Integrated adrenal, somatotropic, and immune responses of growing pigs to treatment with lipopolysaccharide

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ABSTRACT: The objective of this research was to provide an integrated look at systemic adrenal, somatotropic, and immune responses of growing pigs to challenge with lipopolysaccharide (LPS). Weaned pigs were challenged intraperitoneally with 100 μg/kg BW of LPS or sterile saline, and rectal temperature and blood data were collected for 72 h. Daily feed intake also was monitored. Plasma was analyzed for concentrations of cortisol, tumor necrosis factor α (TNFα), the acute phase protein haptoglobin, growth hormone (GH), insulin-like growth factor I (IGF-I), and prostaglandin E2 (PGE2). As expected, LPS decreased feed intake, stimulated a febrile response, and activated the hypothalamic-pituitary-adrenal (HPA) axis as demonstrated by increased cortisol levels. Cortisol reached maximum elevation 2 h after treatment (P < .001) and remained elevated through 12 h (P < .001). Circulating TNFα was increased by LPS at 2 and 4 h after treatment (P < .001), and an apparent (not statistically significant) increase in haptoglobin also occurred in challenged animals. The LPS injection suppressed IGF-I by 2 h following treatment (P < .01), and circulating IGF-I remained reduced relative to controls through 44 h. Overall, GH was increased in LPS-treated pigs (P < .05), although the treatment × time interaction was not significant. Plasma PGE2 was increased transiently at 2 h (P < .05) and then subsequently suppressed at 4, 8, and 12 h following LPS (P < .05). This study provides a comprehensive view of systemic effects of LPS on components of the HPA, growth, and immune axes. In addition, these are the first data to document changes in circulating PGE2 in unrestrained animals during the early hours of the acute phase response to LPS.

Key Words: Disease Models, Hydrocortisone, Insulin-like Growth Factor, Pigs, Prostaglandins, Tumor Necrosis Factor

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Introduction

Reduced growth performance caused by infectious disease processes can result in significant economic loss to livestock producers. Recent research supports the existence of collaboration between the neuroendocrine, growth, and immune axes to effectively combat infection. The proinflammatory cytokines tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), as well as cortisol, are elevated in plasma in response to peripheral lipopolysaccharide (LPS) injection in weaned pigs (Webel et al., 1997). These cytokines are synthesized and secreted in response to infection and may be detrimental to growth in swine. Sickness behaviors, including reduced food intake, inactivity, and fever are induced in pigs by LPS (Johnson and von Borell, 1994). Prostaglandin E2 (PGE2) is a putative mediator of the febrile response to LPS (Kluger et al., 1995; Blatteis and Sehic, 1997), yet the time course of circulating PGE2 in response to LPS has not been determined.

Relatively little is known about the effects of inflammatory challenge on the somatotropic axis in weaned, rapidly growing pigs. In studies on the effects of LPS on pituitary hormone release in prepubertal pigs, systemic levels of growth hormone (GH) were increased within minutes after challenge (Parrott et al., 1995b). A recent study also demonstrated an elevation in serum GH immediately following LPS challenge, but pulsatile GH was decreased in the initial hours after treatment (Hevener et al., 1997). In that same study,
LPS treatment reduced serum concentrations of insulin-like growth factor I (IGF-I) by 2 h following treatment, and IGF-I remained suppressed for the remainder of the 96-h sampling period.

Major objectives of the current study were to evaluate systemic GH and IGF-I in weaned pigs challenged with LPS and to track peripheral changes in PGE$_2$ in response to LPS. Changes in feed intake, rectal temperature, and plasma haptoglobin, cortisol, and TNF$\alpha$ were measured as reliable markers of systemic inflammatory challenge.

