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ENDOCRINE MODULATION OF PHYSIOLOGICAL RESPONSES TO CATABOLIC DISEASE

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Disease or endotoxemia alters the plasma concentrations of anabolic hormones, particularly growth hormone (GH) and insulin-like growth-factor I (IGF-I). In general, these hormones are inhibited during the catabolic disease state. A hypothesis has evolved that anabolic hormones might be useful in patients' recovery under these and other catabolic circumstances. The treatment of cattle with GH has provided significant improvement in the physiological response of the animals to the subsequent injection of bacterial lypopolysaccharide (LPS), perhaps via inhibition of tumor necrisis factor (TNF) release. However, this improved response to disease was not observed with animals treated with GH and infected with one of two parasitic organisms, *Sarcocystis cruzi* or *Eimeria bovis*. Recent attempts with other anabolic hormones, estradiol and progesterone, have proven remarkably effective in improving the adaptive physiological responses of calves to either *E. bovis* infection or to the injection of LPS. All animals displayed signs of infection, but the intensity and duration of symptoms were reduced. Although a mechanism is not yet known, there were no effects on TNF; cortisol; the percentages of lymphocytes expressing CD2, 4, or 8 antigens; or the production of antibodies. © Elsevier Science Inc. 1998

INTRODUCTION

Lean tissue mass wasting in catabolic disease is associated with diminished immune function, increased infection rates, delayed tissue repair, decreased wound healing, and diminished skeletal muscle function (1). Catabolic disease processes also produce fat and protein mobilization (2) accompanied by a reduction in anabolic hormones such as growth hormone (GH) and insulin-like growth factor-I (IGF-I). Because the GH-IGF-I axis favors protein deposition and increased lean body mass, this suggests a hypothesis that anabolic hormones might prove effective in assisting in the recovery from catabolic states such as disease, trauma, and surgery.

METABOLIC CONSEQUENCES OF DISEASE

Tissue wasting is observed in many diseases and represents a complex interplay between anorexia, the need for additional energy, reprioritization of nutrients and the changing endocrine and cytokine environment. Immediately after a lipopolysaccharide (LPS) injection in cattle, there is a transient hyperglycemia (with a delayed insulin release) followed by hypoglycemia (3). Initial hyperglycemia results from increased glycogenolysis and gluconeogenesis in excess of tissue demand for glucose (4). Of interest is the increased uptake of glucose in tissues rich in macrophages (5), presumably to support

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antimicrobial activity (6). Additional energy for insulin-dependant tissues is supplied by free fatty acid and amino acid mobilization during catabolic diseases such as Sarcocystosis, Coccidiosis, or models of endotoxemia

Protein wasting is a serious consequence of anabolic disease and results in loss of key functions (see above; 1). A widely used indicator of protein catabolism is blood urea nitrogen (BUN) levels, which are elevated in endotoxemia (2,7,8) and in other disease models. This becomes particularly important in chronic catabolic disease where there is a progressive tissue wasting leading to reduced growth rates and lowered body weights. However, although there is generalized protein catabolism, all tissues are not equally affected. Elsasser et al. (9) reported that in cattle, protein accretion for rectus femoris muscle (a locomotor, fast twitch muscle) is relatively unaffected, whereas psoas major (postural, slow twitch fibers) was catabolized.

GH-IGF-1 RESPONSES TO DISEASE

Increased lipid and protein mobilization leading to progressive tissue wasting cannot be accounted for solely based on reduced food intake (1). One feature underlying changes in body protein and fat are the disease-induced alterations in GH and IGF-I. Accompanying the acute phase response to *Sarcocystis cruzi* infection in cattle is a significant reduction in plasma GH (10). Similar reductions in plasma GH were observed in cattle injected with LPS (11). In additional experiments, Elsasser et al. (12) demonstrated an effect of tumor necrosis factor (TNF) α to inhibit thyrotropin-releasing hormone-stimulated GH release in cattle, a similar response to that observed with LPS. Moreover, TNF receptors were found in the pituitary gland and TNF inhibited growth hormone-releasing hormone (GRH)-stimulated GH release from cultured bovine pituitary glands (12). These studies suggest that TNF may mediate some of the effects of LPS on GH regulation in cattle. Similar reductions were observed in GH release in response to LPS in rats and birds (13,14), however the mechanism for LPS inhibition of GH release in rats has been associated with interleukin-1 (IL-1)α (15) rather than TNF as in cattle (11,12). On the other hand, free fatty acids (16) and cortisol (17) can reduce plasma GH concentrations and both substances are elevated after LPS injection (3,9,11,12,15)

