Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Domestic Animal Endocrinology 32 (2007) 79–92
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

Temporal response of liver signal transduction elements during in vivo endotoxin challenge in cattle: Effects of growth hormone treatment☆

Cong-Jun Li *, Stanislaw Kahl,
Donald Carbaugh, Theodore H. Elsasser

Growth Biology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Building 200, Room 209, Beltsville, MD 20705, USA

Received 22 November 2005; received in revised form 5 January 2006; accepted 6 January 2006

Abstract

We quantified the changes in abundance of inducible nitric oxide synthase (iNOS) and associated tissue signal transduction pathway elements (STPEs) in the bovine liver in response to lipopolysaccharide (LPS) challenge and further assessed the impact on the LPS-driven variable responses as affected by daily treatment with recombinant growth hormone (GH) prior to LPS challenge. Twenty-four crossbred beef steers were divided into GH-treated (recombinant bovine GH, Monsanto Inc., St. Louis, MO; 0.1 mg/kg BW, i.m., daily for 12 days) and non-GH-treatment (control) groups ($n = 12$/group). Liver biopsy samples were obtained from all animals at 0, 3, 6, and 24 h after LPS challenge (*E. coli* 055:B5, 2.5 μg/kg BW, i.v. bolus) for Western blot analyses of iNOS and STPEs. In response to LPS, tissue levels of iNOS increased significantly ($P < 0.001$) in the first 3 h and persisted at levels greater than those at time 0 until 24 h. GH further augmented levels of iNOS at 0, 3, and 6 h resulting in an overall significant increase in the iNOS protein level ($P < 0.01$). AKT/protein kinase B (AKT/PKB) phosphorylation levels at time 0 were not different between GH-treated and control animals; LPS increased the phosphorylation of AKT/PKB with GH treatment stimulating a four-fold further increase of AKT/PKB phosphorylation. Effects similar to those on AKT/PKB were also observed on signal transducer and activator of transcription 5b (STAT5b). The family of mitogen-activated protein kinase (MAPK) showed different pattern of response. ERK1/2 phosphorylation increased 3 h after LPS challenge but

☆ Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

* Corresponding author. Tel.: +1 301 504 7216; fax: +1 301 504 8623.
E-mail address: cli@anri.barc.usda.gov (C.-J. Li).

0739-7240/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.domaniend.2006.01.001

only in GH-treated group ($P < 0.01$). Compared to 0 h, SAPK/JUN phosphorylation increased in both experimental groups 3, 6 h ($P < 0.01$), and 24 h ($P < 0.05$) after LPS. However, at 3 h the increase was greater ($P < 0.01$) in GH-treated than in control animals. No effect of LPS challenge or GH treatment on $p38^{MAPK}$ was observed. These results suggest that GH treatment has a significant impact on the differential activation of STPEs in the clinical response to LPS.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Growth hormone; iNOS; LPS; MAPK; Signal transduction

1. Introduction

Over the past few years, exogenous growth hormone (GH) treatment has been explored as one potential adjunct for managing the catabolic processes that challenge the host response to infection stress [1,2]. Research suggested that GH might have positive effects on the host response to infection stress based on reports of improved immune function when GH was administered to animals [2–4], perhaps in conjunction with more efficient elimination of the pathogen. However, specific concerns about an increased negative outcome associated with the administration of GH to patients during critical illness [5] as well as poor outcome in several experimental models [6,7] has prompted a reevaluation and termination of the practice in clinical scenarios.

The effects of GH are mediated through coordinated changes in gene expression that are the outcome of interactions between hormone-activated signal transduction pathways and specific feedback loops. The systemic introduction of inflammatory stimuli as modeled with the administration of lipopolysaccharides (LPS) to a host, results in a series of coordinated changes in expression of hepatic genes, many of which participate in the hepatic acute-phase response. These responses are important in the restoration and maintenance of homeostasis after injury [8–10] by cytokines released by monocytes and hepatic macrophages in response to these stimuli. While cytokines stimulate increased expression of one subgroup of hepatic genes, expression is decreased in another subgroup, referred to as negative acute-phase reactants [11,12]. Several GH-responsive genes are among the negative acute-phase reactants [11,12], suggesting that factors responding to GH may be specifically targeted during acute-phase response. The literature documents the extreme situation in a relatively limited case occurrence of severe illness coupled with GH treatment that resolved with mortality. There are few data that address the impact of GH administration on host response to the much more frequently encountered, low-level proinflammatory stress [6]. Few definitive in vivo studies in cattle were directly aimed at identifying the signal transduction pathways that may be involved in the proinflammatory response in animals undergoing GH treatment. We specifically focused on the modulation of NO production and the development of protein tyrosine nitration as a reaction to a low-level LPS challenge [13]. In that study, we suggested pivotal locations in the host response pathway where GH might have influence on biochemical components central to the generation of reactants needed to produce tyrosine nitration in vivo. We also demonstrated the effects of GH administration on components of the nitric oxide (NO)-generating cascade to account for increase in NO production and protein nitration after an immune challenge. Liver protein nitration increased more than

10-fold 24 h after LPS, and an additional two-fold in animals treated with GH before LPS [13]. The data suggested that an increased production of nitrated protein develops in the liver during low-level, proinflammation stress, and nitration is increased by GH administration through a direct effect on the competing activities of NOS and arginase, modulating critical control points in the proinflammatory cascade.

