Lipopolysaccharide-Induced Sickness Behavior in Pigs Is Inhibited by Pretreatment with Indomethacin

Rodney W. Johnson1 and E. von Borell

Swine Nutrition, Growth and Behavior, Department of Animal Science, Iowa State University, Ames 50011

ABSTRACT: Many of the behavioral responses following acute bacterial or viral infection are now considered important for maintaining homeostasis during inflammation. In the present study, we extend this concept to pigs (16 crossbred barrows) by demonstrating that lipopolysaccharide (LPS, 5, 5, or 50 µg/kg BW) from Escherichia coli injected i.p. reduces feed intake, decreases activity, and elevates body temperature. To determine whether any of these effects could be mediated via a prostaglandin (PG)-dependent mechanism, a second experiment with 16 crossbred barrows was conducted. Barrows were pretreated with indomethacin (IND, 5 mg/kg BW [a cyclooxygenase inhibitor]), and their behavior and body temperature following a challenge i.p. injection of LPS (5 µg/kg BW) were assessed. Pretreatment with IND inhibited the anorexia and inactivity caused by LPS, suggesting that the behavioral effects of LPS are dependent on activation of a PG system. Lipopolysaccharide alone, however, did not elevate body temperature in this case; thus, the involvement of PGs in this response was not determined. Collectively, these data indicate that pigs respond to LPS by reducing feed intake, decreasing activity, and becoming febrile. The ability of IND to inhibit behavioral effects of LPS is consistent with the hypothesis that a PG system is involved in mediating sickness behavior. Perhaps, by altering the activity of cyclooxygenase it is possible to enhance or inhibit the behavioral symptoms of sickness in pigs.

Key Words: Pigs, Behavior, Indomethacin, Lipopolysaccharide, Prostaglandins

Introduction

Reduced food intake, inactivity, and sleep are behavioral states observed in animals with an acute bacterial or viral infection. These nonspecific behavioral responses are known collectively as sickness behavior, and in association with other acute-phase responses, are critical for maintaining homeostasis during inflammation (Hart, 1988; Kent et al., 1992b).

A number of infectious diseases of swine are characterized by fever, cachexia, inactivity, and anorexia (see Hart, 1988). Pigs therefore seem to employ many of the systemic acute-phase responses that are used by other mammals and birds to maintain homeostasis during infection. A well-documented system for inducing sickness in laboratory animals is injection of lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria. The effects of LPS are attributed to a cascade of cytokine synthesis and release (Dinarello, 1984), and a subsequent increase in prostaglandin (PG) synthesis (Hashimoto et al., 1988; Uehara et al., 1989; Crestani et al., 1991).

The objectives of the present study were to quantify the behavioral and febrile response of pigs to a challenge dose of LPS and to determine whether PG are involved in mediating these acute-phase responses.

Materials and Methods

Animal Management. Sixteen crossbred barrows were used in each of two experiments (32 barrows total). Average BW (mean ± SD) of barrows for Exp. 1 and 2 was 46 ± 2.3 kg and 32 ± 1.0 kg, respectively. They were individually housed in pens with a total of 1.1 m² floor space. Each pen was equipped with a feeder and a nipple waterer. The room was maintained at 21°C and lit continuously. At 0800, barrows were fed a corn and soybean meal-based diet (15% CP; 3,200 kcal/kg of ME) to provide 2.5 times their maintenance requirement for energy. All procedures

1To whom correspondence should be addressed. Current address: 390 Anim. Sci. Lab., University of Illinois, 1207 West Gregory Drive, Urbana 61801.

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met or exceeded the recommendations in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Consortium, 1988).

Treatment Solutions. Lipopolysaccharide and indomethacin (IND) were purchased from Sigma Chemical (St. Louis, MO). Lipopolysaccharide from *Escherichia coli* serotype K-235 (phenol-extracted) was dissolved in saline (.9% NaCl). Indomethacin was dissolved in a minimal amount of 100% ethyl alcohol and then added to a saline-sodium bicarbonate solution. All treatments were injected i.p in 5 mL of solution.

Behavior. Behavior was monitored directly by scan-sampling (see Lehner, 1979) at 1-min intervals for 30 min at 0, 1, 2, 4, and 8 h after injection. Locomotor activity, resting, and feeding behavior were assessed. Barrows were considered to be engaged in locomotor activity if they were standing, but not eating. Resting behavior was recorded if barrows were lying or sitting, and barrows with their heads in the feeder were considered to be engaged in feeding behavior. For analysis, the time spent engaged in locomotor activity and feeding behavior were summed to estimate total activity. Food intake was determined at 1, 2, 4, 8, and 24 h.

