microbial contribution to gut catecholamine levels, as it is still unclear what constitutes a normal NE or dopamine luminal load. However, it is clear that the presence of bacterially derived NE and dopamine combined with contributions from dietary and ENS sources indicates that the gut is a catecholamine-rich environment, and suggests that the microbes that inhabit it would be accustomed to the presence of catecholamines, and thus have a cause to develop sensors for their detection.

Several animal studies have demonstrated that the psychological and physical stress of a host can markedly affect its gut microflora. Psychologically stressing mice in the form of a social conflict were found to enhance the growth of gut bacteria present *in vivo* (Dréau *et al.* 1999). Physical stress caused by a short-term period of starvation significantly increased the numbers of *E. coli* adhering to the caecal mucosa of the stressed mice compared with the non-hungry controls (Alverdy *et al.* 2000). Meddings & Swain (2000) showed that restraint stress in mice resulted in increased intestinal permeability to bacteria, which was associated with an increase in corticosterone stress hormone levels. Bailey *et al.* (2006, 2011) found that subjecting mice to a psychological stressor significantly altered their gut microflora diversity as well as increasing translocation of gut commensals to the mesenteric lymph nodes. Bailey *et al.* (2010) also demonstrated that stress could alter the mouse gut microbiome diversity to such an extent that it aided an invading enteric pathogen (*Citrobacter rodentium*) to establish an infection. Spill-over of catecholamines from the systemic circulation into the GI tract has been shown to occur during stress that is distant from the gut, and increased release of catecholamines by the gut nerves during non-gut stress has also been shown (Aneman *et al.* 1996, Lyte & Bailey 1997), suggesting that bacterial responses to stress-released chemicals may be the source of these gut microbiome changes.

How could the stress of the host cause such dramatic changes in the behaviour of its gut microflora? Stress via the sympathetic nervous system can modulate levels of gastric acid, reduce gastric motility and stimulate defecation (Lenz *et al.* 1988), which could by changing local physical parameters affect the resident gut microbes. It is also possible that via their ability to sense stress hormones, the gut microbes are directly responding to the stress experienced by their host. A study from Lyte & Bailey (1997) investigated the effect of an acute stress on the diversity of the gut microbiome using the selective neurotoxin 6-hydroxydopamine (6-OHDA), which selectively ablates the nerve terminals of sympathetic neurons and causes a rapid but short-term release of stored NE into the systemic circulation. Following the toxin administration to mice, analysis of the animal's gut microbiome 24 h later showed that the acute stress had significantly changed the profile of the microflora. The 6-OHDA-treated mice showed more than a 1000-fold increase in numbers of Gram negative bacteria such as *E. coli*, over controls.

Even more striking was that the host stress stimulated both attachment of the microflora to the gut wall and their translocation to the mesenteric lymph nodes, which are events preceding development of a gut-associated systemic infection. Within 2 weeks, the time typically required for regeneration of the nerves ablated by the neurotoxin, the gut microbiome composition had returned to normal. Related to this is the finding by Freestone *et al.* (2002) that intestinal *E. coli* isolates respond to NE, dopamine and their metabolites, with growth increases of up to five logs over controls. An acute stress study involving *Salmonella* found that 6-OHDA treatment of pigs pre-colonised with *Salmonella enterica* increased plasma NE levels and significantly enhanced faecal excretion of the pathogen (Pullinger *et al.* 2010*a*,*b*). Collectively, although the Lyte and Bailey and Pullinger studies did not determine whether there were any direct changes in gut catecholamine levels following acute stress, they did reveal that increases in stress hormone release at sites distant from the gut can markedly affect the behaviour of the enteric microflora.