The purpose of the present study was to examine in vivo responses of iNOS and the potential signal transduction pathway elements of bovine liver cells to LPS challenge and the modification of this response by daily treatment with recombinant GH prior to LPS administration. Changes in mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK1 and 2), AKT/protein kinase B (AKT/PKB), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), $p38^{MAPK}$, and signal transducer and activator of transcription 5b (STAT5b) were studied.

2. Materials and methods

2.1. Animals and experimental design

Animals used in this study were young (7–9 months old), sexually immature Angus × Hereford calves born and reared at the USDA Beef Cattle Nutrition Location at Beltsville, MD. Use of LPS and growth hormone was governed by the Investigational New Animal Drug (INAD) application filed with and approved by the Center for Veterinary Medicine, U.S. Food and Drug Administration. The USDA Beltsville Animal Care and Use Committee approved all protocols and procedures for this research. Animals were housed in individual pens with ad libitum access to feed and water. At the time of use, animals were in a fully anabolic state, gaining an average of 1.27 kg live weight per day. When used in study, each calf was acclimated to close human handling and gentle halter restraint in its own pen; one day prior to immune challenge and blood sampling, a sterile Teflon 23 cannula (Abbocath-TT, 14 ga., Abbot Laboratories, No. Chicago, IL) was inserted into the jugular vein and secured in place. Jugular cannula insertion was performed under local anesthesia using an ethyl chloride topical refrigerant spray (Gebauer Chemical Co., 3 Cleveland, OH). Following the conclusion of each sampling period, calves were returned to the nutrition herd.

2.2. LPS challenge, blood sampling, liver biopsy

Experimental protocols previously reported [13] were followed for the LPS challenge study. In this protocol, animals ($n = 24$) were divided equally into two groups: GH-treated (+GH) and non-GH-treated (control or −GH, i.m. injections of placebo vehicle buffer) calves. GH (0.1 mg/kg BW, i.m., recombinant bovine GH, Monsanto Inc., St. Louis, MO) was administered for 12 days prior to sampling and LPS challenging. Blood and liver biopsy samples were collected at 0, 3, 6, and 24 h after LPS administration ($n = 6$/time point). Three GH-treated and three non-GH-treated calves were used at each time point.

Lipopolysaccharide (LPS) challenge was imposed by the intravenous administration of LPS (*E. coli* serotype 055:B5, Sigma Chemicals, St. Louis, MO, 2.5 μg/kg live weight).

This level of LPS was previously shown in our laboratory to produce a transient rise in rectal temperature and an increase in plasma NO [14,15].

Liver tissue samples were obtained under local anesthesia (lidocaine 0.5%, Butler Co., Columbus, OH) by intercostal transcutaneous biopsy as previously described in detail [13,14]. Three 25–30 mg biopsy cores were obtained from each animal using the Tru-cut biopsy needle (14 ga., Allegiance Healthcare Corp., McGraw Pk, IL) for each animal at specified time points. Samples were frozen in liquid N_2 and stored at −80 °C until analyzed.

2.3. Liver tissue homogenate preparation and Western analysis

Liver homogenate was prepared in homogenate buffer containing 10 mM Hepes, pH 7.4; 10 mM KCl; 1.5 mM $MgCl_2$; 0.25 M sucrose; 10% glycerol; 1 mM dithiothreitol (DTT); protease inhibitor cocktail for use with mammalian cell and tissue extract (Sigma) and 0.05% Triton X-100. The liver biopsy samples were homogenized in above buffer (1:5, w/v) using a microcentrifuge Teflon pestle (Kontes, Vineland, NJ) and sonicator. The sonicator was set at level 3 with 3 × 10 s bursts and 50% pause. All procedures were performed on ice. The obtained homogenates were briefly centrifuged at 4 °C for 2 min at 16,100 × *g* to eliminate the debris. The collected homogenate was aliquoted and stored in liquid N_2 until analyzed.

Homogenate proteins were separated for identification by standard SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 10–20% gradient Tris-glycine gels (Invitrogen, Carlsbad, CA) under the reducing conditions as suggested by the manufacturer. Pre-stained molecular weight standards (SeeBlue-plus2, Invitrogen) were included. Separated proteins were eluted from the gels and transferred to pure nitrocellulose membranes (Protran, 0.2 μm, Schleicher & Schuell, Dassel, Germany) by semi-moist electro-transfer. Non-specific binding of reagent antibodies to the nitrocellulose matrix was blocked by incubating membranes with 5% fat-free dry milk in PBS for 1 h prior to incubation with antibodies specific to each desired protein (1:1000 diluted in PBS plus 0.1% Triton 100). Western blot analyses were performed using anti-iNOS antibody (BD Biosciences, San Diego, CA), anti-AKT/PKB, anti-phospho-AKT/PKB, anti-phospho-MAPK family antibodies (Cell Signaling Technology, Beverly, MA), anti-STAT5b, and anti-phospho-STAT5b (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Membranes were then washed five times with PBS containing 0.1% Triton 100. Secondary antibody (Immuno-Pure, anti-mouse-IgG or anti-rabbit-IgG conjugated with horseradish peroxidase, Pierce Biotechnology, Rockford, IL; 1:25,000 diluted with PBS) was added and incubated for 1 h. Membranes were washed five times with PBS and 0.1% Triton 100. Immunoblots were exposed to SuperSignal West Pico Stable Peroxide solution with luminol/enhancer (Pierce Biotechnology) according to the manufacturer's instruction. Western blots were then scanned and analyzed using NIH Image software (National Institutes of Health, Bethesda, MD) to quantify the density of the bands.

2.4. Statistical analysis

Data were statistically analyzed by one-way analysis of variance using GraphPad Prism 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± S.E.M.

