Comparative Aspects of the Endotoxin- and Cytokine-Induced Endocrine Cascade Influencing Neuroendocrine Control of Growth and Reproduction in Farm Animals

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Contents
Disease in animals is a well-known inhibitor of growth and reproduction. Earlier studies were initiated to determine the effects of endotoxin on pituitary hormone secretion. These studies found that in sheep, growth hormone (GH) concentration was elevated, whereas insulin-like growth factor-I (IGF-I) was inhibited, as was luteinizing hormone (LH). Examination of the site of action of endotoxin in sheep determined that somatotropes expressed the endotoxin receptor (CD14) and that both endotoxin and interleukin-1β activated GH secretion directly from the pituitary. In the face of elevated GH, there is a reduction of IGF-I in all species examined. As GH cannot activate IGF-I release during disease, there appears to be a downregulation of GH signalling at the liver, perhaps related to altered nitration of Janus kinase (JAK). In contrast to GH downregulation, LH release is inhibited at the level of the hypothalamus. New insights have been gained in determining the mechanisms by which disease perturbs growth and reproduction, particularly with regard to nitration of critical control pathways, with this perhaps serving as a novel mechanism central to lipopolysaccharide suppression of all signalling pathways. This pathway-based analysis is critical to the developing novel strategies to reverse the detrimental effect of disease on animal production.

Introduction
Gram-negative bacterial infections such as Escherichia coli (E. coli), Salmonella, Pseudomonas and Proteus tend to occur frequently in farm animals. Common portals of entry for these Gram-negative (endotoxin-containing) bacteria include, but are not limited to, the gastrointestinal system, the reproductive tract (especially uteri of postpartum animals), and the mammary gland. Infections of these systems or organs result in some of the most common and costly diseases of production/farm animals such as enteritis, endometritis/metritis and mastitis. Regardless of the chronicity, infections in farm animals with bacteria containing endotoxin, and the cytokine and endocrine changes that result, inevitably impact growth, metabolism and reproduction in a negative manner.

Acute diarrhoea, often caused by Gram-negative bacteria such as enterotoxigenic E. coli and Salmonella, is a common disease in newborn calves and accounts for more than 50% of pre-weaning deaths in intensively raised calves (USDA 1996). However, pre-weaning mortality of farm animals may be just the beginning of economic losses secondary to enteritis caused by Gram-negative bacteria. Other consequences of neonatal diarrhoea are as follows: (1) greater morbidity secondary to increased susceptibility to other pathogens, (2) impaired growth rates due to reduced food intake and metabolic disturbances and (3) delayed puberty and decreased reproductive productivity (production of offspring and milk) as a result of poor growth rates and inadequate energy stores.

The outcomes of bacterial infection associated with the postpartum uterus include puerperal metritis, clinical endometritis, pyometra and subclinical endometritis (Sheldon et al. 2006). Arcanobacterium pyogenes (A. pyogenes), E. coli and other Gram-negative bacteria, namely Fusobacterium necrophorum (F. necrophorum) and Bacteroides spp., are predominant in the uterus of clinically diseased animals (Hirvonen et al. 1999). These common forms of reproductive tract diseases in farm animals (especially dairy cows) may delay the complete regeneration of endometrium, disrupt the resumption of cyclic ovarian function resulting in postponement of the first insemination, increase the numbers of inseminations per conception and thus prolong the calving interval and decrease the calving rate (Hussain and Daniel 1991). It is clear that uterine infections and consequential diseases have detrimental effects on reproductive performance of dairy cows. As most clinical and reproductive consequences are attributed to the presence of A. pyogenes in combination with organisms like E. coli and other Gram-negative bacteria, a better understanding in pathogenesis and the mechanisms involved is of great practical and economic importance.