There is evidence that bacteria within the gut microbiome have evolved specificity in their recognition of host hormones. Adrenaline is not produced within the gut, and gut microbes are most likely to encounter NE and dopamine, the catecholamines utilised by the ENS (Costa *et al.* 2000, Goldstein *et al.* 2003, Furness 2006). Investigation of the catecholamine specificity of several gut bacteria (*E. coli*, *Salmonella* and *Yersinia enterocolitica*) found a significantly greater preference for NE and dopamine over adrenaline. Indeed, in the case of *Y. enterocolitica,* which tends not to colonise extra-intestinal sites, analysis of 11 strains revealed that there was no responsiveness at all to adrenaline, which even competitively antagonised *Y. enterocolitica* responses to NE and dopamine. This suggests that bacteria have evolved sensory systems that are specific for the hormone they will encounter within their host niche. In terms of what in bacteria may be recognising the catecholamines, there is no bacterial evidence for eukaryotic-like adrenergic and dopaminergic receptors (Freestone 2013). However, for *E. coli* O157:H7, it has been reported that NE and adrenaline can bind to the two-component regulator sensor kinase QseC, leading to the proposal that this was the bacterial receptor for these catecholamines (Clarke *et al.* 2006). A later work by Pullinger *et al.* (2010*a*,*b*) found that it was possible to delete QseC without affecting NE and adrenaline responsiveness. In addition to adrenergic catecholamines, QseC has been shown to respond to a microbial signal (termed AI-3) whose synthesis is thought to be associated with LuxS, a key enzyme in the AI-2 quorum sensing signal synthesis pathway (Waters & Bassler 2005, Clarke *et al.* 2006, Walters & Sperandio 2006). However, despite more than 10 years passing since its announcement, the structure of AI-3 has yet to be revealed (Sperandio *et al.* 2003). Interestingly, a recent report from Karavolos *et al.* (2013) has found that a catechol-containing compound, 2,3-dihydroxybenzoylserine could activate the AI-3 reporter, suggesting that this may be the compound motif recognised by QseC. Moreover, Haigh *et al.* (2013) found that deleting LuxS, whose presence contributes to AI-3 production, did not reduce the ability of *E. coli* to respond to NE or adrenaline. Collectively, these studies suggest that bacterial system(s) for the recognition of catecholamines exist, which are additional to QseC and which do not involve factor(s) whose synthesis is dependent on LuxS.

Catecholamines within the gut may also shape the genetic composition of the microbiome bacteria. Peterson *et al.* (2011) found *in vitro* that NE increased the interspecies transfer efficiencies of a conjugative plasmid from *Salmonella typhimurium* to an *E. coli* recipient. Enhancing the rate of local genetic exchange could speed adaptation of the gut microflora to the gut environment.

For possibly food safety reasons, most studies of stress hormone responsive bacteria have focused on gut-associated pathogens such as *E. coli* (Lyte & Ernst 1992, Lyte *et al.* 1997, Freestone *et al.* 1999, 2000, 2002, Vlisidou *et al.* 2004, Dowd 2007, Toscano *et al.* 2007), *Salmonella* (Freestone *et al.* 1999, Pullinger *et al.* 2010*a*,*b*), *Helicobacter* (Doherty *et al.* 2009), *Listeria* (Coulanges *et al.* 1997, Freestone *et al.* 1999), *Campylobacter* (Cogan *et al.* 2007), and *Yersinia* (Freestone *et al.* 1999, 2007*a*,*b*; Fig. 1). However, catecholamines are widely utilised within all the organs and tissues of the mammalian body (Goldstein *et al.* 2003), indicating that it is likely that most microbiome residents will at some point come into contact with their host's catecholamines, and thus have a cause to evolve sensory systems for their detection.

Stress hormone effects on gut bacteria growth and virulence

Given the Lyte & Bailey (1997) finding that NE spill-over into the gut caused the microflora to over grow, the question that arises is the nature of the molecular mechanism by which NE (and other catecholamine) stress hormones induced such bacterial increases. Most investigations of catecholamine growth effects have been conducted *in vitro* using serum- or blood-containing