Body Temperature. The body temperature (*Tb*) was recorded at 10-min intervals with an automated radio-telemetry monitoring system (DATACOL, Mini-Mitter Co., Sunriver, OR). Barrows were anesthetized with sodium thiamylal (20 mg/kg BW i.v.) and maintained with halothane. A 6-cm longitudinal incision was made in the ventral-cervical region and a temperature-sensitive VHF transmitter was implanted adjacent to the jugular vein. Eight barrows for each treatment solutions were fit with transmitters. At least 7 d were allowed for recovery before treatments were initiated. All procedures were approved by Iowa State University's Committee on Animal Care.

Experiment 1. Sixteen barrows were used in a completely randomized design. At 1000, barrows were injected i.p. with saline, .5, 5, or 50 µg/kg BW of LPS; and food was available ad libitum. Seven days later, the barrows were randomly reassigned to treatments and the trial was repeated.

Experiment 2. To investigate the involvement of PG in the behavioral effects of LPS, 16 barrows were used in a completely randomized design. The four treatments comprised the 2^3 factorial combination of IND (vehicle [VEH] or 5 mg/kg BW) and LPS (saline [SAL] or 5 µg/kg BW). At 1000, barrows were injected i.p. with VEH or IND, followed immediately by SAL or LPS; food was available ad libitum. The barrows were randomly reassigned to treatments and the trial was repeated 7 d later.

The dose of IND was selected based on a preliminary study in which 16 pigs similar to those used in this experiment received VEH, 2.5, 5, or 10 mg/kg of IND. The 5 mg/kg dose was the maximum dose that did not suppress feed intake over an 8-h period (data not shown). Similar doses of IND inhibit the behavioral effects of peripherally administered LPS and interleukin-1 in chickens, mice, rabbits, and rats (Hashimoto et al., 1988; Crestani et al., 1991; Johnson et al., 1993b).

Statistical Analysis. All data for Exp. 1 and 2 were analyzed by ANOVA procedures appropriate for a completely randomized design (Steel and Torrie, 1980). For Exp. 1, when ANOVA revealed a significant effect of treatment, differences between treatment means were tested using paired *t*-tests. For Exp. 2, the main effects of LPS and IND as well as the main factor interactions were tested by ANOVA. When appropriate, data were subjected to ANOVA for repeated measures. All statistical analyses were conducted using GLM procedures (SAS, 1990).

Results

Behavioral and PyrogenicEffects of Lipopolysaccharide. The first experiment was conducted to compare several behavioral and physiological effects of LPS injected at various doses. At 1 to 2 h, pigs injected with LPS consumed less feed (*P* < .001; Figure 1). This effect was still evident at 2 to 4 h, but the duration of this effect was dose-dependent (Figure 1). For example, at 2 to 4 h, although barrows injected with .5 µg/kg of LPS consumed less feed than control barrows (*P* < .001), they consumed more than those injected with 50 µg/kg (*P* < .02). At 4 to 8 h, only the barrows receiving the highest dose of LPS (50 µg/kg) experienced a reduction in feed intake (Figure 1). And in fact, barrows administered the lowest dose (.5 µg/kg demonstrated a compensatory increase in feed intake (Figure 1), suggesting that although LPS is a potent inhibitor of feed-motivated behavior, its effects are short-lived. The ANOVA of the total feed consumed in the 24-h period revealed a dose effect for LPS (*P* < .01). Barrows injected with saline, .5, 5, and 50 µg/kg of LPS consumed 2,838, 2,460, 2,634, and 1,953 g/barrow (pooled SE = 142.7), respectively.

