Microbial endocrinology: how stress influences susceptibility to infection

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Abstract

The need to take a more holistic approach in understanding the mechanisms by which stress influences the pathogenesis of infectious disease has resulted in the development of the field of microbial endocrinology. This trans-disciplinary field represents the intersection of microbiology with mammalian endocrinology and neurophysiology, and it has as its basis the tenet that microorganisms have evolved systems for utilizing the neurohormones, that are widely distributed throughout nature, as environmental cues to initiate growth and pathogenic processes. This review reveals that responsiveness to human stress hormones is indeed widespread in the microbial world and documents the advances in microbial endocrinology that have taken place in recent years.
Introduction

**Microbial endocrinology: a holistic approach to understanding how stress can influence susceptibility to infection**

Current perceptions of how stress influences the outcome of infections centre upon the immunology and leave the microbe largely as a bystander. The immune system and central nervous system (CNS) are known to be involved in functionally relevant cross-talk to maintain homeostasis under both normal and disease conditions, and perception of stress by the CNS leads to release of a variety of hormones, neurochemicals and neuropeptides, which can directly affect immune function, usually resulting in impairment [1]. Nearly all immune cell classes possess receptors for stress-related neurohormones adrenaline and noradrenaline [1]. Sympathetic nerve fibres also extensively innervate lymphoid organs, such as bone marrow, thymus, spleen and lymph nodes, and terminate in close proximity to lymphocytes. This intimate connection of immune and nervous systems could simply and mechanistically explain why stress can influence susceptibility to infection [1], but does it explain everything?.

Recent work from the field of microbial endocrinology, a trans-disciplinary research area that represents the intersection between microbiology and neurophysiology [2], suggests a holistic approach, in which the microbe is seen as active participant, is needed for a more complete understanding of how stress affects the progression of infectious disease. Over the past 15 years a clearer picture has emerged of how infectious microbes can actively use the neurohormonal products of their host’s physiological response to stress, such as the release of noradrenaline, to their own advantage. [2] This review seeks to understand the ability of stress to influence susceptibility to infectious disease through the “lens” of microbial endocrinology. Evidence is presented that shows microbes have evolved specific detection systems for sensing the neuroendocrine outflow from a host
stress event, and that this can be used to the microbe’s advantage as an environmental cue to initiate growth and pathogenic processes.

**Review Structure**

Catecholamine stress hormones can influence the outcome of an infection

Happy pigs and cows may be safer pigs and cows

Catecholamine modulation of bacterial infectivity 1: augmentation of host tissue attachment

Catecholamine modulation of bacterial infectivity 2: stimulation of growth

Stress hormone responsiveness is widespread amongst pathogenic bacteria

Bacterial catecholamine specificity and response systems

Global analysis of bacterial catecholamine responsiveness

**Catecholamine stress hormones can influence the outcome of an infection**

Catecholamines are a group of effector compounds derived from tyrosine and comprising a benzene ring with two adjacent hydroxyl groups and an opposing amine side chain (Figure 1). Catecholamines have a central role in a myriad of stress-related phenomena, from acute psychological stress (fight or flight) to actual traumatic injury. Catecholamines mediate many different neuroendocrine signalling phenomena in multicellular organisms, and the catecholamine hormones adrenaline and noradrenaline are an integral part of the acute ‘fight and flight’ (response to a threat) stress response in higher animals [1]. Several experimental models of traumatic stress have shown that hormones elaborated during the stress event can markedly influence the outcome of an infection. In a mouse surgical stress model (partial hepatectomy) gut lumen levels of noradrenaline significantly increased following surgery [3]. Importantly, adhesion to gut mucosa of *Pseudomonas aeruginosa*, an opportunistic pathogen often involved in post-surgical complications was significantly enhanced when inoculated into hepatectomised mice. Furthermore, elaboration of the PA-1 lectin/adhesin (a key *Ps. Aeruginosa* host binding protein) was increased *in vitro* and *in vivo* in the presence of noradrenaline [3].
The finding that intestinal expression of tyrosine hydroxylase, the enzyme catalysing the rate-limiting step in noradrenaline synthesis (Figure 1), is significantly unregulated in response to host stress [4] could explain the stress-associated elevation in gut noradrenaline levels. Sepsis due to the overgrowth and translocation of gut bacteria, particularly Gram-negative species such as *Escherichia coli*, is a well recognised surgical complication [5]. Evidence for a role of catecholamines in the pathophysiology of gut-derived sepsis was provided by the *in vitro* demonstration that noradrenaline, adrenaline, and dopamine or their pharmacologically inactive metabolites induced four log or greater increases in the growth of commensal *E. coli* [6]. Further evidence for the contribution of catecholamines to gut-derived sepsis was obtained in mice administered with the neurotoxin 6-hydroxydopamine; this resulted in an immediate, systemic release of catecholamines which resulted within hours in a concomitant several log-fold specific increase in numbers of *E. coli* within the gut [7]. Significantly, a return to normal gut flora paralleled the time-dependent regeneration of catecholamine-containing nerves within the gut [7].

