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BACTERIA AND HORMONES: WHY THE SCIENCE OF MICROBIAL ENDOCRINOLOGY MATTERS TO DISEASE AND WELL-BEING

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SUMMARY

Nearly 95% of the cells that constitute an individual are prokaryotic and reside in the gut. Additionally, bacteria are as well abundantly present on external surfaces such as the skin. The recognition that these microorganisms, whether commensal or pathogenic, can actively synthesize and respond to neuroendocrine hormones that had previously been viewed as belonging to eukaryotic systems, offers the potential to revolutionize our concept of not only disease causation, but also maintenance of homeostasis. This convergence of seemingly disparate fields, microbiology, endocrinology and neurophysiology, is the emerging translational medicine discipline known as microbial endocrinology and had at its beginnings the demonstration that elaboration of stress-related neuroendocrine hormones by the nervous system innervating the intestinal tract could be utilized as environmental signals by bacteria whether within the gut or on surfaces such as the skin to initiate growth and produce virulence factors as part of the infectious disease process. Although initially met with scepticism, this interdisciplinary concept that a *direct* bacterial-neuroendocrine hormone interface (based on evolutionary inter-kingdom signalling) could contribute to infectious disease pathogenesis has now begun to shape medical thought regarding infectious disease and maintenance of general health.

INTRODUCTION

The ability of stress to alter the pathogenesis of infection has been documented in human and animal studies since the early 1900's (*Cohen* and *Williamson*, 1991; *Peterson* et al., 1991). Depending on the stressor employed and the infectious agent chosen for challenge, stress has been shown to either increase, decrease or not affect survival (*Peterson* et al., 1991). Since stress-induced activation of the neuroendocrine system has been amply documented to affect immune competence (*Bateman* et al., 1989), it has been generally concluded that the ability of stress to alter the pathogenesis of infection must be mediated by neuroendocrine-immune interactions. In the majority of publications the infectious organism is perceived to be somewhat of a bystander since any observed differences in infectivity due to stress are explained in terms of neuroendocrine suppression or enhancement of immune system function. That the infectious organism itself may be equally respond-

ing to the neuroendocrine outflow resulting from the stress event is usually not considered. Indeed, in the current conceptual framework of psychoneuroimmunology as a triad composed of stress, endocrine and immune components (*Ader* et al., 1995), the infectious agent is not generally included.

It is not the intent of this paper to obviate a role for stress-induced modulation of immunity in the pathogenesis of infection. The collective data demonstrating neuroendocrine mediation of immune responsiveness is overwhelming (*Ader* et al., 1995; *Bateman* et al., 1989; *Cohen* and *Williamson*, 1991; *Peterson* et al., 1991). Indeed, the development of the present microbial endocrinology theory regarding direct, non-immune, interactions between the neuroendocrine system and bacteria in the pathogenesis of infectious disease is a direct outgrowth from work in my laboratory on the ability of social conflict stress to modulate immune responsiveness (*Lyte* et al., 1990a, 1990b, 1991). The results from these studies have demonstrated that the immune compartments most affected by social conflict stress are those concerned with first-line protection against infectious challenge, notably the mononuclear phagocyte system. A dramatic increase in the phagocytic ability of splenic and lung macrophages was observed in stressed mice as compared to handled and home cage controls (*Lyte* et al., 1990a, 1990b). From these results it was expected that susceptibility to infection should be decreased in stressed mice since components of the first line defence against infection were enhanced by the stress of social conflict. Surprisingly, this was not found to be the case (*Lyte*, 2010a). Stressed mice were more susceptible to infection with the rapid proliferation of bacteria occurring within hours upon entrance into the social conflict stressed, but not handled control, host (*Lyte*, 2010a).

These seemingly contradictory results led to the consideration that microorganisms may be equally capable of responding to the neuroendocrine outflow resulting from the stress event as compared to immune cells (*Lyte*, 1993). Could direct effects of hormones on the pathogenicity of the infectious microorganism provide as equally compelling an explanation for stress induced-alterations of infectivity as that currently obtained with the effects of stress on immune cell function? This question essentially represented the beginning of the microbial endocrinology theoretical framework (*Lyte*, 2010a).