## Materials and Methods

Thirteen crossbred barrows, approximately 21 d of age, were moved into individual elevated pens shortly after weaning. Initial weights were taken 5 d later, and average body weight was 10.2 ± .9 kg (9.0 to 12.4 kg). Pigs were housed in an environmentally controlled building with constant lighting, and temperature was maintained at approximately 27°C. Each plastic pen was equipped with a nipple waterer and feeder to allow for ad libitum access to feed and water. After an acclimation period of 4 d, venous catheters were placed surgically in the pigs (Takken and Williams, 1981). Catheters were flushed with saline containing 3.5% sodium citrate approximately every 12 h during the 72 h following catheter placement. At 96 h after catheterization, seven animals were injected intraperitoneally with sterile saline to serve as controls, and the remaining six pigs were given 100 µg/kg LPS endotoxin (E. coli 055:B5; Sigma Chemical Co., St. Louis, MO; catalog L-6529). This dosage of LPS was chosen because it was intermediate between the 150 µg/kg i.p. given to neonatal pigs (Matteri et al., 1998) and 50 µg/kg i.p. given to older, heavier pigs (Warren et al., 1997).

Blood sample collection began 24 h prior to challenge and was continued every 4 h during this time to establish pretreatment plasma levels. Samples were collected every 2 h during the 8 h following LPS administration and then at 4-h intervals until 72 h after the challenge. Rectal temperatures were obtained with digital electronic thermometers immediately after blood collection from –24 to 36 h relative to challenge. Feed intake was recorded daily from 24 h prior to challenge until 72 h after challenge and was estimated as the change in feeder weight from the previous weighing.

**Plasma and Serum Analyses.** Blood was collected via the catheters with 5-mL syringes and placed in chilled glass collection tubes containing EDTA and 5.7 mg aprotinin. Samples were centrifuged immediately for 10 min at 5,000 × g. Centrifugation was repeated, and plasma was dispensed into duplicate 12-× 75-mm polypropylene tubes. Tubes were frozen rapidly in liquid N$_2$ and stored at –20°C until analysis. Serum was collected for haptoglobin analyses. Blood was allowed to clot at room temperature and then was stored at 4°C overnight prior to harvest of serum by centrifugation.

Serum was analyzed for haptoglobin using a colorimetric, enzymatic assay as described previously (Dritz et al., 1996). Plasma was analyzed for cortisol (Griffith and Minton, 1992) and GH (Matteri et al., 1994) using RIA. Concentration of IGF-I in plasma was determined using a commercially available two-site immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX; catalog DSL-5600). The assay used antibodies against human IGF-I and human IGF-I as the standard. Insulin-like growth factor I was extracted from binding proteins according to the manufacturer’s protocol for detection of total IGF-I in the sample. To validate the assay for porcine plasma, we evaluated the concentration of IGF-I estimated by the assay in volumes of extract ranging from 25 to 100 µL. When the volume-corrected concentrations of IGF-I were regressed on volume of extract assayed, the resulting slope of the regression line had a 95% confidence interval that included zero. Human IGF-I also was recovered quantitatively when added to porcine plasma prior to extraction. The slope of the regression of IGF-I measured in the assay on IGF-I added to samples had a 95% confidence interval that included 1.0. The limit of detection of the assay was 4 ng/mL.

Plasma TNF$\alpha$ and PGE$_2$ were analyzed using commercially available ELISA. The TNF$\alpha$ assay was developed for measurement of swine TNF$\alpha$ (Endogen, Woburn, MA; catalog EP-TNFA) and was used previously for pigs treated with LPS (Webel et al., 1997). The PGE$_2$ ELISA (Assay Designs, Ann Arbor, MI; catalog 90101) was validated for use in porcine plasma. In the assay of samples from the present study and for evaluation of both recovery of added PGE$_2$ and dilutional parallelism, samples of plasma were diluted 10-fold in assay buffer as recommended by the manufacturer. The PGE$_2$ was recovered quantitatively when added to porcine plasma over a range of 78 to 2,500 pg/mL. The regression of PGE$_2$ measured in the assay on concentration added had a 95% confidence interval that included 1.0. In addition, volumes of 50, 75, and 100 µL of diluted pig plasma were evaluated in assay. When the volume-corrected concentrations of PGE$_2$ were regressed on volume of diluted plasma assayed, the resulting slope of the regression line had a 95% confidence interval that included zero. The limit of detection of the assay (lowest standard in the standard curve) was 39.1 pg/mL.