Psychological stress appears to be as important as physical trauma in its ability to affect the outcome of an infection. Social stress had a marked impact on susceptibility to enteropathogen challenge in a mouse social conflict stress model, even when stress enhanced certain aspects of the immune response such as phagocytosis [8]. In a rodent stress model, stressors such as conflict or restraint caused translocation to systemic sites of both the gut and cutaneous microflora [9].

**Happy pigs and cows might be safer pigs and cows**

Colonisation of farmed animals by enteric pathogens such as *E. coli* O157:H7 and *Salmonella enterica*, and their subsequent dissemination into the human food chain is a major health and economic concern for the meat production industries. The demonstration
that social stress can affect susceptibility to both infection by pathogens [8, 9], and the virulence potential of commensal microflora [5-6], has important implications for farm animal management practices such as transportation, handling, and housing. A mild handling stressor (single daily weighing) was sufficient to markedly alter the balance of the microflora of pigs [10]. Profiling of shed enteric bacteria showed a significant increase in numbers of \textit{E. coli} and other coliforms in faeces of test groups compared to controls [10]. This demonstrates that a common animal management practice considered low in stress impact by humans could be perceived differently by livestock, leading to stress-induced changes in the gut environment that would influence shedding of any pathogen subsequently acquired by livestock. In addition, the environment experienced by a pathogen prior to its entry into a host may markedly affect its subsequent ability to initiate an infection. Toscano \textit{et al.} examined the effects of \textit{in vitro} pre-treatment of \textit{Salmonella typhimurium} with noradrenaline prior to infection of the pathogen into young pigs [11]. Examination of the tissue distribution of \textit{Salmonella} revealed that stress hormone treated bacteria were present in greater numbers and more widely distributed in gut tissues than control bacteria. This has considerable implications for food safety, given the widespread nature of catecholamines and related compounds in human foods [12-14]. There are, however, some methodological issues highlighted by the Toscano \textit{et al.} investigation that typify the experimental design challenges central to the study of microbial endocrinology (see Box 1).

\textbf{Catecholamine modulation of bacterial infectivity 1: augmentation of host tissue attachment}

That environmentally induced stress might influence pathogen shedding in farm animals is known, but the mechanisms underlying this have not been fully elucidated. Equally unclear are the specific host factors that contribute to the colonisation of ruminants and humans by enteric pathogens. The mammalian gut is home to around 10\textsuperscript{12} microorganisms
comprising many hundreds of species that occupy every possible niche. Thus an invading pathogen must overcome the competition of the local microflora, in addition to the myriad of innate and adaptive host immune defences. Anatomically, the mammalian gut is extensively innervated with noradrenaline and dopamine containing sympathetic nerve terminals, which are distributed throughout the enteric nervous system (ENS) [15]. In fact, the total amount of enteric nerves is now estimated to be at least 500 million, which is as many in number as present in the spinal column [16], and the term ‘brain in the gut’ has often been applied to the ENS. It is also not widely appreciated that half of the noradrenaline present within the mammalian body is synthesised and utilised within the ENS. Thus the gut is an environment in which there is a significant presence of catecholamines even under normal conditions. Furthermore, during episodes of acute stress, catecholamines are released by the ENS, or spill over from the systemic circulation [17, 18], causing significant local increases; these changes can then markedly affect the behaviour of the resident microflora [4,6,8-9], as well as pathogens colonising the gut such as *E. coli* [19-23], *Salmonella* [22-23], *Campylobacter* [24] or *Vibrio* [25-26].