Mastitis is one of the major bacterial diseases in postpartum farm animals, especially dairy cows. In the early weeks of lactation, Gram-negative bacteria may be the predominant mastitis pathogen. Clinical cases of Gram-negative mastitis load the hosts with endotoxin. In lactating cows, marked changes in plasma levels of certain energy-related metabolites were reported simultaneous with the endotoxin-induced endocrine alterations. Concentrations of glucose tended to increase initially then subsequently declined and there was a tendency for increased non-esterified fatty acid values, whereas plasma β-hydroxybutyrate (BHB) decreased linearly in a dose-dependent manner after lipopolysaccharide (LPS) infusion (Waldran et al. 2003).

In addition to endotoxin from Gram-negative bacteria causing metabolic perturbations, abnormal metabolic status of farm animals can influence their response to infection. Epidemiological studies have demonstrated interrelations among negative energy balance-related metabolic disorders (hepatic lipidosis and ketosis), the increased incidence of clinical mastitis and the
subsequent decrease in reproductive performance in high-producing postpartum dairy cows (Valde et al. 1997; Washburn et al. 2002). Some trials confirmed the indirect negative impact of clinical and subclinical mastitis on reproductive performance (Barkter et al. 1998), whereas others revealed direct mastitis-induced abnormalities in ovarian function (Moore et al. 1991; Hockett et al. 2000; Huszenicza et al. 2005). Understanding the mechanisms involved in how endotoxin interacts with reproduction, metabolism and endocrinology may lead to unique strategies to reverse the negative effects of infectious disease on farm animals and humans.

Understanding Endotoxin and Neuroendocrine Regulation

While the term 'endotoxin' is often mistakenly applied to any toxin derived from the microbes, the technical definition is specific for the group of LPS complexes extractable from or released from the outer membrane of Gram-negative pathogenic and non-pathogenic species of bacteria such as E. coli, Salmonella, Neisseria, Mannheimia, Pseudomonas and others. For the most part, LPS complexes are rather stable and confined within the membrane of these bacteria under states of what might be called 'bacterial good health'. For example, upon infection with a Gram-negative pathogen, several interactions between the invading bacteria and cellular and biochemical factors in the immune system lead to the degeneration of the outer bacterial membrane with the consequent release of LPS into the host's internal environment. The most common causes for this release of LPS are bacterial autolysis, phagocytosis and digestion of bacteria by prowling activated immune cells such as macrophages and neutrophils, and exogenous lysis facilitated by LPS activation of the complement cascade and lysosome activity. Of concern in human clinical medicine is the recent confirmation that massive septic crisis can be further complicated acutely with the administration of certain antibiotics because of the mode of action of these drugs to facilitate cell wall breakdown with the resulting release of LPS. In this situation, untimely administration of drugs with modes of action like that of the penicillins might precipitate an acute crisis through the initiation of multiple organ failure. Though not a common occurrence, the scenario should be recognized as a potential confounding factor in the treatment of sepsis.

Endotoxin challenges metabolism through direct and indirect mechanisms. The direct effects of LPS, in the absence of cytokine production, on the release of pituitary hormones critical to the maintenance of metabolism as well as reproduction were demonstrated by Coleman et al. (1993). In this regard, some non-immune cells such as hepatocytes (Liu et al. 1998), adipocytes (Daniel et al. 2003) and pituitary cells (Daniel et al. 2005) differentially express the LPS binding receptor CD14 on the plasma membrane, providing a mechanistic explanation as to how cells might respond directly to LPS. Moreover, we have preliminary immunohistochemical evidence that the Toll-like Receptor 4 is also expressed on somatotropes (Elsasser and Sartin, unpublished). While hepatocyte CD14 was shown to upregulate following LPS challenge (Liu et al. 1998), somatotrope expression decreased and constitutive presence on gonadotropes, lactotropes and corticotropes was unchanged (Daniel et al. 2005). This recent discovery of the downregulation of CD14 on somatotropes after LPS release is consistent with an activation of the endotoxin receptor on the pituitary. In regard to the so-called indirect mechanisms of actions, the classical pattern of proinflammatory cytokines released from a challenged immune system functions in both an endocrine and paracrine manner to interact with specific receptors for these cytokines. This affects not only the metabolic character of target cells (liver, muscle and adipose), but also metabolic regulatory organs, the pituitary and pancreas in particular.