Interestingly, there are significant species differences in GH responses to LPS. Sheep (18) and humans (19,20) increase rather than decrease GH release after iv LPS injection, whereas pigs have a change in pulse frequency, but no change in mean plasma levels of GH (15). The LPS effect on GH release in sheep is mimicked by intracerebroventricular injection of TNF (21), suggesting LPS stimulates the release of central TNF to regulate GH release. Data also indicate that LPS can stimulate GH release from cultured sheep pituitary cells (22). Cytokines were examined for an ability to release GH from cultured sheep pituitaries and only IL-1β and IL-1α could release GH. IL-2, interferon-γ, and TNF did not influence basal release of GH from cultured sheep pituitary cells (22), although TNF did inhibit GRH-stimulated GH release as reported for cattle (12).

Many of the anabolic actions of GH are mediated by IGF-I. IGF-I concentrations are uniformly decreased by diseases and by endotoxin injection (1,9,10,20). For example, in cattle, plasma IGF-I concentrations were significantly reduced by infection with *S. cruzi* or *Eimeria bovis* (10,23). The reduction in plasma levels of IGF-I in Sarcocystis infected cattle were accompanied by reduced hepatic IGF-I mRNA content, but no changes in GH receptor binding or GH receptor mRNA (9). This suggests changes in gene regulation or an effect on GH receptor signal transduction mechanisms. Interestingly, plasma IGF-I and IGF-I mRNA remained reduced at 56 d postinfection, long after reduced food intake and other acute phase responses to infection had resolved (9). This suggests that the regulation of appetite and body weights are separable events and that disease can uncouple the

normal tight association between appetite and growth. The delay in IGF-I recovery from Sarcocystis infection relative to the recovery of appetite probably explains the stunting often observed with severe disease.

Sarcocystis miescheriana infection in pigs is also associated with reduced plasma concentrations of IGF-I (24). These experiments also examined IGF binding proteins (IGFBP) and found there was an increase in IGFBP-1, IGFBP-2, and IGFBP-4 (a similar response is observed in humans; 20). These authors suggested a role for increased IGFBP concentrations in altered anabolic responses during disease.

LPS stimulation also produces reduced plasma IGF-I concentrations in humans and rats (1,2,15). The mechanisms for LPS-associated reductions in IGF-I in the rat were related to the large glucocorticoid response to LPS (25). A glucocorticoid receptor antagonist was shown to attenuate the inhibition in plasma IGF-I in rats (25) but not the increase in IGFBP-1, indicating cortisol inhibited IGF-I during disease states. Although this may be the case in other catabolic disease models, in cattle infected with Sarcocystis, the failure of IGF-I to normalize long after other indices of infection had recovered (9) suggests that this may not be the complete picture for all diseases or species.

ANABOLIC HORMONES AND DISEASE

Tissue wasting, reduced growth rates, and reduced plasma concentrations of GH and IGF-I characterize catabolic disease models. An attractive approach to improving animal health would be to administer anabolic hormones to partially increase anabolic reactions and reduce catabolic activity in the animal. Indeed, similar approaches have been under study with human patients (1,7,8). GH treatment of critically ill patients has improved protein anabolism and enhanced nitrogen retention (1,7). There is further evidence for enhanced protein synthesis, reduced urea generation, improved wound healing and shortened hospital stay (1). In addition, there are immunostimulatory activities associated with GH administration in human patients (7). One mechanism for GH actions is to enhance IGF-I concentrations. However, GH had no effect or was only able to partially recover (by 50%) plasma IGF-I concentration (26). In addition, in head trauma patients infused with IGF-I, plasma levels of IGF-I could not be maintained in the presence of a constant infusion and the nitrogen retention effect of IGF-I was consequently diminished (1)

In addition to GH and IGF-I, some studies have investigated use of other anabolic agents. For example, insulin and anabolic steroids can reduce urinary nitrogen loss (26) and an α_2-agonist (clonidine) improved nitrogen balance in esophageal cancer resection (27).