RELEVANCE OF MICROBIAL ENDOCRINOLOGY FOR UNDERSTANDING THE ROLE OF STRESS IN INFECTIOUS DISEASE

The range of hormones and the variety of microorganisms in which they have been identified is very large (*Lenard*, 1992). The presence of insulin in microorganisms has been the most extensively documented with its biological activity demonstrated in every microorganism examined to date (*Lenard*, 1992). Other hormones isolated from microorganisms which have been demonstrated to show biological activity in mammalian cells include corticotropin from *Tetrahymena pyriformis* (*LeRoith* et al., 1982), somatostatin from *Bacillus subtilis* (*LeRoith* et al., 1985) and *Plasmodium falciparum* (*Pan* et al., 1987) and progesterone from *Trichophyton mentagrophytes* (*Schar* et al., 1986). Numerous other hormones identified by radioimmunoassay and chromatographic behaviour, as well as the presence of the corre-

sponding putative receptor, have also been demonstrated in various microorganisms. A recent comprehensive analysis by Roshchina (*Roshchina*, 2010) of the wide spectrum of neurohormones and related cognate receptors that have been isolated form microorganisms highlights the presence in microorganisms of what are otherwise thought to be more commonly associated with mammalian systems (*Roshchina*, 2010).

Investigators have debated the significance of such hormones in microorganisms for decades. The most widely accepted theory concerns the use of such hormones as a form of intercellular communication (*Dohler*, 1986; *Lenard*, 1992; *LeRoith* et al., 1986). Indeed, recent studies have shown that the growth of colonies of *Escherichia coli* involves a high degree of specialization of function by individual bacteria (*Shapiro* and *Hsu*, 1989) and presumably the need for some form of intercellular communication to accomplish this goal. This concept of microbial hormones serving in some capacity as a primitive vertebrate nervous system can further be expanded to the realm of infectious disease in man (*Lyte*, 2010b). According to a microbial endocrinology-based approach, microorganisms entering into a stressed host could utilize the hormones produced during the stress event as environmental cues by which they would sense their surroundings. The development of pathogenicity would then, in part, depend on the ability of the particular microorganism to respond to the type of hormonal environment that it encounters upon entrance into the host.

EVOLUTIONARY CONSIDERATIONS IN MICROBIAL ENDOCRINOLOGY

The evolution of microorganisms preceded that of vertebrates such as man. It is perhaps somewhat surprising to learn that the presence of what are thought to be almost exclusively vertebrate neurotransmitters are in fact widely dispersed throughout nature. For example, in addition to its presence in vertebrates, norepinephrine has been additionally identified in plants (*Smith*, 1971), insects (*Pitman*, 1971), and fish (*Guerrero* et al., 1990). The ubiquitous distribution of norepinephrine throughout nature suggests that microorganisms in general have had ample time preceding the evolution of man to come into contact with a wide spectrum of hormones and develop mechanisms by which to synthesize as well as recognize hormones.

The widespread presence of neurohormones in the environment and in the microorganisms themselves therefore suggests that recognition of mammalian hormones might serve as a type of environmental signal by which microorganisms may sense their surroundings and initiate pathogenic processes. Considering that the gastrointestinal tract has abundant neuronal innervation with a high amount of elaborated neurochemicals such as the stress-related catecholamine dopamine (*Eisenhofer* et al., 1997) and that the majority of infections are acquired via the per oral route, it would seem therefore reasonable to suggest that the hormonal environment that a microorganism encounters upon entrance into the host may play a role in determining susceptibility to infection.

The possibility has been suggested that many of the mammalian cell-to-cell signalling systems that involve

small rapidly diffusible molecules such as the compounds that comprise communication within the neuroendocrine system arose from horizontal gene transfer from bacteria (*Iyer* et al., 2004). As such, it should not be surprising to learn that the complete biosynthetic pathway for catecholamines that is well known in eukaryotic systems is also present in bacteria (*Iyer* et al., 2004).