A number of reports have shown that catecholamines can act as potent stimuli for bacterial attachment to gut tissues. Noradrenaline increases expression of the K99 pilus adhesin of enterotoxigenic *E. coli* [27] and type 1 fimbriae of commensal *E. coli* [28]. Vlisidou et al. examined the effect of noradrenaline on the adherence and enteropathogenicity of *E. coli* O157:H7 in a bovine ligated ileal loop model of infection [21]. Noradrenaline enhanced *E. coli* O157:H7-induced intestinal inflammatory and secretory responses as well as adherence to intestinal mucosa. Noradrenaline modulation of enteritis and adherence was dependent on the ability of *E. coli* O157:H7 to form attaching and effacing lesions [21]. Noradrenaline was also effective in promoting caecal adherence of a non-O157 *E. coli* strain or *E. coli* O157:H7 eae (intimin, host cell tight attachment protein) and espA (type III translocator protein) mutants incapable of intimate mucosal attachment [22]. Using tissue
culture assays and a rat ileal loop model, Nakano et al. [25] examined the effect of noradrenaline on the pathogenicity of *Vibrio parahaemolyticus*. Catecholamine enhanced both the cellular cytotoxicity and enterotoxicity of *V. parahaemolyticus*, and was also found to increase transcription of *vscQ* and *vscU*, genes involved in the TTSS1 (type III) secretion system responsible for cytotoxicity [25].

As well as their direct effects on enteric bacteria, noradrenaline and other hormones released in response to acute stress may also act at the intestinal mucosa to alter interactions between luminal microorganisms and epithelial cells. Ussing chamber analyses of porcine gut explants showed that contra-luminal application of noradrenaline [22-23] or the non-catecholamine adrenocorticotrophic hormone [29] can lead to significant enhancement of luminal attachment of *E. coli* O157:H7 to colon via interactions with mucosal α-adrenergic and melanocortin receptors, respectively. It is noteworthy that the noradrenaline mediated increase in adhesion of *E. coli* O157:H7 to caecal epithelia could be prevented by prior treatment of tissue explants with adrenergic receptor antagonists such as phentolamine [22].

**Catecholamine modulation of bacterial infectivity 2: stimulation of growth.**

The relationship between iron, bacterial growth and virulence has been known for many years, with the limited availability of free iron in the host environment presenting a major obstacle to the growth of most microbial pathogens. One strategy that infectious bacteria often employ to scavenge nutritionally essential iron is the production and utilisation of siderophores, secreted low molecular weight catecholate or hydroxamate molecules that possess high affinity for ferric iron. However, in the presence of high affinity host ferric iron binding proteins, such as transferrin (Tf) in blood and lactoferrin (Lf) in mucosal secretions, siderophores are often ineffective at retrieving host iron. In such situations, host stress responses leading to the release of catecholamines will become important as
catecholamine hormones noradrenaline, adrenaline and dopamine can all mediate the removal of iron from host Tf and Lf [6, 30-32].

The catechol moiety in catecholamine stress hormones, inotropes and even their metabolites complex with the ferric iron sequestered by Tf and Lf, reducing the iron binding affinity of the proteins [6, 30-32], and rendering them susceptible to bacterial ‘theft’ by siderophores such as enterobactin [32]. The role of the siderophore is to facilitate internalisation into the bacterial cell of the Tf or Lf iron liberated by the catecholamine [32, 33]. The precise molecular details of the mechanism by which catecholamine stress hormones liberate Tf- and Lf-complexed iron remains to be determined, but for Gram-negative bacteria, such as *E. coli* [32, 33] or *Salmonella* [34], siderophore synthesis and uptake systems are integral elements in the mechanism by which stress hormones induce growth.

Catecholamine-mediated access to sequestered host iron significantly enhances growth of commensal species such as the coagulase-negative staphylococci [31, 35-36] and therefore may contribute to biofilm formation in intravenous lines [36]. Catecholamines enable pathogenic bacteria such as *E. coli* [30, 37, 38], *Salmonella* [37, 38], *Yersinia* [37, 38], *Vibrio* [26], *Campylobacter* [24] and *Bordetella* [39] that lack specific acquisition systems for Tf or Lf iron to grow in normally bacteriostatic iron-restricted environments, such as blood or serum (Table 1). In serum- or blood-supplemented media, the magnitude of growth stimulation possible with catecholamines can be up to 5 log orders higher than unsupplemented controls in less than 24 hours [6, 30, 31, 35, 37-38]. The co-localisation in the gut of bacteria and Lf may explain why large elevations in noradrenaline levels during acute stress can catalyse the overgrowth and translocation of gut microflora [6, 7, 9] (Figure 2). It is therefore perhaps not surprising that mammals have evolved mechanisms to tightly regulate levels of gut catecholamines and that catecholamine-degrading enzymes
are present throughout the entire length of the GI tract [40]. Analysis of the gut tissue distribution of the human phenol sulfotransferase family of catechol-inactivating enzymes show a close correlation with bacterial presence and numbers, with expression lowest in the stomach and greatest in the large intestine and colon [40].