There exists a general agreement that extrametabolic effects of GH serve to modulate aspects of the immune system (28). For example, there are well documented situations where the experimental supplementation of animals with exogenous GH reversed thymic atrophy in rats, modulated respiratory bursts of neutrophils in vitro and increased the survival rate of hypophysectomized animals challenged with live bacterial infection (28,29). Few data definitively address the effects of GH on in vivo immune response in cattle, and fewer yet address the specific action of GH on the release of inflammatory cytokines in actual disease or diseases simulated with the use of an endotoxin challenge. Burton et al. (30) concluded that although characteristic changes in immunoglobulin distribution were apparent in cows treated with GH, the overall conclusion was that there were no detrimental effects of GH on immune function. Also, GH was shown to increase the blastogenic response of peripheral blood mononuclear cells isolated from cows treated with GH, but that the response was slow in developing and highly dependent on the in vitro conditions of mitogen stimulation (31). Edwards et al. (29) were among the first to investigate the capacity for GH to modulate the inflammatory cytokine response to

endotoxin and merged an in vivo model of pituitary ablation and GH resupplementation with an in vitro endotoxin challenge of peripheral blood immune cells harvested from these animals. The conclusion reached by these investigators was that the GH treatment augmented the TNF response. Elsasser et al. (32) used short term (4–5 d) GH treatment of pituitary intact beef calves growing at a rate averaging 1 kg/d, injected a low level (0.2 microgram/kg, iv) endotoxin bolus, and demonstrated that this use of GH (a) decreased both the TNF response to endotoxin challenge, (b) decreased hepatic microsomal membrane content of TNF receptors, and (c) did not compromise the natural down response in TNF elaboration afer a second endotoxin challenge 4 d later. Further data using this beef calf model demonstrated that the ability of GH to modulate the TNF response to endotoxin followed the same nutritional paradigm as that which affects the ability of GH to regulate IGF-I production in vivo. When the plane of nutrition of calves was reduced from a level that facilitated good growth to that of maintenance of body weight, the effect of GH to alter the TNF response to endotoxin challenge was lost (2), similar to the uncoupling of the GH-IGF-I regulation seen in undernutrition (33).

Attempts to use GH to counter catabolic responses in Sarcocystis infected calves were not successful (9). GH treatment delayed the IGF decline observed with infection, but did not prevent the decline nor moderate the magnitude of reduced plasma IGF-I concentrations. There were no GH-induced improvements on food intake, plasma urea nitrogen, or average daily protein gain. Furthermore, GH treatment caused accelerated fat depletion from fat pads and protein depletion from the intestine, which may further compromise the animal's recovery from the disease insult. There was also a reduction in average daily gain for the infected calves treated with GH. A similar lack of a positive metabolic improvement effect for GH (Figure 1) was observed in young calves infected with another parasitic infection, *E. bovis* (34).

Experiments with another catabolic parasitic disease model in calves used anabolic steroids to improve responses to disease (23). Calves were implanted with an anabolic steroid (EP; Synovex C; 10 mg estradiol-benzoate, 100 mg progesterone; Ft. Dodge Laboratories, Ft. Dodge, KS, USA) at 8 wk of age and infected with *E. bovis* (or coccidiosis) at 11 wk of age. Data indicate an improved food intake in infected animals treated with EP compared with infected calves. This was accompanied by an improved body weight in EP treated, infected calves versus infected calves (+12 kg versus −6 Kg at 28 d postinfection), fewer days of fever for EP treated, infected calves (2 versus 5 d), and fewer days of diarrhea in EP treated, infected calves (5 versus 9 d). In each of the acute phase responses there was an improvement in animals treated with the steroid. In addition, there was an increase in the percentage of lymphocytes expressing CD4 antigens (normally associated with immunity to coccidiosis), which may partially explain the improved responses due to EP. Attempts to use GH in this model were without success. However, these young calves did not release IGF-I in response to GH (attributable to a low GH receptor density in young calves), which may have an effect on the anabolic responses.