NEUROPHYSIOLOGICAL ALTERATIONS ACCOMPANYING INFECTION

Numerous reports have documented that dramatic elevations in the levels of plasma catecholamines occur during the course of infection with Gram-negative bacteria (*Benedict* and *Grahame-Smith*, 1978; *Groves* et al., 1973; *Jones* et al., 1988). Gruchow (*Gruchow*, 1979) observed that elevated levels of stress-related catecholamine activity as measured by 3-hydroxy-4-methoxy mandelic acid excretion preceded the development of acute infectious disease episodes. Groves and colleagues (*Groves* et al., 1973) observed that the levels of norepinephrine and epinephrine were significantly higher in postoperative patients that developed severe septic conditions than in patients with uncomplicated postoperative recovery. Benedict and Grahame-Smith reported that although plasma norepinephrine levels were elevated in patients with septic shock, these levels did not directly correspond with alterations in blood pressure or heart rate as would have been expected (*Benedict* and *Grahame-Smith*, 1978). Animal studies have also documented that several-fold elevations in catecholamines occur as a consequence of infection. For example, following challenge of conscious rats with live *E. coli* survival was negatively correlated with increasing levels of norepinephrine (*Jones* et al., 1988).

The origin of the increased peripheral sympathetic outflow during infection is complex and not yet well understood. Both adrenal and postganglionic sympathetic sources are believed to contribute to the overall rise in the catecholamines with the adrenal medulla contributing the major portion of epinephrine. The proportion of norepinephrine contributed by the adrenal medulla versus the amount released by peripheral sympathetic nerve endings is not certain since the majority of reports have solely examined plasma catecholamine levels. Complicating the understanding of the relative contribution of each to the overall increase in norepinephrine has been the fact that animal models employing live infection have often yielded different results than that employing endotoxin administration. Further, it should be remembered that although the majority of human and animal studies have documented the levels of catecholamines present during sepsis by determination of plasma concentrations, these levels reflect a spillover phenomenon and as such grossly underestimate the amount of catecholamines that may be present within an organ (*Kopin* et al., 1984; *Kovarik* et al., 1987).

Considering, then, that within the microenvironment of the tissue adequate levels of neurohormones may be available to an infectious microorganism upon entrance into the host could the pathogenesis of the resulting infection somehow related to the neurophysiological response of the host (*Lyte*, 1993)? Can the infectious agent

actively utilize the hormonal products of the host's neurophysiological response to stress, such as the elaboration of norepinephrine, to its own advantage?

HISTORICAL EVIDENCE SUGGESTING A ROLE FOR MICROBIAL ENDOCRINOLOGY IN INFECTIOUS DISEASE

The ability of neuroendocrine hormones to influence the *in vivo* growth of pathogenic bacteria was first observed in 1930 (*Renaud* and *Miget*, 1930). Prior to the advent of disposable syringes, metal needles and glass syringes were reused constantly between patients with only a cursory cleaning in alcohol. Patient to patient transmission of infectious disease was frequently encountered due to the inadequate alcohol treatment of syringe needles, which could only marginally kill actively growing (vegetative) bacterial cells, but not bacterial spores. As is well understood today, certain vegetative bacteria such as *Clostridium perfringens*, the causative agent of gas gangrene, can undergo sporulation. Such spores, which are formed from the vegetative cells under conditions of nutritional deprivation, are totally resistant to alcohol treatment and can only be killed by autoclaving.