**Statistical Analyses.** All data were analyzed using a split-plot analysis of variance for repeated measures (Gill and Hafs, 1971). The model included the effect of treatment in the main plot (tested by the animal within treatment variance) and effects of time and the treatment × time interaction in the subplot. Comparisons between treatments within sampling times were made only when a significant F-test ($P < .05$) for the treatment × time interaction was found. All end points measured, except serum haptoglobin and plasma GH, had a significant interaction. Least squares means for each treatment were compared using LSD tests. The response to LPS treatment created an approximately 60-
Fold increase in circulating TNFα. Thus, data for plasma TNFα were normalized by log transformation for analysis, and least squares means of transformed data were compared between treatments. Data presented in Figure 4 are the actual means ± SEM.

Results

Feed intake was depressed \((P < .001)\) in LPS-treated pigs in the period from 0 to 24 h following challenge (Figure 1) but did not differ among treatment groups at other times. Rectal temperature was elevated in LPS-treated pigs at 2 \((P < .001)\), 4 \((P < .001)\), 6 \((P < .01)\), 8 \((P < .05)\), and 12 \((P < .05)\) h after treatment (Figure 2).

Rectal temperatures did not differ among treatment groups at other times. Plasma cortisol (Figure 3) also was increased in LPS-treated pigs at 2, 4, 6, 8, and 12 h after LPS \((P < .001)\). Cortisol concentrations reached a maximum at 2 h \((160.9 ± 6.8 \text{ vs } 25.2 ± 6.3 \text{ ng/mL in controls})\) and returned to control levels by 16 h after treatment. Stimulation of the acute phase response was supported further by a significant rise in plasma TNFα (Figure 4) concentration at 2 and 4 h after LPS \((P < .001)\). Plasma TNFα levels did not differ among treatment groups at other sampling times. Plasma haptoglobin also was analyzed as a marker of the acute phase response.
response (Figure 5). Although haptoglobin concentration in LPS-treated pigs seemed to be elevated above that in controls, no significant treatment $\times$ time interaction was found, and, consequently, no within-time comparisons were made. In addition, no significant treatment effect was observed for haptoglobin. Administration of LPS suppressed IGF-I (Figure 6). In general, plasma IGF-I was reduced in LPS-treated pigs from 2 to 44 h after challenge ($P < .05$) and reached maximal suppression at 6 h after challenge (38.9 ± 6.8 vs 7.5 ± 7.4 ng/mL; $P < .01$). In addition, IGF-I was greater in control pigs at 60 and 64 h after LPS ($P < .05$). Only a trend ($P = .12$) for treatment $\times$ time interaction was observed for GH. However, the overall mean GH concentration for LPS-treated pigs was increased compared to controls (10.3 ± .9 vs 7.6 ± .9 ng/mL; $P < .05$; Figure 7). Initial analysis of the plasma PGE$_2$ concentrations for all pigs in the study revealed no significant treatment $\times$ time interaction. Upon further evaluation of these data, one pig was noted to have extremely high baseline (∼3 to 4 ng/mL) and peak (10.9 ng/mL) plasma PGE$_2$ levels relative to the overall mean baseline (∼2 to 3 ng/mL) and peak (4 ng/mL) for LPS-treated pigs. Consequently, this pig was eliminated from the PGE$_2$ data set (Figure 8 inset), and the data were reanalyzed.
Figure 8). Unexpectedly, PGE$_2$ was greater in LPS pigs in the first sample collected at $-24$ h ($P < .01$), before LPS or saline was given. However, PGE$_2$ was similar between groups at other times prior to treatment. Lipo polysaccharide evoked a transient peak in plasma PGE$_2$ ($3.0 \pm 2 \text{ng/mL} \ vs \ 2.4 \pm 2; P < .05$) at $2$ h after treatment. By $4$ h following LPS, mean PGE$_2$ of challenged pigs began to decline and was suppressed below control concentrations in the $6$ to $12$ h following LPS ($P < .05$). Plasma PGE$_2$ concentrations in LPS-treated and control pigs did not differ through the remainder of the experiment.