culture media to more closely reflect the host environment in which the microbe will encounter the catecholamine (Freestone *et al.* 2008*a*,*b*, Freestone 2013). Blood and serum are bacteriostatic through sequestration of available Fe by the high-affinity ferric iron-binding protein transferrin (Lambert *et al.* 2005). As iron is so essential for the *in vivo* growth of bacterial pathogens, its limitation within the mammalian body represents an important innate immune defence (Ratledge & Dover 2000). In terms of the mechanism of catecholamine of growth stimulation, it has been demonstrated that NE, dopamine and adrenaline can function as a kind of enterobactin-like pseudo-siderophore (Freestone *et al.* 2003, Sandrini *et al.* 2010; Fig. 2B). The ability of the stress hormone to complex Fe enables bacteria to use the catecholamine to acquire the normally unavailable Fe within transferrin and lactoferrin and use it for growth (Freestone *et al.* 2000, 2002, 2003, Sandrini *et al.* 2010, 2013). Sandrini *et al.* (2010) showed that catecholamines can complex with the ferric Fe within transferrin (and also the structurally related mucosal iron-binding protein lactoferrin), resulting in the reduction of the bound Fe(III) to Fe(II), an iron valency for which there is a much reduced affinity (Lambert *et al.* 2005). The reduction of the ferric Fe results in iron release, which can then be taken up by bacteria by either ferric (siderophore based) or ferrous iron uptake systems (Freestone *et al.* 2003, Sandrini *et al.* 2010). This bacterial iron theft is relevant to the infectious disease process, as catecholamine-released iron enables fewer than 100 bacteria in <24 h to increase their cell numbers by more than 100 000-fold (Freestone *et al.* 2000, 2002, 2003, Neal *et al.* 2001, Sandrini *et al.* 2010, 2013). For gut bacteria such as *E. coli* and *Salmonella,* exposure to catecholamines also induces synthesis of a novel autoinducer of growth (AI) (Lyte *et al.* 1996, Freestone *et al.* 1999). The AI is produced only after a few hours of exposure to any of the catecholamines and stimulates growth by a mechanism independent of transferrin (Freestone *et al.* 2003). The AI is rapidly acting in its growth enhancement and resuscitative effects and is recognised by a wide range of gut bacterial species (Freestone *et al.* 1999, Reissbrodt *et al.* 2002). Thus, the effects of the stress hormone exposure could be manifest for some time after gut stress hormones have returned to normal.

A recent work by Sandrini *et al.* (2013) demonstrated that, for *E. coli* and *Salmonella,* binding of transferrin is via the outer membrane porin proteins OmpA and OmpC and is integral to the mechanism by which catecholamines can remove and deliver iron from transferrin. Porin anchoring of the transferrin brings it close to the bacterial cell surface, which enables improved uptake of the transferrin-Fe released by the catecholamine. Importantly, the porins were also found to be the routes by which catecholamine–Fe complexes, and the catecholamines NE and dopamine enter the Gram-negative bacterial cell (Sandrini *et al.* 2013).
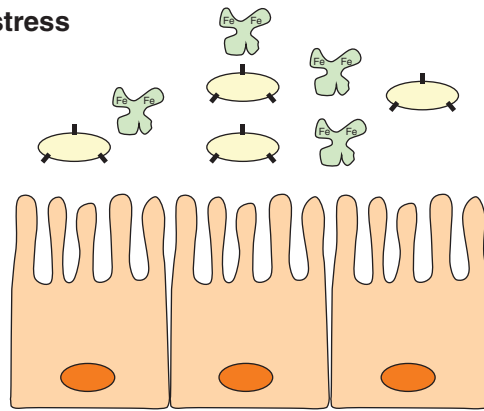
The combined presence within the GI tract of bacteria, lactoferrin and transferrin may explain why increases in NE levels that spill over into the gut during acute stress can catalyse potentially dangerous changes in the behaviour of the gut microbiome (Lyte & Bailey 1997). It is therefore not surprising that the mammalian evolutionary response to curb a stress-related overgrowth of the gut microflora appears to involve the control of gut catecholamine levels. Harris *et al.* (2000) showed that mammals express an array of catecholamine-degrading enzymes throughout the length of the GI tract, particularly in the colon where the gut microbiome is most populous.

In addition to stimulating bacterial growth, catecholamines can also enhance expression of genes required for virulence. NE increased expression of Shiga toxins produced by *E. coli* O157:H7 (Lyte *et al.* 1996). A number of *in vitro* reports have shown that stress hormones can enhance bacterial attachment to host gut tissues. Vlisidou *et al.* (2004) found that NE increased the intestinal mucosa adherence and enteropathogenicity of *E. coli* O157:H7. Green *et al.* (2003) and Chen *et al.* (2003, 2006) also demonstrated that catecholamines increase the attachment and invasiveness of *E. coli* and *Salmonella* to mammalian gut tissues. NE was also found to increase the virulence gene expression of several pathogenic *Vibrio* species (Nakano *et al.* 2007).
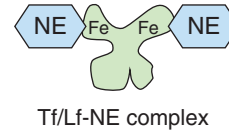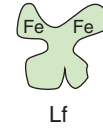
Catecholamine levels *in vivo*