Starting in 1930 reports associating the use of contaminated needles with administration of epinephrine solutions in the development of rapidly disseminating infections began to appear (*Brocard*, 1940; *Cooper*, 1946; *Renaud* and *Miget*, 1930). A previously used syringe needle to treat a gas gangrene patient was then used to administer epinephrine to a patient for urticaria (*Renaud* and *Miget*, 1930). Within 6 hours a fatal fulminating gas gangrene infection developed. These reports noting the rapidity of infectious spread in patients receiving epinephrine injections with contaminated needles led A.A. Miles in 1948 to begin a series of experiments examining the role of catecholamines in bacterial pathogenesis (*Evans* et al., 1948). In these experiments, the ability of epinephrine to modulate the *in vivo* growth of both Gram-positive and Gram-negative bacteria in a guinea pig model was conclusively demonstrated in tissue slices with enhancement of growth of bacteria co-injected with epinephrine that was log orders greater than that for control slices co-injected with saline (*Evans* et al., 1948). The authors concluded that the ability of epinephrine to dramatically enhance bacterial growth was due to some protective coating of the bacteria by epinephrine or an epinephrine-induced inhibition of immune cell function. The testing of each of these possible mechanisms, however, met with failure (*Evans* et al., 1948). Significantly, at no time did these authors or others suggest that the action of epinephrine on bacterial growth was due to a direct, non-immune effect as is currently proposed (*Lyte*, 1993, 2010a).

Interestingly, one of the frequently used techniques by microbiologists to enable gas gangrene infections to "take" in mice has been the co-injection of epinephrine along with *C. perfringens* (*Traub* et al., 1991). It can reasonably be inferred that this practice dates back to the decades old reports described above. It should also be appreciated that much of the historical evidence that has been proposed as the basis for microbial endocrinology rests in reports that have shown over the last 70 years that the intersection of bacteria with endocrinology, and for that

matter neurophysiology, can provide unexpected results. For example, it is well known that the emergence of the worldwide market for anxiety reducing drugs such as diazepam owe their development directly to the first muscle relaxant mephenesin (*Berger*, 1969). What is probably less well recognized is that mephenesin was originally developed as an antibiotic for use against Gram-negative bacteria during World War II and only through serendipity was it observed to cause muscle relaxation in mice (*Berger*, 1969).

RECENT EVIDENCE TO SUPPORT A ROLE FOR MICROBIAL ENDOCRINOLOGY IN INFECTION

The possibility that an infectious organism may respond to such neuroendocrine signals should not seem that unlikely. Work starting in the early 1990's demonstrated that the catecholamines can profoundly influence the *in vitro* growth of Gram-negative bacteria (*Lyte* and *Ernst*, 1992, 1993). Norepinephrine, in particular, was shown to increase the growth of members of the *Enterobacteriaceae* and *Pseudomonadaceae* families. Importantly, this effect of catecholamines on bacterial growth was shown to non-nutritional in nature. If the effect was simply a nutritional one, then epinephrine which has one more carbon atom than norepinephrine and hence can serve as a better energy source should have at least, if not better, ability to modulate bacterial growth when compared to norepinephrine. The results, however, conclusively demonstrated that an order of potency for the effect of the various catecholamines on growth existed for some of the Gram-negative bacteria such as *Y. enterocolitica* responding only to norepinephrine (*Lyte* and *Ernst*, 1992). Studies involving the use of α and ß adrenergic agonists and antagonists, as well as the less physiologically active enantiomer of norepinephrine, (+)-norepinephrine, suggested that a non-α, non-ß adrenergic receptor mediated process may play a role in norepinephrine-induced growth of Gram-negative bacteria (*Lyte* and Ernst, 1993).

More recent studies by a number of groups have confirmed and extended these early reports of direct stimulation of microbial growth by stress-related neurochemicals in both *in vitro* as well as *in vivo* systems (*Bearson* and *Bearson*, 2008; *Burton* et al., 2002; *Freestone* et al., 2000; *Kinney* et al., 2000; *Lyte*, 2004; *Oneal* et al., 2008; *Pullinger* et al., 2010a, 2010b; *Rahman* et al., 2000; *Reissbrodt* et al., 2002; *Sperandio* et al., 2003; *Vlisidou* et al., 2004). However, it should be noted that not all biogenic amines have similar effects on bacterial growth. For example, norepinephrine and dopamine, but not the indoleamine serotonin, increased the growth of a number of Gram-negative enteric pathogens (*Lyte* and *Ernst*, 1992; *Lyte*, 1997). The ability of stress-related biogenic amines to influence virulence-related properties of bacteria was also observed in the enterotoxigenic *E. coli* B44 strain where the production of the K99 pilus adhesin, which is involved in attachment and penetration of the bacterium into the intestinal mucosa, was shown to be increased in the presence of norepinephrine (*Lyte* et al., 1997).