As we have reviewed earlier (Elsasser et al. 2000; Daniel et al. 2002; Elsasser and Kahl 2002; Carroll 2008), LPS elicits a well-timed elaboration of proinflammatory cytokines, prostaglandin derivatives, catecholamines and free radicals from neutrophils, monocytes and macrophages which, depending on the severity of the response, largely halt anabolic processes and initiate catabolic breakdown of tissue reserves. Most influential on metabolic processes and prototypical of the anti-anabolic character of the endotoxin proinflammatory response is the release of tumour necrosis factor-α (TNF-α). This relatively short-term effector has a unique influence on metabolism, but can also be considered as an initiator of further inflammatory response. Data indicate that the TNF-α response to infused or bolus-administered LPS is largely turned off after approximately 4–6 h. Regardless of the administration model, the TNF-α event initiates progression of additional proinflammatory cytokines such as IL-6, and also elaborates anti-inflammatory cytokines such as IL-4, γ-interferon and IL-10 (Fig. 1). Significant in this process are the homeostatic survival mechanisms associated with the development of what is characterized as early and late endotoxin tolerance (West and Heagy 2002; Elsasser et al. 2004). The response is dependent on

Fig. 1. Effect of LPS on plasma TNF-α, IL-6, IL-1 and INF-γ in calves. Calves were treated with endotoxin (arrow; 0.6 μg/kg BW), n = 5. Data are redrawn from unpublished data by the authors for illustrative purposes

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the timely generation of nitric oxide (NO) as driven by TNF-α and accompanied by translocation of NFκB to the nucleus which in turn attenuates transcription of the TNF-α gene. The result is a necessary turn-down or suppression of proinflammatory signalling elements that, if left unchecked, leads to free radical tissue damage (Zeisberger and Roth 1998).

**Sites of Endotoxin Action on Growth Hormone (GH) and Luteinizing Hormone (LH)**

Both GH and LH are produced and secreted by the pituitary under the influence of releasing hormones from the hypothalamus. Thus, LPS may influence circulating concentrations of GH and LH directly by altering production and secretion at the pituitary, or indirectly by altering production and secretion of releasing hormones at the level of the hypothalamus.

Endotoxin was reported to impair adenohypophysial LH release in rats (Rettori et al. 1994) and sheep (Coleman et al. 1993; Fig. 2). In cycling heifers receiving an experimental challenge 42 h after the PGF<sub>2α</sub> (dinoprost)-induced luteolysis (Suzuki et al. 2001), LPS reduced the pulse frequency of LH for 6 h, and increased the mean concentration and pulse amplitude of LH. Plasma concentrations of cortisol and progesterone were simultaneously and transiently increased due to the adrenocortical over-production of these hormones. Plasma oestradiol concentrations were decreased and the preovulatory LH pulse was delayed or completely blocked. A similar disruption was demonstrated in the preovulatory rise of oestradiol and in the secretory pattern of LH in ewes following endotoxin challenge (Battaglia et al. 2000). Endotoxin absorbed from the uterine lumen was reported to suppress the formation of the preovulatory LH peak and to induce the cystic degeneration of dominant follicles in post-partum cows (Peter et al. 1989). Also of particular importance in farm animals is the finding that neonatal exposure to LPS actually programs long-term sensitivity of the GnRH regulatory system, such that post-natal responses to LPS produce a greater inhibition of GnRH and LH (Li et al. 2007).

Endotoxin suppresses circulating concentrations of LH at the level of the hypothalamus (Coleman et al. 1993). This is supported as follows by three primary pieces of evidence. Firstly, portal vein cannulation indicates that LPS results in reduced secretion of GnRH from the hypothalamus, thus suggesting endotoxin inhibits circulating concentrations of LH by inhibiting hypothalamic stimulation of LH secretion (Battaglia et al. 1997). Secondly, the increased secretion of LH in response to LPS by dispersed pituitary cells (Coleman et al. 1993) further suggests that LPS acts at the level of the hypothalamus to inhibit circulating concentrations of LH. Finally, LPS challenge reduces electrical activity in areas of the hypothalamus associated with the generation of GnRH and thus LH pulses (Takeuchi et al. 1997; Yoo et al. 1997).