Most microbial endocrinology studies, including those involving animal infection models, have typically utilised catecholamine levels in the 50–2000 µM range (Freestone *et al.* 2008*a*,*b*). In terms of determination of the *in vivo* levels of catecholamines, some tissues have posed considerable technical challenges. For the gut, variations in food catecholamine content, rapid enzymatic turnover of gut catecholamines, and the adsorbent nature of faecal matter have all posed technical barriers to a definitive statement of active catecholamine levels (Pullinger *et al.* 2010*a*,*b*). The study of Asano *et al.* (2012) determined that there was a measurable catecholamine content within the gut lumen of mice, although it was not clear whether the compounds isolated were representative of the total catecholamine presence. In terms of measurement of
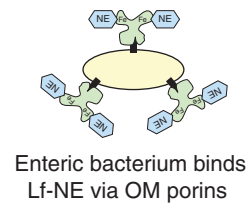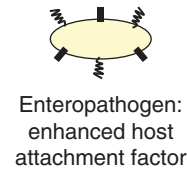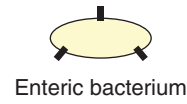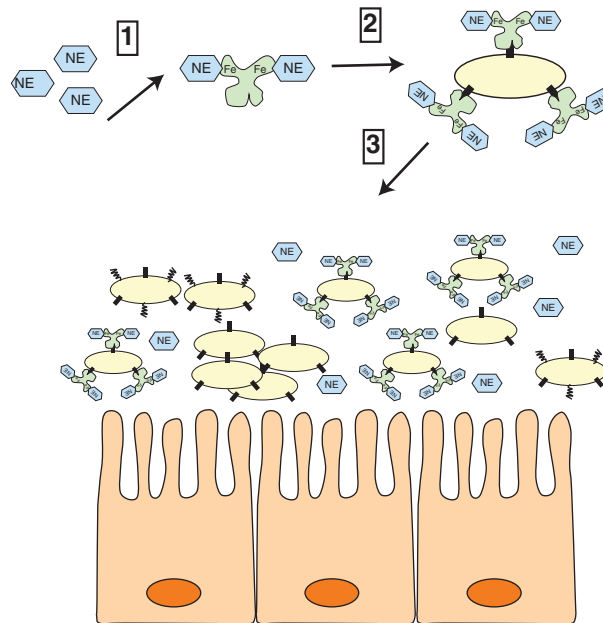
Non-stress

NE
Noradrenaline

Lf

Tf/Lf-NE complex

Acute-stress

1

2

3

Enteric bacterium

Enteropathogen:
enhanced host
attachment factor

Enteric bacterium binds
Lf-NE via OM porins

AI
Noradrenaline
Induced AI

Post-stress

i

ii

iii

iv

Mesenteric
lymph nodes
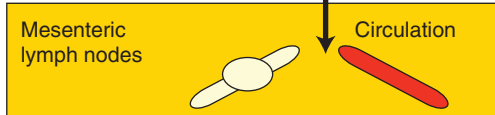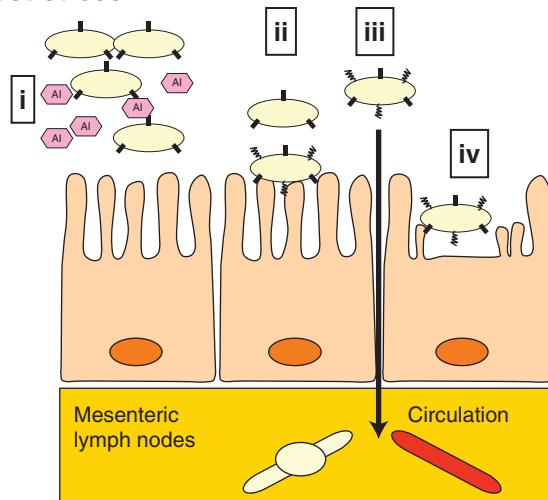
Circulation