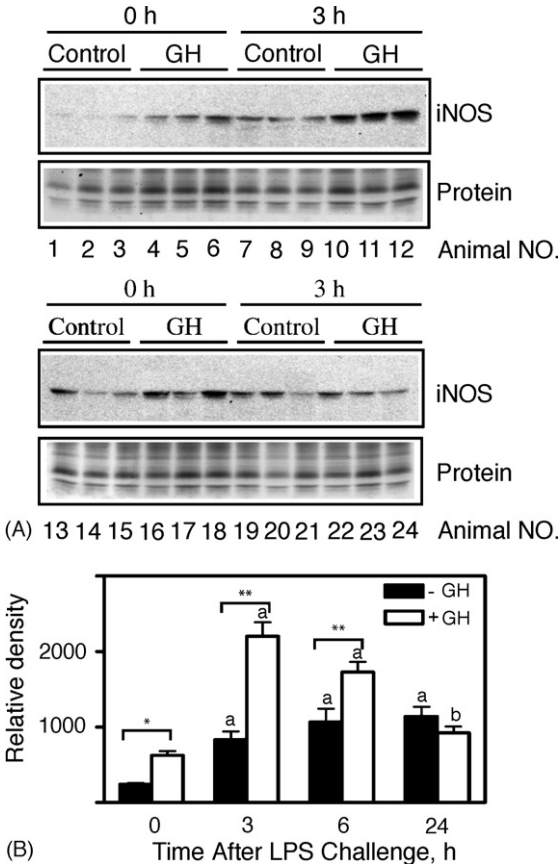

Fig. 1. Protein level of iNOS in liver cells after LPS challenge (*E. coli*, 055:B5, 2.5 μg/kg, i.v.) in control (−GH) and GH-treated (+GH) calves. Western blot of liver homogenates of the 24 individual calves are presented with the respective times and treatment groupings indicated by the horizontal lines (panel A). Values summarized in the panel B represent the mean ± S.E.M. scanned OD for each group of three calves sampled. $^{a}P < 0.01$ and $^{b}P < 0.05$, indicated time point vs. time 0 h in the same group; $^{**}P < 0.01$, control (−GH) vs. +GH; $^{*}P < 0.05$, control (−GH) vs. +GH.

3. Results

3.1. GH augmented the level of iNOS induced by LPS challenge

The effects of LPS and GH treatment on iNOS as determined by Western blot are presented in Fig. 1. Administration of LPS to calves resulted in a significant increase ($P < 0.01$) in iNOS protein level in liver cells (Fig. 1) indicative of the development of the immunological proinflammatory response. In control animals, iNOS level increased 3 h after LPS challenge and maintained an elevated level at 6 and 24 h. LPS challenge in GH-treated animals also resulted in a significant increase in iNOS protein content in liver homogenates ($P < 0.01$) which reached a peak at 3 h after LPS treatment; the level of GH-associated

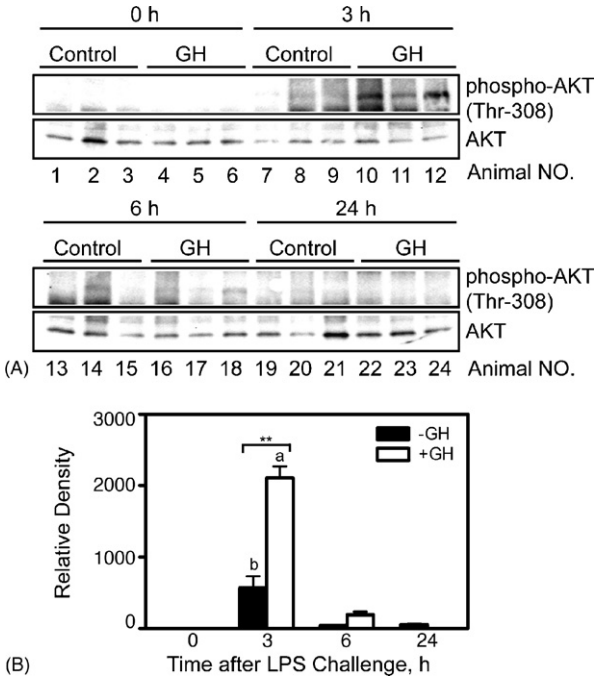

Fig. 2. AKT/PKB phosphorylation in control (−GH) and GH-treated (+GH) calves after LPS challenge. Western blot of liver homogenates with anti-AKT/PKB and anti-phospho-AKT/PKB antibodies (panel A). The 24 individual calves are presented with the respective times and treatment groupings indicated by the horizontal lines. Values summarized in the panel B represent the mean ± S.E.M. scanned OD for each group of three calves sampled. $^{a}P<0.01$ and $^{b}P<0.05$, indicated time point vs. time 0 h in the same group; $^{**}P<0.01$, control (−GH) vs. GH treated (+GH); $^{*}P<0.05$, control (−GH) vs. GH treated (+GH).

induction was still maintained past 3 h though it decreased slightly, compared to 3 h, at 6 and 24 h. The ANOVA indicated that the effects of LPS and GH to increase the iNOS protein level were significant over time ($P<0.01$). In comparison to control animals, GH augmented levels of iNOS protein at 0, 3, and 6 h and resulted in an overall significant increase in the iNOS protein level ($P<0.01$).