Iron delivery from Tf and Lf is not the only mechanism by which catecholamines can induce bacterial growth, at least in Gram-negative bacteria. Catecholamine-induced growth of enteric bacteria in a serum-based medium leads to the production of a non-LuxS dependent autoinducer of growth [35, 41]. This novel autoinducer is heat stable, cross-species acting, and induces increases in growth of a magnitude similar to that achievable with the catecholamines [35]. Interestingly, this activity works independently of Tf or Lf, and is able to stimulate recovery of viable but non-culturable *E. coli* O157:H7 or *Salmonella* [32, 35, 42, 43] as well as increasing rates germination of *Bacillus anthrax* spores [44]; it is also recognised by periodontal pathogens [45]. Induction of the autoinducer in Gram-negative bacteria requires only a 4-6 hour exposure to noradrenaline [35, 41], which suggests that the effects of catecholamine release during acute stress could have lasting and wide acting effects on the gut microflora long after catecholamine levels have returned to normal (Figure 2).

**Catecholamine stress hormones are recognised by many pathogenic bacteria**

Although most investigations of catecholamine induction of growth and virulence have been carried out with gut-associated bacteria, catecholamines are ubiquitous in the mammalian body, and it is becoming increasingly clear that bacterial species occupying other anatomical regions can similarly respond to changes in the stress hormone levels of their host’ environment (Table 1). In humans, stress is a known risk factor in initiation and progression of gum (periodontal) disease, and stress hormones such as cortisol, noradrenaline and adrenaline have been isolated from saliva [46]. Noradrenaline and
adrenaline increased the growth of human oral bacteria implicated in periodontal disease in a serum based medium and anaerobic culture conditions [45, 47]. Of 43 species tested, over half showed significant catecholamine responsiveness (Table 1), providing additional insight into why stress can exacerbate gum disease. Catecholamines were also recently shown to be growth factors for *Bordetella* species, including the respiratory pathogens *B. bronchiseptica* and *B. pertussis* [39]. Under iron-limited conditions, or in serum-based media, noradrenaline stimulated growth of *B. bronchiseptica* and induced expression of BfeA, a siderophore receptor important for growth in vivo [39]; this suggests that *Bordetella* species may also perceive catecholamines as a host environmental cue.

Table 1 illustrates the bacteria that currently identified as being catecholamine stress hormone responsive. Most of the species shown, *Borrelia* [48], *Bordetella* [39], *Campylobacter* [24], *E. coli* [30, 37, 38, 49, 50], and *Salmonella* [37, 38], will be familiar for the diseases they cause in mammals; however, *Aeromonas hydrophila* is a pathogen of frogs as well as man [51], and while *Vibrio* is best known for its pathogenic burden to humans, it can also cause infections in oysters. Indeed, stress of juvenile oysters led to increased susceptibility to infection with *Vibrio* species that was specifically linked to increased levels of noradrenaline in the oyster [52]. Furthermore, injection of noradrenaline into unstressed oysters also increased infection-induced mortality [52].

**Bacterial catecholamine specificity and response systems**

While it should be apparent that bacteria colonising stressed hosts will be exposed to catecholamines, it is less well appreciated that bacteria outside of mammalian hosts are potentially exposed to catecholamines made by protozoa [53] and plants [54]; and that catecholamines and structurally similar compounds are widespread in foods [11-14].

Noradrenergic and dopaminergic neurons are abundant in the ENS, but the lack of neurons containing phenylethanolamine N-methyltransferase (Figure 1a) means that
adrenaline is not synthesised within the ENS [15]. Certain aspects of \textit{E. coli} O157:H7 virulence appear to be regulated by adrenaline [55-57], which has led to the suggestion that adrenaline, in addition to noradrenaline, might be a specific host hormonal cue for enteropathogenic bacteria. Other studies have examined whether specificity exists in enteric bacterial species for the catecholamine they are likely to encounter in the gut. Comparative analyses of catecholamine growth responsiveness of three enteric pathogens characterized by their propensity to primarily inhabit the gut (\textit{Y. enterocolitica}), or to colonize extra-intestinal sites (\textit{E. coli} O157:H7 and \textit{S. enterica}) revealed a distinct preference for noradrenaline and dopamine over adrenaline [37]. Indeed, \textit{Y. enterocolitica} isolates showed no significant growth responsiveness to adrenaline, and adrenaline actually antagonised \textit{Y. enterocolitica} responses to noradrenaline and dopamine [37]. Similarly, examination of growth responses of several \textit{Vibrio} species to a range of catecholamines and their metabolites revealed that adrenaline was less potent than noradrenaline or dopamine at inducing growth [26]. In a separate study, adrenaline was found less effective than noradrenaline at enhancing pathogen attachment to tissue culture cells [58].