In contrast to the effects on LH, endotoxin’s effect on circulating concentration of GH is more complicated. In species where LPS decreases, the circulating concentration of GH (cattle and rat), the effect is primarily at the level of the hypothalamus. However, in species where LPS increases circulating concentrations of GH (sheep and human), the effect appears to occur primarily at the pituitary. In pigs, an acute increase in circulating concentrations of GH following an LPS challenge has been reported (Parrott et al. 1995; Hevener et al. 1997). However, this effect was only short-lived, and subsequent LPS-induced uncoupling of the GH/IGF-I axis persisted. Pituitary production and secretion of GH is primarily under the regulation of GH releasing hormone (GHRH) and somatostatin from the hypothalamus. In species where GH concentration is decreased by LPS, the effect is likely mediated through cytokine-induced stimulation of somatostatin production (Scarborough 1990). Sheep injected with LPS secrete GH during the same period when LH secretion is reduced (Fig. 2; Coleman et al. 1993). Challenge with LPS also results in an increase in somatostatin concentrations in hypophysial portal blood with no change in the concentrations of GHRH in sheep (Briard et al. 1998). Under normal physiological conditions, an increase in somatostatin accompanied with no change in GHRH would be expected to result in decreased circulating concentrations of GH. However, Briard et al. (1998) observed increased circulating concentrations of GH associated with increased hypophysial portal blood concentrations.

![Fig. 2. Effects of LPS on pulsatile patterns of LH and GH in sheep treated with LPS (Coleman et al. 1993)](image-url)
of somatostatin in sheep challenged with LPS (Briard et al. 1998). Thus, the effect of LPS to increase circulating concentrations of GH was not explained by altered hypothalamic function, and the effects of LPS to increase circulating concentrations of GH appear to be mediated at the pituitary. Further supporting a pituitary site of action, LPS challenge also results in increased secretion of GH from dispersed pituitary cells (Coleman et al. 1993).

Possible Mechanisms by Which Endotoxin Alters LH and GH

Multiple changes occur in response to endotoxin challenge which could potentially affect the decrease in circulating concentrations of LH. One key response to a disease challenge and stress in general is activation of the hypothalamic–pituitary–adrenal axis with an associated increase in glucocorticoids, particularly cortisol. Indeed, administration of cortisol will reduce circulating concentrations of LH observed following challenge with LPS (Debus et al. 2002) and GH (Thompson et al. 1995). However, inhibition of cortisol synthesis during the LPS challenge does not prevent LPS suppression of pulsatile GnRH and LH secretion (Debus et al. 2002). Thus, while cortisol may play a role in LPS-induced suppression of circulating concentrations of LH, other factors are also likely involved.

The endogenous opioid system may also be involved in LPS suppression of circulating concentrations of LH. Central administration of the opiate antagonist naloxone blocked LPS suppression of circulating concentrations of LH in monkeys (Xiao et al. 2000). In addition, administration of naloxone to heifers following LPS treatment resulted in increased circulating concentrations of LH (Kujjo et al. 1995). However, naloxone did not prevent the decrease in circulating concentrations of LH observed following challenge with E. coli in sheep (Leshin and Malven 1984). The inability of an opiate antagonist to block E. coli-induced LH suppression may be associated with the differential proinflammatory cytokine profiles observed in LPS vs E. coli challenge studies. For instance, in pigs, LPS is commonly known to induce the primary proinflammatory cytokines TNF-α, IL-1β and IL-6 (Carroll et al. 2003). However, in pigs challenged with live E. coli, TNF-α concentrations are not elevated (Strauch et al. 2004) indicating that the activation of the acute phase immune response is dependent upon the immunological challenge. Thus, while the endogenous opioid system may play a role in LPS suppression of circulating concentrations of LH, other systems are involved in the effect of Gram-negative bacterial challenge to suppress LH.