3.2. GH augmented the LPS-induced phosphorylation of AKT/PKB

In looking into the possible mechanisms through which immune challenge affects GH actions on signal transduction elements, the serine/threonine kinase AKT/PKB was studied with regard to its activation by examining the phosphorylation attributes using specific anti-phospho-AKT/PKB antibody (Fig. 2). In GH-treated animals and control animals the phosphorylation was detectable at 3 h after LPS challenge ($P<0.05$), but in GH-treated animals, AKT/PKB was significantly phosphorylated at 3 h after LPS challenge ($P<0.01$). The phosphorylation of AKT was eliminated at 6 h after the LPS challenge.

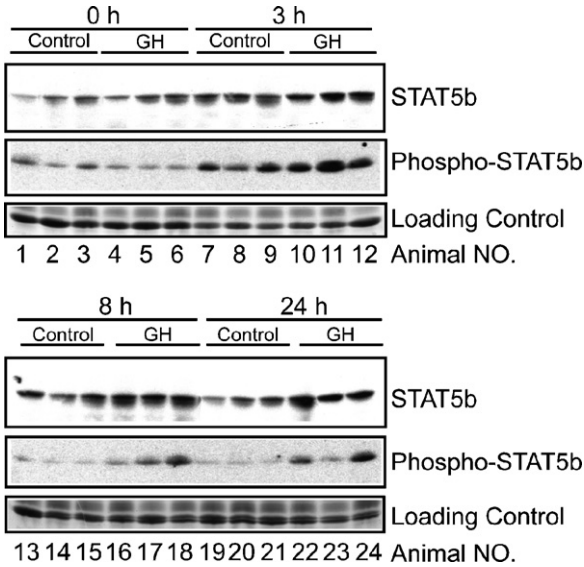

Fig. 3. Differential response of STAT5b protein level and phosphorylation. Western blot of STAT5b and phospho-STAT5b. The 24 individual calves are presented with the respective times and treatment indicated by the horizontal lines. Loading control: SimpleBlue (Invitrogen) stained identical SDS gel.

3.3. Differential response of STAT5b protein level and phosphorylation

Fig. 3 shows the Western blots of STAT5b and phospho-STAT5b in the liver cell homogenates from GH-treated and control animals. Fig. 4 summarizes the quantified results. Compared to 0 h, LPS challenge increased protein levels of STAT5b in control animals at 3 and 6 h ($P < 0.05$) and in GH-treated animals at 3, 6, and 24 h ($P < 0.01$). However, the magnitude of the increase at each time was significantly higher ($P < 0.01$) in GH-treated than in control animals. On the other hand, the phosphorylation of STAT5b showed a different temporal pattern between GH-treated and control animals. As summarized in Fig. 4, in both experimental groups the phosphorylation of STAT5b increased after LPS administration and reached highest level at 3 h, thereafter decreased but still remained higher, compared to 0 h, in GH-treated group ($P < 0.01$). At each time after LPS challenge, however, STAT5b phosphorylation level was greater in GH-treated than non-treated animals ($P < 0.01$).

3.4. Differential response of mitogen-activated protein kinases family

The family of MAPK, ERK, SAPK/JNK, and $p38^{MAPK}$ showed very different patterns of response in the liver of cattle after LPS challenge. ERK1/2 were phosphorylated in response to LPS challenge in GH-treated animals ($P < 0.001$) at 3 h after LPS challenge and were dephosphorylated at 6 and 24 h (Fig. 5). There were no significant changes in control animals. SAPK/JNK responded to LPS in both GH-treated and control animals (Fig. 6). The phosphorylation of SAPK/JNK increased in both experimental groups ($P < 0.01$) but was

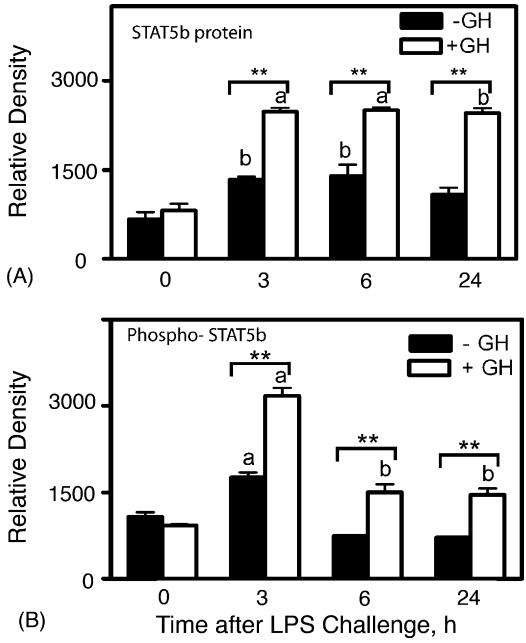

Fig. 4. (A and B) Quantified data for STAT5b protein level and phosphorylation. Values summarized in the graph represent the mean ± S.E.M. scanned OD for each group of three calves sampled. [a]$P < 0.01$ and [b]$P < 0.05$, indicated time point vs. time 0 h in the same group; [**]$P < 0.01$, control (−GH) vs. GH treated (+GH); [*]$P < 0.05$, control (−GH) vs. GH treated (+GH).

greater ($P < 0.01$) in GH-treated than in control steers 3 h after LPS challenge. In both groups, the highest response was observed 3 h after LPS administration and then phosphorylation of SAPK/JNK decreased to initial phosphorylation value at 0 h. Phosphorylation of $p38^{MAPK}$ showed no temporal response to LPS challenge in both groups (Fig. 7). The magnitude of the animal-to-animal variability in phosphorylation of $P38^{MAPK}$, however, was much higher than variability observed in response of other proteins tested in this study.

