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STRESS, BACTERIA AND THE HPA AXIS

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The effect of gut microbiota on a wide variety of systemic and behavioural activities is well characterised. Gut microbes are even responsible for locust swarming (*Dillon* et al., 2000): Three organisms commensal to the host all produce guiacol, an essential component of the locust aggregation pheromone, from vanillic acid derived from plants on which they feed. Many other examples exist and include the fact that conventionalisation of germ-free mice increases fat storage by 60% through suppression of synthesis of a natural epithelial inhibitor of lipoprotein lipase (fasting intestinal adipocyte factor) (*Backhed* et al., 2004). Monoassociation of germ-free animals completes full maturation of T and B lymphocyte immune populations as well as organised lymphoid tissue (*Jiang* et al., 2004). Monoassociation of germ-free mice stabilises an exaggerated HPA axis response to stress (*Sudo* et al., 2004).

Bacteria communicate with each other through signalling molecules. Quorum sensing is a mechanism whereby bacteria can sense their environment including the bacterial biomass. These signalling molecules (auto-inducers) were first identified in marine bacteria such as *Vibrio fischeri* in the Hawaiian squid (*Euprymna scolopes*) (*Visick* et al., 2000). A particular auto-inducer (A1-2) is essential for the generation of the bioluminescent light organ in the squid. Moments after hatching the *V. fischeri* monospecifically colonises the light organ of its symbiotic partner at high density. The bacteria are essential for normal development of the light organ and the bacteria must cause swelling of the epithelium lining the crypts of this structure since mutants that colonise but do not cause swelling induce poor light organ development (*Koropatnick* et al., 2004). A fragment of the bacterial surface peptidoglycan, tracheal cytotoxin and LPS are responsible. This cytotoxin is pathogenic in humans and causes extensive damage to local tissues in whooping cough and gonorrhoea. The A1-2 acts as a cell density sensing signal and regulates expression of up to several hundred genes. Pathogenic *E-coli* 0157 also make A1-3 which regulates flagellar motility and secretion of proteins and restores a mutant *V. fischeri* (LUX S mutant), deficient in A1-2 and A1-3 to full protein secretion and motility.

It is therefore particularly interesting that catecholamines, part of the HPA axis response to stress, have been known since the 1930's to promote bacterial growth (*Lyte*, 2004). Nor-epinephrine restores the LUX S mutant to full capacity and this action is blocked by both ∀ and ∃ adrenergic receptor antagonists but this blockade did not affect growth (*Lyte*, 2004; *Sperandio* et al., 2003; *Winzer* and *Williams*, 2003). Since these adreno-receptors are not found in the organisms, A1-3 and nor-epinephrine are presumed to be recognised by the same bacterial receptors but these must be distinct from the ∀ and ∃ adrenergic receptors. *Lyte* (2004) has for a number of years suggested that activation of the HPA axis and production of catecholamines produces up-regulation of growth of potentially pathogenic organisms and activation of

their virulence genes, which in turn, through a complex cascade may lead to sepsis in susceptible individuals such as in patients undergoing surgery or in intensive care.

The stress response in germ-free mice is exaggerated relative to conventional animals (*Sudo* et al., 2004). Monocontamination of these animals leads to a relatively blunted, but normal, HPA axis response with respect to ACTH and corticosterone levels when animals are subjected to restraint stress. This exaggerated response can be restored to normal by monocontamination with certain organisms or with faeces from SPF animals. This restoration only occurs in animals which have been so conventionalised within 3 weeks of birth. Similarly handling of neonates dampens the adult HPA axis in response to stress if this occurs in the same neonatal period (*Meaney* et al., 1988). Maternal deprivation in conventional neonates however results in an increased HPA axis response to stress in adults. The effect of maternal deprivation in monkeys has been studied by *Bailey* and *Coe* (1999) who have shown a decrease in the number of aerobic lactobacilli in faeces of such animals within 3 days of separation. Prenatal maternal stress alters intestinal microbiota in Rhesus monkeys and this has been shown to occur particularly if the stress occurs late in pregnancy, i.e., between 15 and 24 weeks (*Bailey* et al., 2004). Anaerobic bifidobacteria were only reduced if stress occurred late in life whereas anaerobic lactobacilli were reduced in number both in early as well as in late stress.

The role of the HPA axis in susceptibility to experimental arthritis induced by streptococcal cell wall has been shown to be crucially important by *Sternberg* et al (1989). Lewis rats are susceptible to induction of arthritis whereas Fischer rats are not. The Lewis strain has impaired plasma corticotropin releasing hormone (CRH) as well as corticosterone responses to streptococcal cell wall, IL-1 and CRH. The hypothalami of Lewis rats respond poorly relative to Fischer hypothalami in CRH development to IL-1, acetylcholine, norepinephrine and a 5HT agonist (*Calogero* et al., 1992). Thus the HPA axis may be blunted because of a genetic background and render the animal susceptible to inflammatory events. These observations may have far reaching implications for inflammatory diseases (*Shanks* and *Lightman*, 2001).

Shanks et al. (2000) has additionally shown that exposure of Sprague Dawley rats to endotoxin in the neonatal period alters the adult HPA and immune responses to stress and inflammation. Specifically, rats injected on day 3 and 5 with endotoxin had greater mean corticosterone levels in adulthood but greater suppression of splenocyte proliferation to LPS. Most importantly Lewis rats were protected from adjuvant arthritis development if pre-treated in the neonatal period with endotoxin, suggesting that early encounters with LPS in the neonatal period could set the "tone" of the HPA axis in adult life.

SUMMARY AND CONCLUSIONS

Thus enteric microbiota can influence the HPA axis at a critical period in neonatal life and cause a permanent change as exemplified by these experiments and even in animals genetically predisposed to lowered HPA axis responses to stress. Similarly psychological stress (maternal deprivation) of neonates results in increased HPA axis responses in adulthood and this can be prevented by handling the animals during deprivation in the neonatal period. Amazingly the

molecular basis for these observations has been recently identified (*Weaver* et al., 2002). *Weaver* et al. (2004) have shown that increased grooming/licking in rats altered the offspring epigenome, at a glucocorticoid receptor gene promoter in the hippocampus. This particular epigenetic effect was reversible by cross-fostering and again persisted into adulthood. The effects of stress both prenatally and postnatally on the numbers of enteric commensal bacteria such as bifidobacteria and lactobacilli have also been observed (*Bailey* and *Coe*, 1999; *Bailey* et al., 2004). Some of the hormones induced by stress such as catecholamines may have both direct and indirect effects on enteric bacterial type and number as well as their functional activation. The known effects of catecholamines on the quorum sensing system of certain bacteria may in part explain this latter phenomenon.

Thus the interaction between bacteria and the HPA axis is bidirectional and should be taken into account when explorations of the HPA axis are undertaken and examined.

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