Catecholamine antagonists have been used extensively in mammalian systems to identify and characterise catecholamine receptors. A pharmacological investigation of the effects of a wide range of \(\alpha\)– and \(\beta\)–adrenergic and dopaminergic receptor antagonists on noradrenaline, adrenaline and dopamine dependent growth induction in \textit{Y. enterocolitica}, \textit{E. coli} O157:H7 and \textit{S. enterica} has demonstrated that there are separate and distinct bacterial response systems for each catecholamine [38]. Alpha– but not \(\beta\)–adrenergic antagonists blocked responses to noradrenaline and adrenaline, but did not inhibit dopamine responsiveness. Conversely, dopaminergic antagonists blocked responses to dopamine, but did not affect growth induction by noradrenaline and adrenaline. Interestingly, the antagonist effects were found to be independent of the ability of the
catecholamine to supply host iron to the bacteria, indicating that the inhibition observed was specifically due to a blockade of the bacteria to the stress hormone [38]. Considered collectively, the specificity and antagonist studies [37, 38] suggest that enteric bacteria have evolved/retained specific response systems for the catecholamine(s) they are likely to encounter in the anatomical region they colonise when in the mammalian body.

The ability of catecholamines to stimulate bacterial growth in higher organisms has been known for over a decade [2], but little is known about the nature of the putative bacterial adrenergic and/or dopaminergic receptor(s) to which noradrenaline, adrenaline and dopamine might bind and exert their effects, or even whether the binding properties of such a receptor are similar between different species. Although there is no evidence for the existence of mammalian-like catecholamine response systems in bacteria, the aromatic amino acid decarboxylases that are responsible for the sequential production of the catecholamine chemical messengers, specifically dopamine, noradrenaline and adrenaline, are present in prokaryotes [59]. Based on the phyletic patterns of such enzymes in the production of rapidly diffusible chemical messengers such as the catecholamines, Iyer et al. have proposed that the modern evolution of cell-to-cell signalling within mammalian neuroendocrine systems actually represents late horizontal gene transfer from bacteria [59]. A recent report [60] suggests we should broaden our idea of what can constitute a bacterial catecholamine-response system. Clarke and co-workers used in vitro constructs to demonstrate that noradrenaline and adrenaline could bind to the E. coli O157:H7 two-component regulator sensor kinase QseC, leading to the proposal that this is the bacterial receptor for these catecholamines [60]. QseC also recognises a novel LuxS dependent autoinducer termed AI-3 [55, 57], which intriguingly suggests a possible intersection of interkingdom (microbial endocrinology) and intercellular (quorum sensing) signalling pathways [55-57, 60]. Interestingly, Y. enterocolitica shows a growth response to noradrenaline, but not adrenaline, and does not contain homologues
for QseC (or the related receptor QseE) indicating that a different response system for noradrenaline must exist in *Yersinia* species [37, 38].

**Global analysis of bacterial catecholamine responsiveness**

Although there have been reports of catecholamine effects on expression of selected virulence genes [25, 39, 55-57], there have been few investigations examining whether catecholamines can modulate bacterial gene expression in a global context. Bansal *et al.* [58] used DNA microarrays to analyse gene expression profiles of biofilm grown *E. coli* O157:H7. Although the culture medium used was not very host-like (Luria broth), (Boxes 1 and 2), both noradrenaline and adrenaline caused upregulated expression of genes involved in surface colonisation and virulence. Furthermore, the profiles of the genes regulated by the two catecholamines were also different to each other [58]; this agrees well with the differential catecholamine responsiveness observed by Freestone and co-workers [37, 38]. A total of 938 and 970 genes were differentially expressed following treatment with adrenaline and noradrenaline, respectively, with only 411 differentially expressed genes common between the two catecholamines [58]. The Bansal study demonstrated that noradrenaline and adrenaline modulated expression of genes involved in a number of cellular processes, but interestingly found none of the *eae* genes showed any significant changes in expression following exposure to either of the catecholamines [58].