4. Discussion

In the present study, we examined the expression of iNOS and the activities of potential signal transduction pathway elements in cattle liver cells in response to LPS challenge and the modification of these responses by daily treatment with recombinant GH prior to LPS challenge. We demonstrated that: (i) GH treatment prior to LPS challenge augmented LPS-induced iNOS content in cattle liver cells and (ii) affected several potential signal transduction pathway elements involved in the mediation and regulation of NO-generating cascade. Firstly, these observations suggest possible mechanisms through which GH may enhance immune response to mild bacterial infection in cattle. Secondly, they may be helpful in interpretation of previously reported increases in NO production and protein nitration after

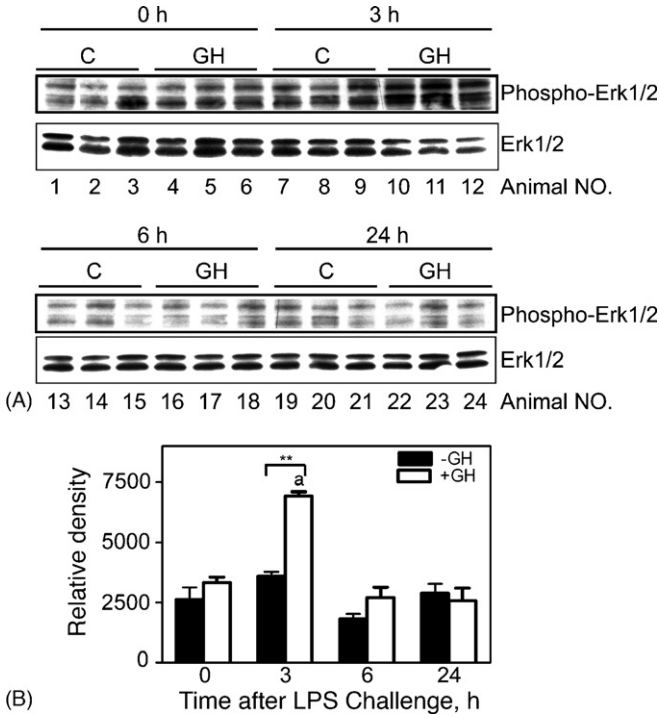

Fig. 5. The temporal response of ERK1/2 to LPS in GH-treated and control animals. Western blot of liver homogenates with anti-ERK1/2 and anti-phospho-ERK1/2 antibodies (panel A). The 24 individual calves are presented with the respective times and treatment groupings indicated by the horizontal lines. Values summarized in the panel B represent the mean ± S.E.M. scanned OD for each group of three calves sampled. $^{a}P < 0.01$, indicated time point vs. time 0 h in the same group; $^{**}P < 0.01$, control (−GH) vs. GH treated (+GH).

immune challenge in cattle treated with GH [11]. Nitric oxide is a free radical produced from the catalytic oxidation of the guanidino group of L-arginine by NOS. Nitric oxide has been shown to have a number of important biological functions, including tumor cell killing, host defense against pathogens, vasodilatation, and neurotransmission [16]. Nitric oxide produced by the constitutive forms of NOS, endothelial and neuronal NOS, functions principally as a vasodilator and neurotransmitter, respectively. The third form of NOS, inducible NOS (iNOS), is generally not present in resting cells but is induced by various stimuli, which include bacterial LPS, tumor necrosis factor-α (TNF-α), interleukin (IL1β), and interferon-gamma (IFN-γ) [17,18]. In contrast to the constitutive forms of NOS, iNOS is regulated at the level of transcription. Increased expression of iNOS has been associated with disorders as diverse as septic and hemorrhagic shock, and chronic infections.

The effect of GH on LPS-induced NO production was examined in our previous report [13]. Growth hormone significantly augmented NO production in LPS-challenged young cattle liver due to the increase in NOS isoform activity. Although the increased expression of iNOS in the liver in response to GH is well characterized, the intracellular signaling pathways that mediate this effect have not been identified. Here, we present data that GH and LPS

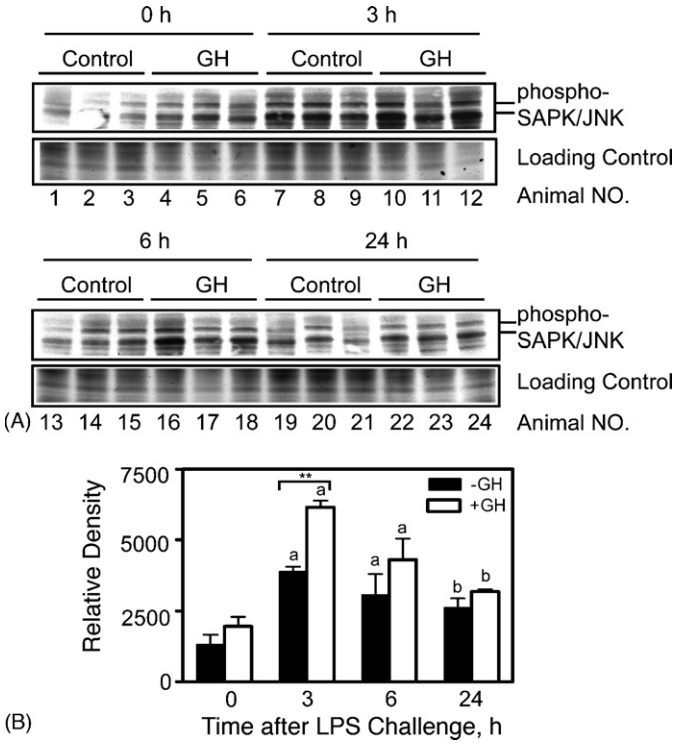

Fig. 6. The temporal response of SAPK/JNK to LPS in GH-treated (+GH) and control (−GH) animals. Western blot of liver homogenates with anti-phospho-SAPK/JNK (p-54/46) (panel A). The 24 individual calves are presented with the respective times and treatment indicated by the horizontal lines. Density of Western blot was corrected with loading control. Values summarized in the panel B represent the mean ± S.E.M. scanned OD for each group of three calves sampled. $^{a}P<0.01$ and $^{b}P<0.05$, indicated time point vs. time 0 h in the same group; $^{**}P<0.01$, control (−GH) vs. GH-treated (+GH) animals.