Other reports have also noted the possible interaction between the host's endocrine environment and the proliferation of microorganisms. Intracellular steroid binding proteins in yeasts such as estradiol in *Coccidioides immitis* has led to the suggestion that mammalian

hormones may influence the proliferation of fungal infections especially in pregnant women (*Powell* et al., 1983). Interestingly, the host neuroendocrine environment may have opposite effects regarding the proliferation of a microorganism. For example, binding of the ß-adrenergic receptor on the surface of the protozoa *Trypanosoma cruzi* leads to an increase in cAMP levels and a subsequent inhibition in its rate of proliferation and differentiation (*de Castro* et al., 1987). As mentioned previously, insulin has been observed in every microorganism examined to date (*Lenard*, 1992). In patients with pre-existing diabetes mellitus, the pathogenesis of the rodent-borne infectious disease melioidosis has been shown to be dependent on serum insulin levels. Binding studies involving the causative agent of melioidosis, *Pseudomonas pseudomallei*, have demonstrated the presence in the bacterium of a specific, high affinity binding site for insulin (*Woods* et al., 1993).

Taken as a whole, these reports strongly suggest that an infectious organism may respond to a wide range of host neuroendocrine signals in an effort to establish a productive infection in the face of a competent immune system. The direct effect of neurochemicals on infectious agent replication may provide as equally compelling an explanation for stress-induced alterations of infectivity as that currently obtained with the effects of stress on immune cell function. Therefore, the consideration of the infectious process from a microbial endocrinological perspective may provide new insights into the mechanisms by which stress can alter host susceptibility to infectious challenge.

MICROBIAL ENDOCRINOLOGY AND INFECTIONS OF INDWELLING MEDICAL DEVICES

One of the foremost applications of microbiology endocrinology to infectious disease is in the intensive care clinical setting. The ability of bacteria to colonize indwelling medical devices, such as central venous catheters (CVCs), is recognized as the most common infection encountered in the intensive care setting (*Rello* et al., 1994). The incidence of nosocomial infections has been estimated at approximately 2 million cases per year with approximately half of those being associated with indwelling medical devices such as CVCs (*Darouiche*, 2004). The majority of catheter-associated nosocomial infections are caused by coagulase-negative staphylococci (C-NS), the normal skin commensal *S. epidermidis* being responsible for 50-70% of reported cases (*Rupp* and *Archer*, 1994).

In an effort to combat CVC-related infections and the possible subsequent progression to catheter-related bloodstream infection (CRBSI) (*Crnich* and *Maki*, 2001), the use of antimicrobial coated catheters has been proposed and evaluated in a number of clinical studies (*Crnich* and *Maki*, 2002, 2004; *Darouiche* et al., 1999; *Geffers* et al., 2003; *Marciante* et al., 2003; *McConnell* et al., 2003). However, concerns about the efficacy of antimicrobial-coated CVCs in the prevention of CRBSI remains (*McConnell* et al., 2003) as well as does their possible contribution to the emergence of drug-resistant clones (*Sampath* et al., 2001; *Tambe* et al., 2001). The environmental factors that may influence *S. epidermidis* adherence and biofilm formation are largely unknown (*Costerton* et al.,