The time spent eating was directly proportional to feed intake (data not shown). The activity (sum of time spent eating and moving) of barrows was influenced by time, dose and a time × dose interaction (*P* < .001). Consequently, the effect of treatment on the activity during each 30-min period was analyzed separately. At 1 h, the activity of barrows injected with LPS was reduced in a dose-dependent fashion (*P* < .001; Table 1). This effect was still evident at 4 h, but the duration was dose-dependent (Table 1). For example, at 4 h, barrows injected with .5 µg/kg of LPS were more active than barrows receiving 5 or 50 µg/kg of LPS (*P* < .001). From Table 1, it is evident that even the control barrows (i.e., saline-injected) experienced a reduction in activity over time. It was anticipated, however, that the motivation for feed
Figure 1. Feed intake of barrows following a challenge dose of lipopolysaccharide (LPS). Barrows were injected i.p. with saline, .5, 5, and 50 μg/kg BW of LPS from Escherichia coli, and feed was available in ad libitum amounts. Bars represent the treatment mean ± SEM (n = 8). Asterisks indicate that a treatment mean is different from the saline-injected control (**P < .01; ***P < .001).

would be higher at 0 h (barrows were feed-restricted prior to injection) than at 1, 2, or 4 h (feed was available in ad libitum amounts following injection).

Another common response to acute bacterial or viral infection is elevated Tb. As hypothesized, repeated measures ANOVA of Tb revealed a time × dose interaction (P < .001). The febrile response to LPS peaked at 4 h (Figure 2). The change in Tb from 0 to 4 h for barrows injected with saline, .5, 5, and 50 μg/kg of LPS was 27, 1.09, 2.06, and 2.30 °C (pooled SE = .354), respectively (P < .02).

Effects of Indomethacin on Lipopolysaccharide-Induced Responses. To determine whether PG are involved in the effects of LPS, barrows were injected with VEH or IND, followed immediately by SAL or LPS. As expected, LPS caused anorexia (Table 2). At 1 to 2 h, LPS reduced feed intake (P < .11). The main effect of IND was to increase it (P < .001). Feed intake at 1 to 2 h, however, depended on both LPS and IND (P < .04). Pretreatment with IND completely inhibited the anorexia caused by LPS (Table 2). At 2 to 4 h, LPS-treated barrows consumed less feed than those treated with saline (P < .01). The effects of IND did not influence feed intake (P > .30), and the interaction between LPS and IND was not significant. Consistent with what was reported for Exp. 1, in Exp. 2 the effect of LPS (i.e., at the 5 μg/kg dose) on feed intake was not significant after 4 h. Cumulative feed intake from 0 to 4 h was reduced by LPS (P < .09) and increased by IND (P < .02). Cumulative feed intake from 0 to 4 h and 0 to 8 h, however, depended on both LPS and IND (P < .09 and P < .05, respectively).

A three-way ANOVA (time × LPS × IND) on the total activity revealed a significant effect of LPS (P < .05), IND (P < .001), their interaction term (P < .001), and the three-way interaction (P < .001). Consequently, total activity during each 30-min time period was analyzed separately. Consistent with results from Exp. 1, LPS caused a reduction in activity (Table 3). At 1 and 2 h, LPS reduced behavioral activity (P < .08 and P < .02, respectively). Activity at 1 h, however, depended on both IND and LPS (P < .04). Pretreatment with IND completely inhibited the reduction in activity caused by LPS. At 2 and 4 h, there was a tendency for activity to depend on both IND and LPS (P < .18 and P < .13, respectively; Table 3). Because IND is a specific inhibitor of the cyclooxygenase pathway (Shen and Winter, 1977), these data suggest that anorexia and inactivity caused by LPS involved activation of a PG system.

Contrary to what was observed in Exp. 1, and despite the expression of behaviors that are indicative of sickness, LPS did not significantly elevate Tb (data not shown). Repeated measures ANOVA of Tb did not detect a time × main factor interaction (P > .20). It should be noted, however, that there were missing cells throughout the data set. The Tb after the LPS challenge peaked at 4 h. But the effects of LPS and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipopolysaccharide, μg/kg BW</th>
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<tbody>
<tr>
<td>Time, h^b</td>
<td>Saline</td>
</tr>
<tr>
<td>0-5</td>
<td>28.4^c</td>
</tr>
<tr>
<td>1-1.5</td>
<td>23.0^x</td>
</tr>
<tr>
<td>2-2.5</td>
<td>15.3^x</td>
</tr>
<tr>
<td>4-4.5</td>
<td>9.3^x</td>
</tr>
<tr>
<td>8-8.5</td>
<td>10.1</td>
</tr>
</tbody>
</table>

^aTime spent active (sum of the time spent eating and moving) during 30-min sampling periods.
^bHour after injection with lipopolysaccharide.
^cTreatment mean based on eight observations.
^x,yTreatment means in same row with different letter superscripts are different (P < .05).
Figure 2. Body temperature (T_B) of barrows following a challenge dose of lipopolysaccharide (LPS). Barrows were injected i.p. with saline, .5, 5, and 50 μg/kg BW of LPS from *Escherichia coli* at 0 h, and feed was available in ad libitum amounts. Each data point represents the mean of four observations. The change in T_B from 0 to 4 h (peak elevation in T_B) for barrows receiving 0, .5, 5, and 50 μg/kg of LPS was .27, 1.09, 2.06, and 2.30 °C (pooled SE = .354), respectively (P < .02).