induction of iNOS is associated with activation (phosphorylation) of ERK1/2, SAPK/JNK, but not $p38^{MAPK}$. Intracellular signaling molecules belonging to JAK2-STAT5b pathway and AKT/PKB are also activated or augmented by GH treatment. The pattern of iNOS expression determined by Western blot presented in this study closely fits the temporal development of protein nitration and plasma concentration of NO_x (nitrate + nitrite) as a measure of NO production in control and GH-treated calves as observed in our previous study [13].

A known main regulator of iNOS gene expression is the transcription factor NF-κB, a DNA-binding protein important for the regulation of various genes involved in immune and inflammatory responses [19]. Phosphorylation of NF-κB inhibitory protein (IκB) at the two serine sites by IκB kinase (IKK)-α and β is in most cases the key step in NF-κB activation. AKT/PKB, a serine–threonine protein kinase which regulates the activation of IKK-α and β phosphorylation, is known to promote cell survival [20]. The phosphoinositide 3-kinase (PI3K)-AKT/PKB pathway also suppresses LPS-induced inflammation and

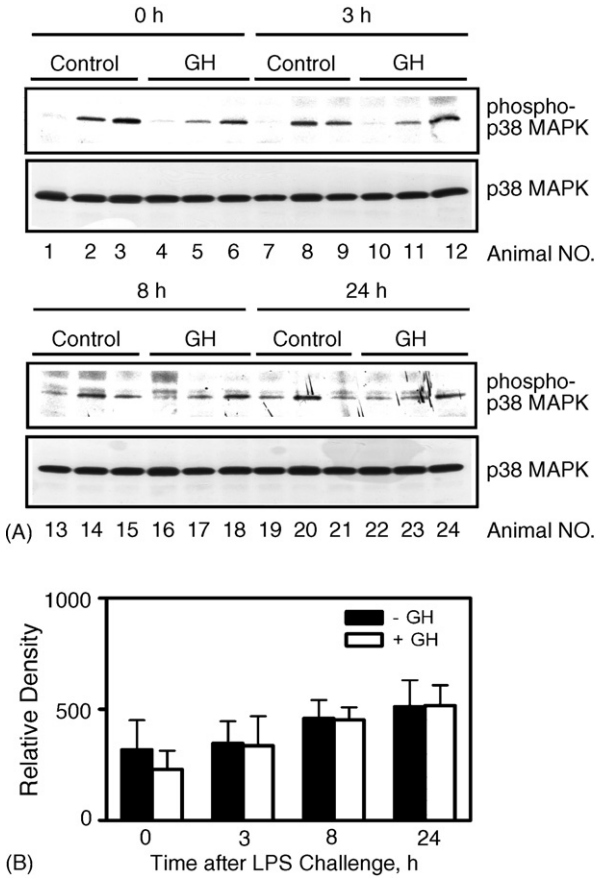

Fig. 7. The response of $p38^{MAPK}$ to LPS in GH-treated and control (−GH) animals. (Panel A) Western blot of liver homogenates with anti-phospho-$p38^{MAPK}$. The 24 individual calves are presented with the respective times and treatment groupings indicated by the horizontal lines. Density of Western blot was corrected with loading control. Values summarized in the panel B represent the mean ± S.E.M. scanned OD.

coagulation in endotoxemic mice [21,22]. AKT/PKB has emerged as the focal point for many signal transduction pathways, regulating multiple cellular processes such as glucose metabolism, transcription, apoptosis, cell proliferation, angiogenesis, and cell motility. In addition to acting as a kinase toward many substrates involved in these processes, AKT/PKB forms complexes with other proteins that are not substrates, but rather act as modulators of AKT/PKB activity and function [23,24]. In this study, GH stimulated the phosphorylation of AKT/PKB molecules in vivo at the early stage of LPS challenge, indicating the involvement of AKT/PKB in the augmentation of LPS-induced iNOS-NO in bovine liver cells. Since this is the first such observation in large domestic animals, we do not know what exact role AKT/PKB may play in the increased production of NO. This is certainly not straightforward question that can be answered in this experiment. More in vitro experiments utilizing cultured cells with specific inhibitors of AKT/PKB as well as specific antibodies are needed.

