Stress and bacteria: microbial endocrinology

Paul Everest

Regulation of virulence of *Campylobacter jejuni* by norepinephrine has implications for the husbandry of food production animals and transmission of infection to man

Bacteria living within the environs of a host (whether human or animal) are subject to stressful conditions and have to overcome them to survive.[1 2] In particular, enteric bacterial pathogens have to tolerate exposure to the acid environment of the stomach, resist the detergent-like activity of bile salts and ever decreasing oxygen concentrations as they descend the gastrointestinal tract, the presence of a competing microbial flora and the antimicrobial peptides of the epithelial surfaces they encounter. Bacteria—whether commensal, obligate or opportunist pathogens—live in a permanent state of stress and regulate their gene expression and, in the case of potential pathogens, virulence gene expression[1 2] in response to these environmental stresses.

The mammalian or avian hosts harbouring these organisms may themselves be subject to conditions that induce stress and the physiological responses that characterise that rather imprecise term. Thus, ill human patients in hospital—whether due to acute illness, infection, any form of accidental/induced trauma or animals reared and transported under some food production conditions—have a neurophysiological response to stress by the local (enteric) and systemic release of catecholamine hormones and, in particular, norepinephrine (noradrenaline) by the enteric nervous system.[3 4]

It has been recognised for some time that norepinephrine potentiates bacterial growth both in vivo and in vitro and induces expression of virulence determinants in enteric pathogens, particularly *Escherichia coli*.[5 6] In this issue of *Gut*, Cogan *et al*[7] (***see page 1060***) provide evidence that norepinephrine also regulates virulence in the important intestinal food-borne pathogen *Campylobacter jejuni*. Norepinephrine, in the presence of iron-limiting conditions, increases *C jejuni* growth, motility and bacterial invasion into cultured intestinal (Caco-2) cells and decreases the time taken for the organism to affect Caco-2 cell tight junction barrier function. The implications for animal husbandry in particular and the physiological state of the organism for transmission and disease causation in a human host are intriguing.

Lyte[3 4] has termed the study of the interaction of microbes with stress hormones ''microbial endocrinology''. There has been an increasing awareness of the role of neuroendocrine hormones in the pathogenesis of infectious disease. Thus, exposure of enteropathogenic *E coli* (EPEC) to norepinephrine for 6 h increases bacterial growth when using a small bacterial inoculum under iron-limiting conditions.[8 9] Intriguingly, the host iron-binding proteins transferrin (TF) and lactoferrin (LF) were required for norepinephrine to stimulate growth of *E coli*. Bacteria require iron for growth in vivo, but iron is limited for bacterial growth by host iron-binding proteins. Norepinephrine at physiological concentrations complexes with these host iron-binding proteins, and the organism can bind and use these complexes as a source of iron in vitro. Radiolabelled iron complexed to TF and LF is taken up by the bacterial cells and norepinephrine was also internalised by the organism. Thus, the effect of norepinephrine on bacterial growth is mediated by its ability to complex with host iron-binding proteins and, in turn, for the bacteria to use the iron in these complexes for growth. This observation has implications for commensal organisms involved in sepsis in hospitalised patients. Using faecal isolates of *E coli*, Freestone *et al*[10] showed that iron removal from LF and TF complexed to norepinephrine required an intact 3,4-dihydroxybenzoyl (catechol) structure, whereas norepinephrine metabolites in which the catechol moiety had been modified were not used as a source of iron by *E coli*. A role for catecholamine-mediated bacterial iron supply in the pathophysiology of gut-derived sepsis was proposed.