As discussed earlier, challenge with LPS and Gram-negative bacteria results in activation of the immune system. Components of the innate immune system may be responsible for the suppression of circulating concentrations of LH. Indeed, investigators have examined the role of the cytokines TNF-α and IL-1β as well as prostaglandins in LPS suppression of LH. Central administration of both TNF-α and IL-1β suppressed circulating concentrations of LH (Daniel et al. 2005). Central administration of IL-1β to monkeys also suppressed circulating concentrations of LH (Xiao et al. 2000). However, neither central nor peripheral administration of the cytokine antagonists TNF-R1 nor IL-1RA prevented the LPS-induced suppression of circulating concentrations of LH (Xiao et al. 2000; Priego et al. 2003). Thus, while increased concentrations of inflammatory cytokines may play a role in LPS suppression of circulating concentrations of LH, the mechanism for LH inhibition is clearly multifactorial.

In response to LPS challenge, prostaglandin production is also enhanced. Treatment with a prostaglandin synthesis inhibitor (flurbiprofen) prevented endotoxin suppression of LH and GnRH (Harris et al. 2000). Thus, prostaglandin formation is a crucial step in LPS suppression of pulsatile secretion of GnRH and LH. In contrast, the oestradiol-induced surge of LH was blocked by LPS, but the inhibition was found to function via prostaglandin independent pathways (Breen et al. 2004).

The mechanisms by which LPS alters circulating concentrations of GH are clearer than the means by which LPS suppresses LH. Early data suggested that in some species (rat), LPS administration results in suppressed circulating concentrations of GH. The reduced GH is due to increased somatostatin release in response to inflammatory cytokines, specifically IL-1, TNF and IL-6 (Scarborough 1990). In contrast, other species increase circulating concentrations of GH in response to inflammatory cytokines and to LPS administration. In this case, the site of action is the pituitary as opposed to the hypothalamus. More recently, this disparate effect of LPS between different species was evaluated by Priego et al. (2003). Rats administered low doses of LPS had increased plasma GH, whereas high doses were inhibitory to GH, indicating that differences observed were due to dose of LPS and not species differences. Thus, the typical model in rats is a model of endotoxic shock, whereas the model in sheep and humans tends to model a less severe disease.

Studies with dispersed ovine pituitary cells have found that treatment with IL-1 stimulated GH synthesis and secretion when TNF-α reduced GRH-stimulated GH release (Fry et al. 1998). Moreover, TNF-α will inhibit GH release from cultured bovine pituitary cells (Elssasser et al. 1991). In addition, peripheral administration of TNF-α and IL-1β resulted in increased circulating concentrations of GH (Daniel et al. 2005). Intravenous (IV) but not intracerebroventricular administration of the cytokine antagonists, sTNF-R1 or IL-1RA, prevented the LPS-induced increase in circulating concentrations of GH (Daniel et al. 2005). The differing effects of TNF-α between in vivo and in vitro models suggest that in vivo, TNF-α activates IL-1β release which in turn is a stimulus to GH. This increase in inflammatory cytokines in response to LPS plays a critical role in endotoxin-induced alterations in circulating concentrations of GH. Finally, Daniel et al. (2005) discovered that CD14 is expressed on somatotropes and is down-regulated in response to LPS injection, whereas the typical dogma suggests that the major effects on the rodent pituitary are via folliculostellate cells. In agreement with previous research (Coleman et al. 1993), these data also provide evidence that GH can be released from the somatotrope in response to direct exposure to LPS.
Another mediator which may be involved in the GH and LH response to LPS is ghrelin. Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor, which stimulates GH secretion (Kojima et al. 1998). In pigs, ghrelin has been reported to increase body weight gain and circulating concentrations of GH and insulin (Salfen et al. 2004). Recent data indicate that LPS challenge results in increased circulating concentrations of ghrelin in humans, a species with increased GH in response to LPS (Vila et al. 2007).