Intracellular signaling molecules belonging to JAK2-STAT5b pathway are activated by GH [25]. STATs proteins comprise a family of transcription factors latent in the cytoplasm that participate in normal cellular events, such as differentiation, proliferation, cell survival, apoptosis, and angiogenesis following cytokine, growth factor, and hormone signaling. STATs are activated by tyrosine phosphorylation, which is normally a transient and tightly regulated process [26]. STAT5b is downstream of GH signaling [27,28]. The JAK-STAT signaling pathways do not usually function autonomously; rather, they are regulated by a vast array of intrinsic and environmental stimuli. These complex mechanisms of regulation can add plasticity to the transcriptional output in a specific cell or tissue [27]. Diverse protein kinases, including several MAPKs, phosphorylate STATs on serine residues, allowing additional cellular signaling pathways to potentiate the primary STAT-activating stimulus. Similarly, it is possible that additional sites of regulated serine phosphorylation or other posttranslational modifications may regulate attenuation of STAT activity [29,30]. In this study, a noteworthy result was the discrimination between increases in protein level and phosphorylation of STAT5b after LPS challenge and GH treatment. It may reflect the dual functions of STAT5b, one as a transcription factor that requires increasing amount of protein when it is activated and one as a signaling molecule that is activated by phosphorylation.

Utilization of particular signaling pathways in regulating cellular function or inducing gene expression appears to be dependent on, among other factors, the type of stimulus and cell examined [31]. The MAPKs are a family of serine/threonine protein kinases widely conserved among eukaryotes and activated by a variety of environmental stresses, inflammatory cytokines, growth factors, and G protein-coupled receptor agonist. This family includes the MAPK/ERKs signaling cascade; SAPK/JNK and $p38^{MAPK}$ [32,33]. The MAPKs have been shown to activate a number of transcription factors, including activator protein-1 (AP-1), activating transcription factor (ATF)-2, cAMP-responsive element binding protein, NF-κB, certain members of the Ets family [34,35] as well as activating transcription of iNOS [31]. Stimulating, inhibitory, and neutral roles for the MAPKs have been reported in signaling gene expression, including that for iNOS. For example, both ERK1 and 2 were shown to be necessary for iNOS induction by IL-1β. In contrast, ERK played a neutral role in iNOS induction by either TNF-α plus IL-1α in astrocytes [36] or IFN-γ plus LPS in glioma cells [37]. Correlation of activation (phosphorylation) of ERK1/2 and JNK/SAPK with increases of iNOS expression in GH-treated animals after a single LPS challenge indicates that LPS and GH induction of iNOS may be modulated by ERK1/2 and JNK/SAPK. There is no significant relation between the induction of iNOS and $p38^{MAPK}$ activity, indicating that $p38^{MAPK}$ pathway may not be involved in this particular situation. However, the magnitude of the animal-to-animal variability was much higher for $p38^{MAPK}$ than for other proteins we have tested. We speculate that the variability of phosphorylation of $p38^{MAPK}$ might reflect the sensitivity of individual animals to either LPS or GH. Nevertheless, these data suggest an important role for MAPK pathways in modulation of signaling by GH using the JAK-STAT signaling pathway.

GH is classically thought of as an anabolic peptide hormone within the context of the endocrine system. However, recent evidence supports a role for GH acting as a cytokine in the immune system under conditions of stress, counteracting immunosuppression by glucocorticoids. Lymphoid cells express the GH receptor, which belongs to the cytokine receptor

superfamily, and immune tissues can produce GH, suggesting an autocrine/paracrine mode of action for GH. GH can act as a cytokine, promoting cell cycle progression of lymphoid cells and preventing apoptosis [38]. While the knockout mouse models argue that GH does not play a major role in the development of the immune system, there is ample evidence for a role of GH and its receptor in immune modulation (see review and references therein [38]). Our previous report and the present data provide evidence further supporting the concept that GH modulates NO production and the development of protein tyrosine nitration as a reaction to a low-level LPS challenge in vivo, and that GH is able to activate signaling pathways similar to those used by cytokines, such as PI-3 kinase, AKT/PKB, and MAPK pathways. The data of the presented study suggests for the first time that many of the immuno-modulatory functions of GH are mediated through differential effects on the signal transduction elements studied herein and that these functions may be dynamic in that there appears to be a temporal component to the order of activations through which the various signal transduction elements are affected.

References

[1] Kelley KW. From hormones to immunity: the physiology of immunology. Brain Behav Immun 2004;18:95–113.
[2] Saito H, Inoue T, Fukatsu K, Ming-Tsan L, Inaba T, Fukushima R, et al. Growth hormone and the immune response to bacterial infection. Horm Res 1996;45:50–4.
[3] Kelley KW. The role of growth hormone in modulation of the immune response. Ann N Y Acad Sci 1990;594:95–103.
[4] Huang Y, Wang SR, Yi C, Ying MY, Lin Y, Zhi MH. Effects of recombinant human growth hormone on rat septic shock with intraabdominal infection by *E. coli*. World J Gastroenterol 2002;8:1134–7.
[5] Takala J, Ruokonen E, Webster NR, Nielsen MS, Zandstra DF, Vundelinckx G, et al. Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med 1999;341:785–92.
[6] Liao W, Rudling M, Angelin B. Growth hormone potentiates the in vivo biological activities of endotoxin in the rat. Eur J Clin Invest 1996;26:254–8.
[7] Liao W, Rudling M, Angelin B. Contrasting effects of growth hormone and insulin-like growth factor I on the biological activities of endotoxin in the rat. Endocrinology 1997;138:289–95.
[8] Baumann H. Hepatic acute phase reaction in vivo and in vitro. In Vitro Cell Dev Biol 1989;25:115–26.
[9] Baumann H, Prowse KR, Marinkovic S, Won KA, Jahreis GP. Stimulation of hepatic acute phase response by cytokines and glucocorticoids. Ann N Y Acad Sci 1989;557:280–95, discussion 295–6.
[10] Fey GH, Gauldie J. The acute phase response of the liver in inflammation. Prog Liver Dis 1990;9:89–116.
[11] Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci 2005;6:1045–56.
[12] Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 2004;168:28–40.
[13] Elsasser TH, Kahl S, MacLeod C, Nicholson B, Sartin JL, Li C. Mechanisms underlying growth hormone effects in augmenting nitric oxide production and protein tyrosine nitration during endotoxin challenge. Endocrinology 2004;145:3413–23.
[14] Kahl S, Elsasser TH, Blum JW. Nutritional regulation of plasma tumor necrosis factor-alpha and plasma and urinary nitrite/nitrate responses to endotoxin in cattle. Proc Soc Exp Biol Med 1997;215:370–6.
[15] Kahl S, Elsasser TH. Tumor necrosis factor-α (TNF-α), nitroc oxide (NO), and xanthine oxidase (OX) responses to endotoxin (LPS) challenge in heifers: effect of estrous cycle phase. J Anim Sci 2005;83:7.
[16] Lorsbach RB, Murphy WJ, Lowenstein CJ, Snyder SH, Russell SW. Expression of the nitric oxide synthase gene in mouse macrophages activated for tumor cell killing. Molecular basis for the synergy between interferon-gamma and lipopolysaccharide. J Biol Chem 1993;268:1908–13.