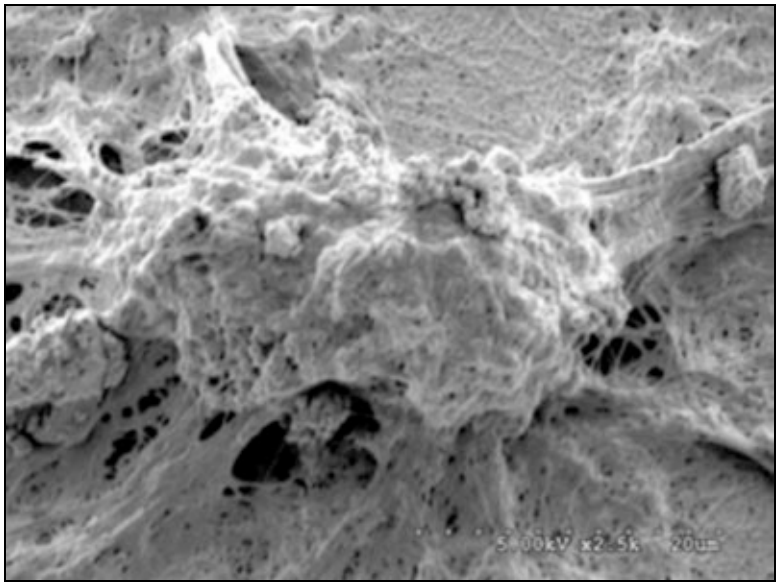

Figure 1: *S. epidermidis* biofilm formation in plasma-supplemented medium in the presence of the stress-related catecholamine norepinephrine. Little, or no, detectable biofilm formation was observed for the strain incubated in plasma-supplemented medium in the absence of catecholamines (control) cultures (not shown).

1999; *Watnick* and *Kolter*, 2000). Factors extend from disinfectants at the bedside (*Knobloch* et al., 2002) to the therapeutic drugs infused through catheter lines (*Lyte* et al., 2003). The examination of this latter environmental factor (*Lyte* et al., 2003) led to the application of the microbial endocrinology hypothesis to the study of the mechanisms governing bacterial colonization of indwelling medical devices. Specifically, could the drugs which are given to critically-ill patients for the maintenance of cardiac and renal function, namely the catecholamine inotropes, themselves be contributing to the ability of *S. epidermidis* to establish biofilms on CVCs as well as to the development of antimicrobial resistance and CRBSI.

Work from my laboratory has shown that catecholamine inotropic drugs may indeed serve through microbial endocrinology-based mechanisms as an aetiological factor in the bacterial colonization of indwelling medical devices due to their ability to stimulate *S. epidermidis* growth and biofilm formation (*Lyte* et al., 2003; *Neal* et al., 2001). The exposure of *S. epidermidis* to pharmacologically relevant concentrations of the widely used inotropic drugs, dobutamine and norepinephrine, resulted in increased biofilm growth and production of exopolysaccharide (the cellular excreted "glue" that holds cells in a biofilm together) as shown by both scanning electron microscopy and immunofluorescence (*Lyte* et al., 2003) and Figure 1. This demonstration of microbial endocrinology-based interactions in the development of medical important infections in patients receiving indwelling medical devices has led to the call for the design of new inotropic drugs that do not stimulate bacterial growth (*Singer*, 2007). Most recent has been the extension of these results in indwelling medical devices to the application of microbial endocrinology

to the design of new treatment modalities for even greater serious medical conditions such as post injury systemic immune response syndrome (*Lyte*, 2009).

CONCLUSION

There is growing recognition that inter-kingdom signalling, such as that exemplified by neuroendocrine hormones that are shared by both prokaryotic and eukaryotic cells, represents an interdisciplinary approach to understanding the biological processes that influence the pathogenesis of infectious disease as well as homeostasis. The recognition of this interdisciplinary field, which has been termed microbial endocrinology, represents the intersection between microbiology and neurobiology. The relevance of microbial endocrinology to health and infectious disease is increasingly being recognized by the medical community (*Everest*, 2007; *Singer*, 2007; *Stewart*, 2003) as well as the publication of the first dedicated to this sub-discipline within microbiology (*Lyte* and *Freestone*, 2010).

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