The observation that adrenaline did not activate expression of *eae* or related genes was in marked contrast to several other studies [55-57]. A recent transcriptional profiling study, which used a smaller array of 610 *E. coli* O157:H7 virulence genes, reported that *E. coli* O157:H7 cultured in serum-SAPI medium containing noradrenaline showed differential regulation of 101 genes compared to similarly grown non-supplemented controls [61]. Genes showing a higher level of expression included *eae*, *espB* and *espA*, and the *stx1*
and stx2 shiga toxin genes. These results agree well with an earlier report that noradrenaline increased shiga toxin production in serum-SAPI [62], and provide supportive evidence that acute stress resulting in elevated noradrenaline levels in the gut could result in enhanced virulence of *E. coli* O157:H7 *in vivo* [19-23].

**Concluding remarks and future directions**

As part of a more holistic approach to understanding how stress alter susceptibility to infection, this review has shown that bacteria can respond to the inevitable neurohormonal outflow that occurs during stress, and that they can have multiple response systems to utilize catecholamines both for growth and induction of pathogenic processes [32-33, 35, 37-38, 55, 60]. This review has also highlighted a number of recent developments relating to the mechanisms by which bacteria ‘sense’ stress-related neurohormones. First is the realisation from the adrenergic and dopaminergic antagonist [38] and catecholamine specificity studies [37] that bacterial growth responses to catecholamines involve much more than just ‘simple’ acceptance of host-sequestered iron Evidence is now available that bacteria have catecholamine response systems that possess extraordinary pharmacological similarity to the mammalian adrenergic and dopaminergic receptors [38]. Similarly, evidence exists for both the evolution of bacterial specificity for the catecholamine most prevalent in the host niche inhabited [37], and a possible intersection of microbial endocrinology with quorum sensing signalling [55-57, 60].

As is common in an evolving scientific discipline, the research described in this review often poses many more questions than it provides answers. The clear future themes in microbial endocrinology will involve identification of bacterial receptor(s) and response system(s) specific to each catecholamine [37-38, 60], and development of novel antimicrobials based on catecholamine antagonists [38]. Obviously, catecholamines are only one part of a complex network of neurophysiology, and there remain a large number
of neurohormones and other effectors released during stress [2] that have not been investigated and that may play a role in infectious disease (as suggested in Ref. [29]). The finding that the complete sequence for B-endorphin receptor is present in E. coli [67] is just one example of how the application of a microbial endocrinology approach could yield new insights in the pathogenesis of infectious diseases.

The continued development of the microbial endocrinology field will depend on the involvement of neuroendocrinologists and neurophysiologists who can bring needed guidance and understanding to the microbiologist’s experimental design. The interdisciplinary nature of microbial endocrinology incorporates a “bench-to-bedside” approach that should ultimately prove of utility in the clinical arena. Indeed, a recent editorial in Lancet debating the therapeutic use of catecholamine inotropes in the treatment of sepsis has cited the ability of catecholamines to affect bacterial growth as further evidence for why new treatment modalities need to be devised [68].

Acknowledgements

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Table 1. Stress hormone responsive bacteria

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<th>Species</th>
<th>Growth</th>
<th>Virulence</th>
<th>Reference</th>
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<tr>
<td>Aeromonas hydrophila</td>
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<tr>
<td>Acinetobacter 1wolffii</td>
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<td>[35]</td>
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<td>Bordetella bronchiseptica, B. pertussis</td>
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<td>+</td>
<td>[39]</td>
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<td>Borrelia burgdorferi</td>
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<td>[48]</td>
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<tr>
<td>Campylobacter jejuni</td>
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<td>Citrobacter freundii</td>
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<td>[35]</td>
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<td>[35]</td>
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<td>[35, 65]</td>
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<td>Listeria monocytogenes</td>
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<td>Vibrio parahaemolyticicus, V. mimicus, V. vulnificus</td>
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<td>Xanthomonas maltophilia</td>
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<td>Yersinia enterocolitica</td>
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<td>[35, 37-38, 49]</td>
</tr>
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</table>

**Periodontal pathogens**

- Actinomyces gerencseriae, +
- A. naeslundii, A. odontolyticus +
- Campylobacter gracilis +
- Capnocytophaga sputigena, C. gingivalis +
- Eikenella corrordens +
- Eubacterium saburreum +
- Fusobacterium periodonticum, +
- F. nucleatum subsp. Vincentii +
- Leptotrichia buccalis +
- Neisseria mucosa +
- Peptostreptococcus anaerobius, +
- P. micros +
- Prevotella denticola, +
- P. melaninogenica +
- Staphylococcus intermedius +
- Streptococcus gordoni, +
- S. constellatus, S. mitis, +
- S. mutans, S. sanguis +
The ‘+’ denotes that catecholamine stress hormones, inotropes or their metabolites have induced enhancement of growth or virulence of the bacterial species shown.
Box 1. Design issues in the study of microbial endocrinology