plasma catecholamines, data are much more abundant, and human circulatory levels of dopamine, NE and adrenaline are typically in the nM range (Goldstein *et al.* 2003). However, plasma catecholamine levels can increase by several log orders following surgery or cardiac inotrope administration. Thompson *et al.* (1999) demonstrated that in dopamine-medicated cardiac surgery patients, plasma NE levels rose to as high as 9.24 μM. Work from Freestone *et al.* (2012) found that levels of NE and dopamine in this range and lower (5 μM) markedly increased *Pseudomonas aeruginosa* growth, biofilm formation and attachment to human ciliated epithelia. A later work by Sandrini *et al.* (2014) demonstrated that 5 μM dopamine and NE were stimulatory to the growth of *S. pneumoniae*, enhancing biofilm formation and expression of genes involved in metabolism and virulence. Interestingly, acutely ill ventilated patients are at significant risk of developing ventilator-associated pneumonia from bacteria such as *P. aeruginosa* and *S. pneumoniae* (Freestone *et al.* 2012), and catecholamine solutions are occasionally directly administered to the airway to reduce inflammation (Stannard & O'Callaghan 2002).

In addition to catecholamines, acute stress also results in glucocorticoid stress hormone release by the adrenal glands (Reiche *et al.* 2004), which is important in the infection context as exposure to adrenocorticotropic hormone significantly enhanced attachment of *E. coli* O157:H7 to gut mucosa (Schreiber & Brown 2005, Brown & Price 2008). Verbrugghe *et al.* (2011) found that social stressing pigs resulted in elevated serum cortisol levels, and that the cortisol released increased intracellular growth of *Salmonella* within porcine alveolar macrophages. Dynorphin is an opioid released during stress into the gut; Zaborina *et al.* (2007) showed that dynorphin enhanced *P. aeruginosa* virulence by activation of the quorum sensing quinolone signalling system. Figure 3 summarises in cartoon form the effect that acute host stress can have on the gut microbiome. The figure shows that psychological stress can result in overgrowth of gut microbes through stress hormone-mediated release of transferrin and lactoferrin iron. Overgrowth of commensals can inadvertently lead to their translocation to the mesenteric lymph nodes, resulting in possibly wider dissemination. In the case of pathogens, stress hormone contact results in increased attachment to host cells resulting in epithelial tissue damage and cell invasion.

Messages to the host (from some) members of the gut microbiome

This review has thus far considered the relevance to health of bacterial sensing of host signalling molecules. However, the gut and other microbiome bacteria 'speak' to one another using a variety of chemical languages, which, in some cases, their host cells can also sense (Waters & Bassler 2005). The homoserine lactone family of bacterial communication molecules are the most intensely studied, as they are produced during infection and are known to interact with the mammalian immune system. Telford *et al.* (1998) found that the *Pseudomonas* N-3-oxododecanoyl homoserine lactone (3-oxoC12HL) inhibited lymphocyte proliferation and downregulated production of the protective cytokines TNFα and IL12. Tateda *et al.*