**Discussion**

Inflammatory challenge is known to provoke a wide variety of physiological responses. Among the well-documented responses to LPS challenge are transient fever, depressed feed intake, changes in plasma concentrations of acute phase proteins, and activation of the hypothalamic-pituitary-adrenal (HPA) axis (see Kelley et al., 1994 for review). Similarly, LPS decreased feed intake and stimulated a marked febrile response in the current study. The duration of fever in studies using $20 \mu$g (Parrott et al., 1995b; Parrott and Vellucci, 1998; Parrott et al., 1998) and $7 \mu$g/kg (Parrott et al., 1997) of LPS given i.v. to prepubertal pigs was shorter than the $12$ h exhibited in this study. However, studies using higher LPS doses ($50 \mu$g/kg) administered i.p. showed peak elevation in body temperature at $4$ h after LPS (Johnson and von Borell, 1994) and an increased duration of fever, with temperature returning to baseline $8$ h after LPS. Thus, the $100 \mu$g/kg dose of LPS used in the current study would be expected to result in an extended febrile response.

The suppression of feed intake by pigs observed in the $24$ h following LPS challenge is consistent with the anorectic effects that accompany immune challenge of animals. Because of the emerging role of cytokines in various physiological axes beyond the immune system, their potential effect on feed intake and, consequently, muscle protein accretion, metabolism, and other components of the growth axis should be considered. Warren et al. (1997) reported a decrease in feed consumed by pigs during the $8$-h sampling period following an i.p. injection of $50 \mu$g LPS/kg. This anorexia also was seen after pigs were given a centrally administered dose of recombinant porcine TNF$_{\alpha}$. However, low doses of LPS did not increase plasma TNF$_{\alpha}$, though sickness behavior still was exhibited by the animals. This suggests that peripheral cytokine levels may not be directly responsible for suppression of feed intake, but rather that peripheral proinflammatory mediators, possibly in concert with each other, either directly or indirectly signal further cytokine production in the brain, which eventually leads to decreased feed intake (reviewed in Johnson, 1998). Our study supports this notion, because feed intake was suppressed for a $24$-h period that extended far beyond the peak elevation of plasma TNF$_{\alpha}$ at $2$ h after LPS challenge (TNF$_{\alpha}$ data discussed below).

Activation of the HPA axis by LPS administration has been well established. In the present study, $100 \mu$g/kg LPS given i.p. evoked the expected rise in cortisol resulting from stimulation of the HPA axis by cytokines. Several previous studies have reported a shorter duration of cortisol elevation than that seen in this study. For example, Parrott et al. (1995b) reported a rise in cortisol in prepubertal pigs peaking at $160$ min when $20 \mu$g LPS was administered i.v., which is similar to the peak observed in our study at $2$ h after LPS. However, cortisol levels in the previous study seemed to return to baseline by $6$ h after treatment, whereas cortisol remained elevated for $12$ h after LPS and then returned to control levels in our study. This inconsistency in duration of cortisol elevation is probably a result of our higher dosage ($100 \mu$g/kg) and route of administration (i.p.). Duration of cortisol elevation also was shorter in another study by Parrott et al. (1997), in which low doses ($7 \mu$g/kg) of LPS were given i.v. to pigs. In studies by Warren et al. (1997), $50 \mu$g LPS/kg was administered i.p. to male pigs and resulted in an abrupt rise in cortisol that lasted throughout the $24$-h sampling period. The peak cortisol levels reported by that group were similar to those seen in our study. Also, neonatal pigs had elevated cortisol levels $(-350 \text{ng/mL})$ at $3$ h following $150 \mu$g/kg LPS given i.p. (Matteri et al., 1998).

Plasma concentrations of TNF$_{\alpha}$ were elevated in the hours immediately following i.p. LPS injection in growing pigs (Warren et al., 1997; Webel et al., 1997). Similarly, we evaluated plasma TNF$_{\alpha}$ in this study as a representative proinflammatory cytokine. The unmistakable TNF$_{\alpha}$ response observed in our experiment peaked at $2$ h after LPS and remained elevated until $4$ h after challenge. To our knowledge, this is the first study to monitor TNF$_{\alpha}$ concentrations beyond $24$ h following challenge. It was confirmed that no further changes in systemic TNF$_{\alpha}$ occurred beyond the initial response.