In comparative analyses the test and control environments should as far as possible be identical. In *in vitro* bacterial virulence studies it is obvious that, where feasible, the media used should mimic the host environment as closely as possible. This consideration led Lyte and Ernst to develop serum-SAPI, a minimal salts medium supplemented with 30% adult bovine serum [49]. Serum-SAPI is a highly stressful bacteriostatic medium that exposes bacteria to conditions similar to experienced inside a host, such as iron limitation, limited nutrient availability, and immune defence proteins such as antibodies and complement. Serum-SAPI, or variants of it [45, 47], have been used extensively in the analysis of stress hormone effects on bacteria, particularly growth responses [6, 11, 24, 26, 30-32, 34-38, 45-50, 65]. Most bacteria grow poorly in serum-SAPI, at least in part because of the iron restriction imposed by the serum iron binding protein transferrin, and generally they achieve only a few log increases in growth over starting inocula (which are usually low – typically around $10^2$ colony forming units (CFU) ml$^{-1}$, which is intended to reflect the numbers of bacteria likely present at the start of an infection) [6, 30, 31, 35-38, 49-50]. Supplementation with catecholamine stress hormone supplementation allows bacteria to access the iron sequestered by transferrin, and enables growth; addition of exogenous iron can also support growth [32-33, 37-38]. The stimulation of growth in the presence of catecholamines can be considerable – up to 5 log-fold over controls [6, 30-31, 35-39, 49-51].

As comparative analyses require that the test and control environments should be virtually identical, the bacteriostatic nature of serum- or plasma-SAPI, and the resultant poor growth of control cultures, can seriously complicate experimental design (the creation of equivalent conditions between the test and control). This problem is well exemplified in the Toscano *et al.* study [11], where the test pre-incubation medium comprised serum-SAPI
supplemented with 2 mM noradrenaline, but because of poor growth in non-catecholamine supplemented serum-SAPI, the control consisted of Luria broth, a traditional rich microbiological media. This disparity in experimental conditions then raises questions as to the origin of the marked differences in virulence phenotype observed between the test and control *Salmonella* cultures [11]. Are the phenotypic changes solely due to the catecholamine, or is there a significant contribution from host factors present within the serum-based culture media?

**Box 2. The ability of catecholamines to bind iron can affect bacteria in different ways**

As highlighted by Lyte [2], the medium chosen to investigate catecholamine responsiveness is critically important in determining the mechanism by which the hormone modulates bacterial growth or virulence. Most analyses of catecholamine responsiveness have been conducted using host-like (serum- or blood-supplemented) iron limited media that contain a sequestered iron source, such as Tf or Lf. In such media catecholamine supplementation leads to a microbiologically important growth stimulus, which has its origin in catecholamine-mediated host iron provision [6, 24, 26, 30-32, 35-39, 45, 47, 49-51, 64-65]. Other analyses have used more traditional laboratory media to analyse catecholamine effects, for example Walters and Sperandio used DMEM (Dulbecco's modified Eagle medium, a nutritionally complete tissue culture media used for culture of eukaryotic cells, but also popular media for analysis of bacterial virulence) to show that 50 μM adrenaline can increase expression of certain *E. coli* O157:H7 virulence genes [55-56]; and Vlislidou *et al.* used 5 mM noradrenaline in Luria broth to investigate catecholamine effects on EHEC adherence [21]. Catecholamines can clearly chelate iron [30-32, 35-39], and in iron-replete media such as Luria broth or DMEM, this may lead to production of a more iron-limited environment. Given the role that iron, and particularly iron restriction, can
play in the regulation of bacterial virulence, consideration should be given to the possibility that catecholamine-induced effects on gene expression observed in laboratory culture media might, at least in part, be due to alterations in available iron levels.