Figure 3

Host stress effects on the gut microbiome. Non-stress: lactoferrin (Lf) and transferrin (Tf) maintain Fe limitation in mucosal secretions, which are therefore bacteriostatic. The number of gut microbes including pathogens during this time of calm is therefore within normal parameters. Acute stress: enteric nervous system activity while experiencing the acute stressor results in the release/spill-over of catecholamines within the gut (noradrenaline (NE) and dopamine). Work from *in vitro* and *in vivo* reports demonstrates that the encounter of the gut microflora, especially pathogens, could result in two major events. The first (1) is that NE acts as a cue to cause pathogens to induce virulence factor expression – such as production of host attachment factors such as adhesins. The second (2) is that the catecholamine interacts with the lactoferrin (and any transferrin that might also be present), converting a normally bacteriostatic set of Fe-chelating proteins into a useful nutritional iron source and providing support for increased bacterial growth in the gut (Freestone *et al.* 2000, Sandrini *et al.* 2010, 2013). Post-stress: several hours after the acute stress has passed and the levels of catecholamines in the gut may have returned to normal; however, stress-related events are still occurring. (i) For gut bacteria, such as *Escherichia coli*, only a short 4 h exposure to catecholamines is sufficient to induce synthesis of a novel autoinducer of growth (AI) (Lyte *et al.* 1996, Freestone *et al.* 1999). The AI is very wide acting in its effects and can stimulate growth of a wide range of gut bacterial species. In the case of pathogens such as *E. coli* O157:H7, the AI also enhances shiga toxin expression (Lyte *et al.* 1996). (ii) *In vivo* studies have demonstrated that acute host stress can increase microflora numbers and attachment to gut epithelia (Lyte & Bailey 1997). *In vitro* studies have demonstrated that catecholamines can increase attachment of enteric pathogens to gut mucosa (Chen *et al.* 2003, 2006, Green *et al.* 2003, Vlisidou *et al.* 2004). (iii) The increasing numbers of pathogens, and possibly also gut microbiome bacteria (Lyte & Bailey 1997, Bailey *et al.* 2006, 2010), could affect gut integrity leading to bacterial translocation either to the mesenteric lymphatic tissue or, in a worst-case scenario, directly into the systemic circulation, where even commensal bacteria could lead to sepsis and multiple organ failure. (iv) Noradrenaline exposure of enteropathogenic and enterohaemorrhagic *Escherichia coli* can also lead to attaching and effacing lesions and destruction of gut tissue. (Adapted, with permission, from Freestone PPE, Sandrini SM, Haigh RD & Lyte M 2008*b* Microbial endocrinology: how stress influences susceptibility to infection. *Trends in Microbiology* **16** 55–64).

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(2003) found that 3-oxoC12HL rapidly induced apoptosis of macrophages and neutrophils. A clinical study by Boontham et al. (2008) investigated whether homoserine lactones influenced the pathophysiology of patients suffering from severe sepsis. *In vitro* studies demonstrated that 3-oxoC12HL inhibited protective pro-inflammatory cytokine expression and T cell activation, and directly induced apoptosis in dendritic and CD4+ T cells. What was most striking about this study was that a positive correlation appeared to exist in the sepsis patients among homoserine lactone leakage from the gut into the circulation, immune cell impairment and patient mortality (Boontham et al. (2008). Collectively, these studies suggest that bacterial homoserine lactone signals convey a false and detrimental message to their host, instructing it to turn off immune defences, which would favour survival of the infecting bacteria.

The gut microbiome can modulate the mood of its host

Establishment of an appropriate gut microflora is one of the most important events in the early life of a human, as evidence is growing that the gut microbiome influences brain functioning (Adlerberth & Wold 2009, Collins et al. 2012). In this respect, it is known that the brain and the gut are closely connected to form a bidirectional neuro-humoral communication system, collectively termed the gut–brain axis. The gut–brain axis comprises the CNS and autonomic nervous systems, the neuroendocrine and immune systems, ENS and the enteric microflora (Cryan & O'Mahony 2011). Within the axis, the vagus nerve plays a central signalling role as it connects the 100 million neurons of the ENS to the brain. It is well understood that via the axis that the brain can regulate gut activity (Cryan & O'Mahony 2011), but other works have focused on the reverse pathway and is indicating that the gut microbes can influence the brain. An investigation by Lyte et al. (2006) using mice demonstrated that the brain responds within hours to the introduction of a pathogen (*C. rodentium*) into the gut, long before manifestation of any infection-related symptoms. This pathogen to brain signalling, which appeared to be mediated by neurons within the vagus nerve, manifested itself in the mice as a display of significantly more anxiety-like behaviour. Colonisation of the gut by the pathogen *Campylobacter jejuni* also induced early (pre-infection symptom) anxiety in mice (Goehler et al. 2005, 2008). In humans, administration of bacterial lipopolysaccharide induces significant anxiety feelings soon after treatment (Reichenberg et al.

2001). In addition to the presence of a bacterial pathogen inducing anxious feeling in its host, the absence of a gut microbiome can also negatively affect mood and behaviour. Crumeyrolle-Arias et al. (2014) examined the general behaviour and response to stress of germ-free rats vs normal microbiome-colonised rats. The absence of an established gut microflora resulted in striking behavioural changes in the rats: germ-free animals spent less time in social interaction activities, were more challenge averse and spent more time in latent behaviours such as non-movement or crouching in corners. In response to stress, the serum cortisol levels of the germ-free rats were nearly three times higher than in microbiome-colonised animals. Consistent with the behavioural findings, the germ-free rats also had a lower dopaminergic turnover rate in the parts of the brain known to control reactivity to stress and anxiety-like behaviour.