Perhaps an increase in ghrelin stimulates the increase in GH observed following an LPS challenge. In addition, ghrelin has been demonstrated to reduce circulating concentrations of LH in rats (Furuta et al. 2001), monkeys (Vulliemoz et al. 2004), sheep (Iqbal et al. 2006) and humans (Kluge et al. 2007). The possible relationship of ghrelin to the LPS inhibition of LH and stimulation of GH should be a focus of future research involving the mechanisms of LPS action in ruminants.

Endotoxin Disrupts GH Signalling

Initial studies demonstrated that the proinflammatory mediators of LPS action within the immune system mediated a reduction in circulating plasma IGF-1 concentrations and this reduction was independent of the decrease in voluntary food intake observed in the LPS-treated calves (Elsasser et al. 1995). The chronic decline of this key anabolic hormone during catabolic disease suggested that calves suffering weight loss from infectious diseases might benefit from the use of anabolic hormones to decrease the catabolic milieu. As such, studies were conducted to evaluate the actions of anabolic agents such as oestradiol and GH (Elsasser et al. 1998; Sartin et al. 1998). Elsasser et al. (1998) examined the possibility that treatment with exogenous GH could be used to overcome the effects of sarcocystis infection in cattle by increasing plasma IGF-1 concentrations and normalizing metabolism. However, GH had no ability to normalize plasma concentrations of IGF-I or other indices of metabolism. Since no problems were found with liver GH receptor functions, this lack of effect of GH was hypothesized to relate to altered GH signalling. In a series of follow-up studies using the LPS model, Elsasser et al. (2004, 2007a,b) determined that the activation of the major signal transduction regulator of GH action, protein tyrosine kinase JAK-2, by GH was reduced by infection, thus explaining the GH resistance observed during disease. In Fig. 3, a representative Western blot of liver homogenate proteins from control (before LPS, lane 1) and after LPS (24 h, 2.5 μg/kg E. coli 055:B5 LPS, lanes 2 and 3) biopsy samples. (Elsasser et al. 2007a)

This nitration response is that while it appears to be a transient phenomenon in states of normal health, the formation of nitrated JAK-2 is an overt and prolonged occurrence in chronic disease states. The demonstrated ability to specifically modulate this nitration response by altering the generation of nitric oxide and superoxide may prove useful to further pharmacological strategies that might be used to re-establish stable metabolism and mitigate the impacts of disease stress on animal health.

Conclusion

Many diseases common to farm and production animals are caused by infections with Gram-negative bacteria. The consequences of these infections are a result of the liberation of endotoxin/LPS from the bacteria and the reaction of the immune system/inflammatory cells. Cytokine (e.g. IL-1 and TNF-α) production by inflammatory cells in response to LPS is a normal and necessary function of the immune system in animals to prevent and alleviate infections. However, the inflammatory cytokines can also initiate a cascade of events that impair hormonal and metabolic homeostatic processes regulating growth, metabolism and reproduction. The purpose for impairing these functions is most likely a consequence of the lofty nutrient/metabolic demands for the immune system’s response to infections. In an attempt to redirect or conserve nutrients for immune functions, growth is impaired by direct or indirect actions of cytokines on the somatotropic axis. In adult animals, nutrients may be conserved by inhibiting energetically risky behaviour [i.e. reproduction (oestrus, pregnancy and lactation)] through manipulation of the hypothalamic–pituitary–gonadal axis at any point. More research is needed to completely understand the mechanisms behind the effects of disease stress on growth, metabolism and reproduction. While previous data on severe proinflammatory-mediated dysfunction were associated with stark pathology, the nitration concept follows closely with perturbations associated with low level responses to immune challenge that do not culminate in death. For example, the implications of the recent data by Elsasser et al. (2007a,b) suggest a novel mechanism for inhibition of endocrine signalling which should be examined as a possible unifying mechanism by which LPS actions suppress key functions in cells. By understanding the intricacies of LPS-induced cytokine release and how cytokines affect farm animal production, we can target more precisely the strategies needed to combat infectious agents as well as stabilize

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host responses to infection in order to speed recovery and improve animal welfare.

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