It has recently been shown that epinephrine/norepinephrine is involved in the quorum sensing of bacteria.[11 12] Quorum sensing is a cell to cell signalling mechanism in which bacteria respond to hormone-like molecules called autoinducers (AIs) produced by other growing bacteria of the same species in the same environment. Enterohaemorrhagic *E coli* 0157:H7 has an autoinducer, AI-3, which is involved in interkingdom signalling with epinephrine/norepinephrine, and it is this signalling that activates transcription of virulence genes. QseC sensor kinase is a bacterial receptor for both AI-3 and epinephrine/norepinephrine, the first identified bacterial adrenergic receptor.[11] Adrenergic antagonists can specifically block these responses and a qseC mutant is reduced in virulence in a rabbit model of infection. Neuroendocrine hormones can also affect expression of attachment organelles in bacteria. Norepinephrine has been shown to increase expression of the K99 pilus adhesin of enterotoxigenic *E coli* in vitro[13] and also of *E coli* type 1 fimbriae in vivo following surgical stress.[14] Use of an ex vivo tissue culture model has shown that norepinephrine or dopamine stimulation of enteric nerves in the tissue increases the adhesion of *E coli* 0157 to caecal epithelium which could be prevented by prior treatment with adrenergic receptor antagonists.[15] Norepinephrine also augments *E coli* O157:H7-induced intestinal inflammatory and secretory responses as well as bacterial adherence to intestinal mucosa in a bovine ligated ileal loop model of infection. Norepinephrine modulation of enteritis and adherence was dependent on the ability of *E coli* O157:H7 to form attaching and effacing lesions.[16] Using a surgical stress model in animals which increased norepinephrine in the gut, animals were subsequently challenged with *Pseudomonas aeruginosa*. This resulted in increased mucosal adhesion of *P aeruginosa* through increased expression of bacterial adhesion.[17] These hormones also increase the growth of *Salmonella* species,[18] *Listeria monocytogenes*[19] and invasion of *Salmonella choleraesuis* into intestinal epithelia.[20]

Food production animals may not live a stress-free lifestyle, particularly animals and poultry intensively reared. The work by Cogan *et al*[7] published in this issue of *Gut*, if extrapolated to the animal or bird intestine in which *C jejuni* are living, suggests that stress in animals may encourage colonisation by *C jejuni*. The pathogenic determinants of *C jejuni* are still relatively little understood, so any insight into virulence regulation is welcome. Motility is an absolute requirement for virulence in *C jejuni* animal models of disease (reviewed by Konkel *et al*[21]) and addition of norepinephrine to organisms

grown in iron-limiting conditions resulted in increased motility and restoration of the iron replete motile phenotype in their studies. Flagella are also important in the colonisation of the chicken gastrointestinal tract.[21] In addition, flagella are also the means by which secreted bacterial virulence factors are delivered into host eukaryotic cells, effecting bacterial invasion into epithelia.[21] Increased motility and invasion also implies increased fitness for transmission within the poultry flock or for causing disease in man. It is well known that poultry abattoir workers during their first period of employment suffer from episodes of acute diarrhoea.[22] Does this reflect intense exposure to organisms with enhanced virulence from stressed animals? Over time the number of episodes decreases, suggesting acquired immune protection from exposure to different serotypes of *C jejuni*. What are the wider implications for the many infections acquired from food and are there differences in the ability of *C jejuni* to cause disease in man if they are present in food from a stressed or stress-free animal or bird?

It has been known for a long time that stress in man and animals increases susceptibility to infectious diseases.[23] What was perhaps unexpected is that bacterial stress responses imposed by the host environment on the organism and the host's norepinephrine stress response imposed by infection both potentiate the growth and virulence of the organism. Bacteria/stress hormone interaction is likely to be one of many variables whereby the virulence of *C jejuni* is regulated. All of us are ''stressed'' at some time, and it is intriguing to speculate whether that translates into a particular susceptibility to gastrointestinal or, indeed, any type of infection due to increased production of enteric norepinephrine. As most *C jejuni* infections in man in the western world are sporadic in nature,[24] are we more susceptible to these organisms in stressful times? The production of stress-free ''happy'' animals for food may have implications for preventing the acquisition and potential transmission of disease adapted *C jejuni*.[25]

Gut 2007;**56**:1037–1038.
doi: 10.1136/gut.2006.117150

Correspondence to: Dr P H Everest, Division of Veterinary Infection and Immunity, Institute of Comparative Medicine, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK; phe3d@udcf.gla.ac.uk

Competing interests: None.