Chemical studies have also shown that in iron-replete media catecholamine interactions with ferric iron can lead to the generation of oxygen-derived free radicals, a cell damaging process that has been implicated in development of neurodegenerative diseases such as Parkinson’s [63]; therefore, particularly at high catecholamine concentrations, the role of oxidative stress influencing bacterial gene expression should also be a consideration.
Figure Legends

Figure 1. Structures of catecholamines and biosynthetic pathway
(a) Catecholamine biosynthetic pathway. Catecholamines are a group of compounds derived from tyrosine and comprising a benzene ring with two adjacent hydroxyl groups and an opposing amine side chain. In mammals, the tyrosine pathway is used for the synthesis of catecholamines, most commonly from food sources. The precursor of dopamine (DOPA) is 3, 4-dihydroxyphenylalanine. For clarity, the various enzyme cofactors needed in the pathway are not shown. (b) Structures of synthetic catecholamine inotropes used in intensive care settings. These drugs are used therapeutically to control heart function and regulate blood pressure; however, they can also enhance increases in growth of both commensal *E. coli* and skin-dwelling coagulase-negative staphylococci [6, 31, 36]. In the latter case, this might lead to biofilm formation within indwelling medical devices such as intravenous lines, with consequent infections in hospitalized patients [36]. (c) Structures of dietary catechol-containing compounds. Catechol-containing compounds, such as chlorogenic acid and catechins, are widespread in beverages (tea and coffee), fruit and vegetables and are much more abundant in foods than catecholamines. The catechol group in these supposedly harmless dietary compounds enables them to function in a similar way to the catecholamine stress hormones, i.e. to induce growth of enteric pathogens by delivering iron from host iron binding proteins, and by inducing production of growth stimulatory autoinducers [14].

Figure 2. Model of enteropathogenic *E. coli* behaviour in a stressed host GI tract
This cartoon shows how an initial few cells of an invading enteropathogen, such as *E. coli* O157:H7, might behave when their host becomes acutely stressed. (a) Host gut during calm times. Lactoferrin maintains iron limitation in mucosal secretions, which are therefore
bacteriostatic. The number of pathogen is low. (b) Acute stress. ENS activity increases with the consequent release of catecholamines (noradrenaline [NA] and dopamine) within the gut. Work from in vitro and in vivo reports shows that the encounter of the pathogen with the stress hormone could result in two major events. The first (i) is that NA acts as a cue to prime virulence factor expression – in this case production of attachment factors such as adhesins [3, 19-22, 27, 28, 56-58]. The second (ii) is that the catecholamine interacts with the lactoferrin/transferrin, converting it to a useful iron source [6, 14, 24, 30-32, 36-38, 64] and providing support for growth in the gut. Bacteria bind lactoferrin [P. Freestone et al., unpublished data], and internalise the iron [6, 14, 30], enabling growth and an increase in pathogen numbers. (c) Several hours after the acute stress has passed and the levels of catecholamines have returned to normal. For enteric pathogens such as E. coli O157:H7, only a transient 4 hr exposure to catecholamines is sufficient to induce production of a novel autoinducer of growth (AI). This AI not only stimulates growth [35, 41] but can also enhance shiga toxin production [43]. The increasing numbers of pathogens, and possibly also endogenous gut bacteria [6-9], could affect gut integrity leading to bacterial translocation (iii) [7, 9], either to the mesenteric lymphatic tissue or, in a worst case scenario, directly into the systemic circulation, where they could lead to sepsis and multiple organ failure. Previous exposure to NA might also markedly increase expression of E. coli virulence genes [27, 58, 61-62], leading to increased attachment of the pathogen to the gut mucosa, either through non-intimate attachments [22] (iv) or through espA-dependent intimate attachment [21, 61] (v and vi), causing the attaching and effacing lesions characteristic of enteropathogenic and enterohaemorrhagic E. coli. As well as stress elaborated catecholamines, E. coli O157:H7 might also respond to signals produced by the intestinal microbial flora, such as the LuxS-dependent AI-3, which also activates transcription of genes involved in attaching and effacing lesion formation [55-57].
Figure 1 Catecholamines

(a) Catecholamine Biosynthetic pathway

Phenylalanine

Tyrosine

DOPA

Dopamine

Noradrenaline

Adrenaline

(b) Synthetic Catecholamine inotropes

Dobutamine

Isoprenaline

(c) Dietary Catechol compounds

Catechin

Chlorogenic acid
Figure 2

Model of how enteropathogenic *E. coli* might behave in the GI tract of a stressed host

(a)

(b)

(c)

Mesenteric Lymph nodes
Systemic Circulation

Enteropathogen
Enteropathogen: enhanced attachment factor expression
Noradrenaline
Lactoferrin
Lactoferrin-NA complex
NA-induced Al