Hepatic production of acute phase proteins is a hallmark of the body’s physiological response designed to maintain homeostasis following injury, trauma, or infection (Heinrich et al., 1990; Baumann and Gauldie, 1994). The acute phase protein haptoglobin has been suggested as an appropriate indicator of the acute phase response to turpentine injection in pigs (Eckersall et al., 1996). Hall et al. (1992) determined that serum haptoglobin concentration also serves as an accurate indicator of tissue damage following *Actinobacillus pleuropneumoniae* in pigs. Additionally, repeated injection of LPS increased haptoglobin concentrations in growing pigs (Dritz et al., 1996). Whereas our data generally are consistent with those of Dritz et al. (1996), no statistically significant elevation of plasma haptoglobin was seen. The single dose of LPS may not have been sufficient to drive prolonged secretion of both IL-1 and IL-6, which are implicated in stimulation of haptoglobin
LPS administration and both cytokines would be necessary to evoke a significant rise in plasma haptoglobin concentration. Repeated LPS administration and \textit{A. pleuropneumoniae} challenge likely provide for more sustained stimulation of IL-1 and IL-6 secretions. However, the single LPS dose used in our study seemed to have changed circulating haptoglobin in the expected direction. Similar to our findings, Webel et al. (1997) reported that a single small dose of LPS in pigs failed to increase $\alpha$-1-acid glycoprotein, another acute phase protein, even though increased levels of TNF-α and IL-6 were apparent.

Consistent with the observed reduction in feed intake in response to LPS, we observed a prolonged reduction in circulating IGF-I. Muscle protein accretion often is affected by disease processes, and normal growth and homeostasis may become secondary to combating infection during these times (Hasselgren, 1993). Reduction of peripheral concentrations of IGF-I by parasitic infections have been detected in both cattle (reviewed in Spurlock, 1997) and pigs (Prickett et al., 1992). Pigs fed the antimicrobial ASP-250 had increased serum IGF-I concentrations (nearly 25%) compared to pigs fed control diets (Hathaway et al., 1996). Also, LPS administered to rats resulted in depressed plasma IGF-I and decreased expression of IGF-I mRNA in the liver and skeletal muscle and increased expression in the kidney (Fan et al., 1994). These studies indicate an integration of the growth and immune axes in response to immunological stress. Treatment of prepubertal gilts with 5 $\mu$g LPS/kg i.p. resulted in depressed circulating IGF-I (Hevener et al., 1997). Here we extend those findings to include young growing pigs, in which plasma IGF-I fell rapidly after LPS administration, concurrent with decreased feed intake. Therefore, we speculate that depressed consumption of feed may contribute to the decreased IGF-I. However, the decrease in IGF-I was quite abrupt, and suppression continued even after normal feed intake resumed. In fact, the rapidity with which IGF-I decreased in treated pigs suggests that clearance of IGF-I, in addition to reduced secretion, may have contributed to the LPS-induced decline in the early hours following treatment. Finally, both control and challenged animals showed an upward trend in IGF-I concentrations until the end of the 72-h sampling period. Although the reason for this trend is not entirely obvious, it is consistent with results from other sequentially sampled pigs (Hevener et al., 1997).