REFERENCES

1 **Raivio T**. Envelope stress responses and Gram negative pathogenesis. *Mol Microbiol* 2005;**56**:1119–28.
2 **Dorrel C**, Lejeune P, Ridrigue A. The Cpx system of Escherichia coli, a strategic signalling pathway for confronting adverse conditions and for settling biofilm communities. *Res Microbiol* 2006;**157**:306–14.
3 **Lyte M**. The role of microbial endocrinology in infectious disease. *J Endocrinol* 1993;**137**:343–5.
4 **Lyte M**. Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol* 2004;**12**:14–20.
5 **Lyte M**, Ernst S. Catecholamine induced growth of gram negative bacteria. *Life Sci* 1992;**50**:203–12.
6 **Lyte M**, Arulanandam BP, Frank CD. Production of Shiga-like toxins by Escherichia coli O157:H7 can be influenced by the neuroendocrine hormone norepinephrine. *J Lab Clin Med* 1996;**128**:392–8.
7 **Cogan TA**, Thomas AO, Rees LE, *et al*. Norepinephrine increases the pathogenic potential of Campylobacter jejuni. *Gut* 2007;**56**:1060–5.
8 **Freestone PP**, Lyte M, Neal CP, *et al*. The mammalian neuroendocrine hormone norepinephrine supplies iron for bacterial growth in the presence of transferrin or lactoferrin. *J Bacteriol* 2000;**182**:6091–8.
9 **O'Donnell PM**, Aviles H, Lyte M, *et al*. Enhancement of in vitro growth of pathogenic bacteria by norepinephrine: importance of inoculum density and role of transferrin. *Appl Environ Microbiol* 2006;**72**:5097–9.
10 **Freestone PP**, Williams PH, Haigh RD, *et al*. Growth stimulation of intestinal commensal Escherichia coli by catecholamines: a possible contributory factor in trauma-induced sepsis. *Shock* 2003;**20**:183–8.
11 **Clarke MB**, Hughes DT, Zhu C, *et al*. The QseC sensor kinase: a bacterial adrenergic receptor. *Proc Natl Acad Sci USA* 2006;**103**:10420.
12 **Kendall MM**, Sperandio V. Quorum sensing by enteric pathogens. *Curr Opin Gastroenterol* 2007;**23**:10–5.
13 **Lyte M**, Arulanandam B, Nguyen K, *et al*. Norepinephrine induced growth and expression of virulence associated factors in enterotoxigenic and enterohemorrhagic strains of Escherichia coli. *Adv Exp Med Biol* 1997;**412**:331–9.
14 **Hendrickson BA**, Guo J, Laughlin R, *et al*. Increased type 1 fimbrial expression among commensal Escherichia coli isolates in the murine cecum following catabolic stress. *Infect Immun* 1999;**67**:745–53.
15 **Chen C**, Lyte M, Stevens MP, *et al*. Mucosally-directed adrenergic nerves and sympathomimetic drugs enhance non-intimate adherence of Escherichia coli O157:H7 to porcine cecum and colon. *Eur J Pharmacol* 2006;**539**:116–24.
16 **Vlisidou I**, Lyte M, van Diemen PM, *et al*. The neuroendocrine stress hormone norepinephrine augments Escherichia coli O157:H7-induced enteritis and adherence in a bovine ligated ileal loop model of infection. *Infect Immun* 2004;**72**:5446–51.
17 **Alverdy J**, Holbrook C, Rocha F, *et al*. Gut-derived sepsis occurs when the right pathogen with the right virulence genes meets the right host: evidence for in vivo virulence expression in Pseudomonas aeruginosa. *Ann Surg* 2000;**232**:480–9.
18 **Williams PH**, Rabsch W, Methner U, *et al*. Catecholate receptor proteins in Salmonella enterica: role in virulence and implications for vaccine development. *Vaccine* 2006;**24**:3840–4.
19 **Coulanges V**, Andre P, Ziegler O, *et al*. Utilization of iron-catecholamine complexes involving ferric reductase activity in Listeria monocytogenes. *Infect Immun* 1997;**65**:2778–85.
20 **Green BT**, Lyte M, Kulkarni-Narla A, *et al*. Neuromodulation of enteropathogen internalization in Peyer's patches from porcine jejunum. *J Neuroimmunol* 2003;**141**:74–82.
21 **Konkel ME**, Monteville MR, Rivera-Amill V, *et al*. The pathogenesis of Campylobacter jejuni-mediated enteritis. *Curr Issues Intest Microbiol* 2001;**2**:55–71.
22 **Cawthraw SA**, Lind L, Kaijser B, *et al*. Antibodies, directed towards Campylobacter jejuni antigens, in sera from poultry abattoir workers. *Clin Exp Immunol* 2000;**122**:55–60.
23 **Peterson PK**, Chao CC, Molitor T, *et al*. Stress and pathogenesis of infectious disease. *Rev Infect Dis* 1991;**13**:710–20.
24 **Tauxe RV**. Epidemiology of Campylobacter jejuni infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, Tompkins LS, eds. *Campylobacter jejuni:current status and future trends*. Washington: American Society for Microbiology, 1992:9–19.
25 **Humphrey T**. Are happy chickens safer chickens? Poultry welfare and disease susceptibility. *Br J Poult Sci* 2006;**47**:379–91.