Besides inducing anxiety, there is some evidence that the gut microflora can reduce the endocrine elements of stress and thus create a more positive mood in their host. Messaoudi et al. (2011) found that administration of a probiotic formulation containing *Lactobacillus helveticus* and *Bifidobacterium longum* relieved symptoms of psychological distress in both humans and rats. Administration of *Lactobacillus rhamnosus* to mice reduced their stress-induced corticosterone levels and also made them more energetic when giving a swimming challenge (Bravo et al. 2011). The probiotic-related improvement of mood disappeared when the mice vagus nerve was severed, suggesting that the effect was of microbial origin and was being communicated along the gut–brain axis signalling pathway. More recent work from Tillisch et al. (2013) has demonstrated that, in human volunteers, the consumption of a fermented milk product supplemented with a probiotic directly changed the activity of several brain areas known to be involved in sensory perception and emotion.

The gut microbiome can modulate its host's appetite

There is growing evidence for a connection between host food desire and the behaviour of its gut microbiome. Norris et al. (2013) was one of the first to propose that gut microflora could affect the appetite of its host. The ability of the gut microbiome to induce feelings of anxiety in its host could explain effects on appetite; however, the emerging picture is one of greater molecular complexity. An investigation in which mice were chronically infected with *Helicobacter pylori* found that the infected animals

displayed changes in feeding behaviour that continued long after eradication of the pathogen and complete resolution of any infection-related changes in the animal's gastric physiology. The persistent reduction in food desire alteration was thought to be due to infection-related changes in levels of the appetite-regulating peptide pro-opiomelanocortin (Bercik *et al.* 2009). In humans, eating disorders such as anorexia and bulimia affect an estimated 5% of women and 2% of men (Tennoune *et al.* 2014). Psycho-social causes have thus far been considered the main explanations although very recently bacteria have been found in the human gut, which apparently stop the body from regulating its appetite. The alpha-melanocyte-stimulating hormone (α-MSH) is involved in control of feeding and emotion (Fan *et al.* 1997). Tennoune *et al.* (2014) identified the commensal *E. coli* ClpB heat-shock chaperone protein as a conformational antigen mimetic of α-MSH, and demonstrated that ClpB-immunised mice produced an anti-ClpB IgG, which was cross-reactive with α-MSH. Intragastric inoculation of ClpB-expressing *E. coli* in mice decreased their food intake and stimulated formation of ClpB- and α-MSH-reactive antibodies, while animals colonised with ClpB-deficient *E. coli* retained a normal appetite. Extending the study to human eating disorder patients revealed that the plasma levels of anti-ClpB IgG cross-reactive with α-MSH were increased. The authors suggest that there is a link between ClpB-expressing bacteria (of which there are many within the gut microbiome) and host regulation of feeding and emotion via inadvertent production of anti-ClpB antibodies that cross-react with α-MSH, depleting internal levels of the appetite-stimulating hormone and thus contributing to the development or continuation of the eating disorder. If a causal connection is proven, then there is potential for treating eating disorders via the use of selective antibiotics to control the numbers of ClpB-expressing gut microflora. Conversely, in the case of obese patients, perhaps encouraging the numbers of appetite-suppressing ClpB-positive species, possibly as a probiotic formulation, might help to improve weight loss.

Conclusion

Microbial Endocrinology can provide a useful conceptual framework on which to develop a holistic understanding of the factors that shape the interactions between microbiome residents and their host during health and disease. It is clear from animal studies that residents within the gut microbiome can sense the emotional status (psychological stress in particular) of their host. It is also equally clear that the gut microflora can influence the emotional state of their host, even to the point of causing stress and anxiety. Understanding the content of the host–microbe dialogue is necessary to appreciate the contributions of the endogenous microbial microflora to human physiology, and also possibly to understand how much the microbiome is influencing our behaviour. A deeper understanding of the intimate and inter-dependent relationship between the gut microbiome and their human host could also open up possibilities for novel microbial-based therapies in the treatment of non-infection-related conditions such as mood and eating disorders.

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