[17] MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annu Rev Immunol 1997;15:323–50.
[18] Nathan C. Inducible nitric oxide synthase: what difference does it make? J Clin Invest 1997;100:2417–23.
[19] Liu W, Kato M, Itoigawa M, Murakami H, Yajima M, Wu J, et al. Distinct involvement of NF-kappaB and p38 mitogen-activated protein kinase pathways in serum deprivation-mediated stimulation of inducible nitric oxide synthase and its inhibition by 4-hydroxynonenal. J Cell Biochem 2001;83:271–80.
[20] Harada N, Hatano E, Koizumi N, Nitta T, Yoshida M, Yamamoto N, et al. Akt activation protects rat liver from ischemia/reperfusion injury(1). J Surg Res 2004;121:159–70.
[21] Xie P, Browning DD, Hay N, Mackman N, Ye RD. Activation of NF-kappa B by bradykinin through a Galpha(q)-and Gbeta gamma-dependent pathway that involves phosphoinositide 3-kinase and Akt. J Biol Chem 2000;275:24907–14.
[22] Schabbauer G, Tencati M, Pedersen B, Pawlinski R, Mackman N. PI3K-Akt pathway suppresses coagulation and inflammation in endotoxemic mice. Arterioscler Thromb Vasc Biol 2004;24:1963–9.
[23] Brazil DP, Park J, Hemmings BA. PKB binding proteins. Getting in on the Akt. Cell 2002;111:293–303.
[24] Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002;296:1655–7.
[25] Davey HW, Xie T, McLachlan MJ, Wilkins RJ, Waxman DJ, Grattan DR. STAT5b is required for GH-induced liver IGF-I gene expression. Endocrinology 2001;142:3836–41.
[26] Calo V, Migliavacca M, Bazan V, Macaluso M, Buscemi M, Gebbia N, et al. STAT proteins: from normal control of cellular events to tumorigenesis. J Cell Physiol 2003;197:157–68.
[27] Horvath CM. STAT proteins and transcriptional responses to extracellular signals. Trends Biochem Sci 2000;25:496–502.
[28] Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. Science 2002;296:1653–5.
[29] Ivashkiv LB. Jak-STAT signaling pathways in cells of the immune system. Rev Immunogenet 2000;2:220–30.
[30] Ahmed ST, Ivashkiv LB. Inhibition of IL-6 and IL-10 signaling and Stat activation by inflammatory and stress pathways. J Immunol 2000;165:5227–37.
[31] Chan ED, Morris KR, Belisle JT, Hill P, Remigio LK, Brennan PJ, et al. Induction of inducible nitric oxide synthase-NO{middle dot} by lipoarabinomannan of mycobacterium tuberculosis is mediated by MEK1-ERK, MKK7-JNK, and NF-{kappa}B signaling pathways. Infect Immun 2001;69:2001–10.
[32] Goldsmith EJ, Cobb MH, Chang CI. Structure of MAPKs. Methods Mol Biol 2004;250:127–44.
[33] Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, et al. MAP kinases. Chem Rev 2001;101:2449–76.
[34] Gupta S, Campbell D, Derijard B, Davis RJ. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science 1995;267:389–93.
[35] Janssen-Heininger YM, Macara I, Mossman BT. Cooperativity between oxidants and tumor necrosis factor in the activation of nuclear factor (NF)-kappaB: requirement of Ras/mitogen-activated protein kinases in the activation of NF-kappaB by oxidants. Am J Respir Cell Mol Biol 1999;20:942–52.
[36] Da Silva J, Pierrat B, Mary JL, Lesslauer W. Blockade of p38 mitogen-activated protein kinase pathway inhibits inducible nitric-oxide synthase expression in mouse astrocytes. J Biol Chem 1997;272:28373–80.
[37] Nishiya T, Uehara T, Edamatsu H, Kaziro Y, Itoh H, Nomura Y. Activation of Stat1 and subsequent transcription of inducible nitric oxide synthase gene in C6 glioma cells is independent of interferon-gamma-induced MAPK activation that is mediated by p21ras. FEBS Lett 1997;408:33–8.
[38] Jeay S, Sonenshein GE, Postel-Vinay MC, Kelly PA, Baixeras E. Growth hormone can act as a cytokine controlling survival and proliferation of immune cells: new insights into signaling pathways. Mol Cell Endocrinol 2002;188:1–7.