The mechanism for EP effects was unclear in the Eimeria infection model. Because GH has proven beneficial in the LPS model, a study was initiated to examine the effects of EP on subsequent LPS effects in cattle. One benefit to EP was found in that the duration of salivation and coughing and wheezing immediately after IV LPS injection was minimal in the EP-treated calves. Moreover, food intake was improved and metabolite and hormonal changes were less severe in EP-treated, LPS injected calves. Although the mechanism for the protective effects of EP were again not readily apparent, comparison of the results with the mechanism for GH protection in endotoxemia (32) proved interesting. GH treatment suppressed LPS induced TNF and cortisol responses, effects not observed in our EP-

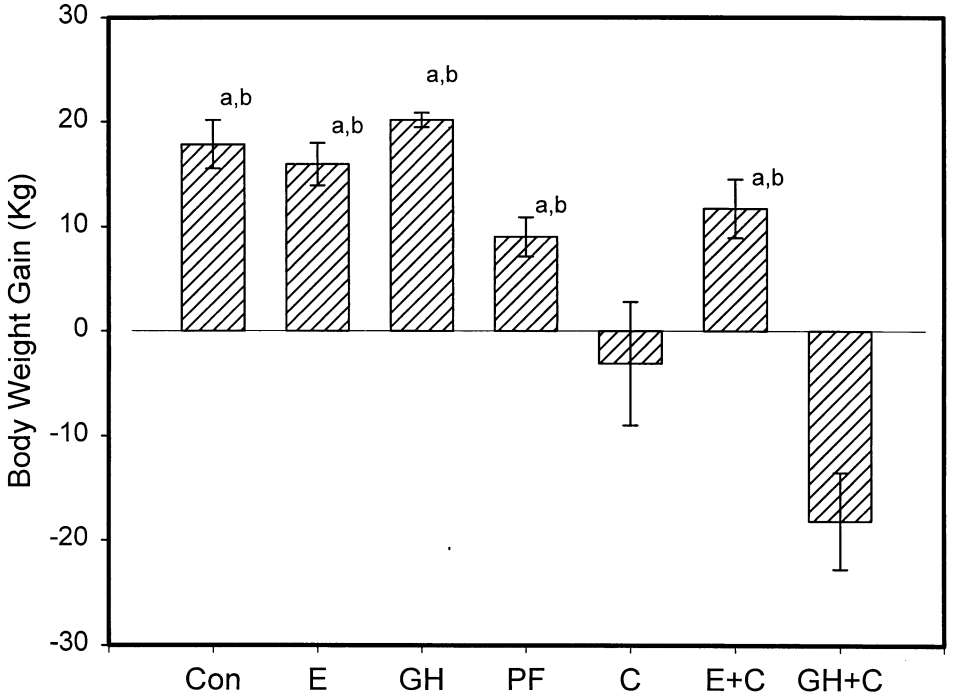

Figure 1. Effect of anabolic hormones on body weight changes in calves from Day 14 to Day 28 post-infection with *E. bovis*. Con, control; EP, estradiol-benzoate + progesterone; GH, growth hormone; PF, pair fed to C, coccoidiosis. There were significant differences in weigt gain between infected and non-infected calves ($P < 0.0001$). a, differences from Group C; b, differences from GH/C calves. Like letters indicate treatments did not differ. Adapted from Amer J Vet Res 58:891–896, 1997.

treated, LPS injected calves. Thus, GH and EP used independent mechanisms to achieve similar protective effects in cattle injected with LPS.

Data for possible mechanisms for steroid protective effects have been published. There is a report of estrogen inhibiting macrophage nitric oxide (NO) production (35). Moreover, estradiol regulates NO synthase synthesis in the hypothalamus (36) and progesterone regulates NO synthase in macrophages (37). Estrogen also can have protective effects on the heart (38). Because NO may play a role in mediating cytokine actions, this may provide some insight into the molecular mechanism(s) for estrogen protection in endotoxemia and coccidiosis.

There is clear evidence that anabolic hormones can play a beneficial role to improve animal responses to disease. Before clinical adoption of this approach, additional progress must be made in determining the factors that can modify the effects of the anabolic hormones, for example, age, nutritional status, hormone selection, dose of hormone, timing of hormone administration relative to disease onset, specific diseases, and others. Current studies in animals have examined a single treatment (hormone) on a limited number of catabolic circumstances. Consideration should be provided to the human literature where GH or other anabolic hormones are administered in concert with parenteral nutrition as well as other supportive therapy (1). The role that hormones may ultimately play as tools in the clinical arsenal will more likely be as an adjunct therapy rather than a primary treatment strategy. Although, current studies have not been uniformly successful, the potentials for anabolic hormones to have positive effects are quite remarkable and hold great promise for clinical intervention in catabolic disease states.

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