In contrast to the effect of LPS on IGF-I, the effects of treatment on circulating GH were far less striking, although overall GH was significantly elevated in LPS-treated pigs. Nevertheless, the overall effect of LPS to increase GH was consistent with the decrease in IGF-I concentrations observed in these animals. Nutrients probably are redirected from normal growth mechanisms toward other necessary defense components during times of immunological challenge (Hasselgren, 1993). In cattle (Elsasser et al., 1988, 1995) and rats (Fan et al., 1994, 1995), inflammatory challenges resulted in decreased GH levels in the periphery. However, GH concentration increased following LPS administration in sheep (Coleman et al., 1993). That observation, along with the overall increase in mean GH levels and the decreased IGF-I levels of challenged pigs in our study, support the theory of an uncoupling of the GH/IGF-I axis during sepsis. One possible explanation of this uncoupling involves a decrease in GH receptor levels in the liver. Incubation of GH-stimulated, cultured rat hepatic cells with IL-1β and TNFα decreased IGF-I mRNA, as well as GH receptor mRNA, in a dose-dependent manner (Wolf et al., 1996). Also, data from calves in the acute phase of parasitic infection with \textit{Sarcocystis cruzi} demonstrated decreased levels of plasma IGF-I, hepatic IGF-I mRNA, and GH receptor mRNA (Elsasser et al., 1998). Thus, circulating GH could not effectively signal the liver to produce and secrete IGF-I, which then would drop. The lack of negative feedback from IGF-I, in turn, might have been the mediator of the apparent increase in GH levels seen in the later hours following endotoxin administration in this study. This provides a reasonable explanation for the increased GH and decreased IGF-I concentrations observed.

We hypothesized that systemic PGE$_2$ would be elevated by LPS treatment in pigs. Indeed, elevated plasma PGE$_2$ was reported in restrained rabbits (Rondono et al., 1988), but restraint stress itself stimulated prostaglandin-dependent hyperthermia (Parrott and Lloyd, 1995). To our knowledge, the current study is the first to report time series data of the effects of LPS injection on systemic PGE$_2$ concentrations in unrestrained animals. The LPS provoked a transient increase in mean plasma PGE$_2$. Because our first plasma sample was obtained at 2 h after challenge, we cannot rule out the possibility that PGE$_2$ was elevated to an even greater extent prior to that time. Even so, the initial transient rise in plasma PGE$_2$ following LPS challenge is consistent with a role for systemic PGE$_2$, perhaps in concert with other pyrogenic mediators, in the early generation of the febrile response (Blatteis and Sehic, 1997). Peripheral administration of PGE$_2$ resulted in fever in pigs (Parrott et al., 1995a), and peripherally administered indomethacin attenuated LPS-induced fever (Parrott and Vellucci, 1998), anorexia, and decreased activity (Johnson and von Borell, 1994). Therefore, these symptoms are generally recognized as prostaglandin-dependent mechanisms.

Following the transient elevation of plasma PGE$_2$, a more prolonged suppression occurred. Although this suppression cannot be explained definitively, we could speculate that concurrent high circulating levels of cortisol might participate in the reduction in plasma PGE$_2$. In fact, recent studies have shown that cyclooxygenase-2 activity was suppressed in pulmonary cell lines by the synthetic glucocorticoid dexamethasone (Ristimaki et al., 1996; Newton et al., 1998).

In the 24 h prior to treatment, we observed falling baselines of PGE$_2$ in both treatment groups. These may
have been associated with increased activity in the animal room or interactions with research personnel at the onset of the study. Regardless of the cause, however, two important points can be made relative to PGE$_2$ during this time. First, basal concentrations (relative to 48 h after treatment) were attained by 4 h prior to treatment, making the posttreatment rise and subsequent fall in PGE$_2$ in treated pigs apparent. Second, at 24 h, before treatments were given to either group of pigs, PGE$_2$ in the LPS group was elevated significantly, and even more so than immediately after treatment. However, pigs were not febrile in the 24 h prior to treatment. Taken together, the data suggest that elevated peripheral PGE$_2$ per se is not pyrogenic. Thus, if peripheral PGE$_2$ supports fever mechanisms, it apparently must be present in the circulation with other pyrogenic mediators.

**Implications**

Data from the current study support and extend current knowledge of the multisystem involvement in response to peripheral inflammatory challenge. We believe that there are two major new implications of the present study. The first relates to the biology of the febrile response and raises the possibility that peripheral prostaglandin E$_2$ may provide upstream signals that support pyrogen elaboration within the brain. The second has important implications for livestock production. We noted that insulin-like growth factor I was reduced by systemic inflammation, even beyond the period of reduced feed intake. This may shed light on why some animals grow poorly in the absence of overt clinical signs of disease. Characterization of the somatotropic axis following bona fide disease challenge will help to confirm this speculation.

**Literature Cited**


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