IND did not influence the change in T_B from 0 to 4 h, and the interaction between LPS and IND was not significant.

Discussion

Nonspecific behavioral symptoms of sickness include anorexia, somnolence, and inactivity. These behaviors are now considered to be integral components of the acute-phase response and are important for maintaining homeostasis during inflammation. In the present study, we took advantage of a well-documented model for inducing sickness in laboratory animals by injecting LPS. The resultant data indicate that pigs respond to LPS by reducing feed intake, reducing activity, and becoming febrile. Pigs, therefore, seem to employ many of the same behavioral and physiological responses that are used by other animals to maintain homeostasis during infection. The fact that we were able to inhibit the behavioral effects of LPS by pretreatment with IND suggests that these responses are dependent on activation of a PG system.

A variety of pathogenic agents induce cells of the immune system to synthesize and release cytokines such as interleukin-1, tumor necrosis factor-α, and interleukin-6. These proinflammatory cytokine molecules amplify the immune response and activate other behavioral and physiological disease defense mechanisms. The new view is that cytokines act as communication signals to coordinate the various components of the acute-phase response. Many of the behavioral and physiological effects of LPS are attributed to the cascade of cytokine synthesis and release. Acute injection of LPS (Calapai et al., 1991), interleukin-1 (Plata-Salaman et al., 1988; Uehara et al., 1989; McCarthy and Daun, 1992), and tumor necrosis factor-α (Plata-Salaman et al., 1988; Socher et al., 1988) reduce both feed and water intake. Feed-motivated behavior and social exploration are reduced following injection of LPS (Johnson et al., 1993a), interleukin-1 (Bluthé et al., 1991b; Crestani et al., 1991; Kent et al., 1992a), and tumor necrosis factor-α (Bluthé et al., 1991a). Both LPS and interleukin-1 induce slow-wave sleep (Krueger et al., 1984, 1986), and LPS, interleukin-1, tumor necrosis factor-α and interleukin-6 induce fever (Kluger, 1991).

Table 2. Effects of indomethacin on the feed intake (g/barrow, as-fed basis) of barrows following a challenge dose of lipopolysaccharide [n = 8]

<table>
<thead>
<tr>
<th>Time, h&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control (no drug)</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>LPS</td>
</tr>
<tr>
<td>0-1</td>
<td>826</td>
<td>831</td>
</tr>
<tr>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>247</td>
<td>53</td>
</tr>
<tr>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>221</td>
<td>31</td>
</tr>
<tr>
<td>4-8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>369</td>
<td>410</td>
</tr>
<tr>
<td>8-24</td>
<td>911</td>
<td>1,113</td>
</tr>
<tr>
<td>Cumulative intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1,073</td>
<td>884</td>
</tr>
<tr>
<td>4 h&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1,294</td>
<td>915</td>
</tr>
<tr>
<td>8 h&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1,654</td>
<td>1,325</td>
</tr>
<tr>
<td>24 h</td>
<td>2,565</td>
<td>2,438</td>
</tr>
</tbody>
</table>

<sup>a</sup>Hour after injection with lipopolysaccharide (LPS).
<sup>b</sup>Main effect of LPS (P < .11) and drug (P < .001). LPS x drug interaction (P < .04).
<sup>c</sup>Main effect of LPS (P < .01).
<sup>d</sup>Main effect of drug (P < .05).
<sup>e</sup>Main effect of LPS (P < .09) and drug (P < .02). LPS x drug interaction (P < .09).
<sup>f</sup>LPS x drug interaction (P < .05).
Table 3. Effects of indomethacin on the behavioral activity of barrows following a challenge dose of lipopolysaccharide (n = 8) 

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Control (no drug)</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>LPS</td>
</tr>
<tr>
<td>0–0.5</td>
<td>30.0</td>
<td>29.3</td>
</tr>
<tr>
<td>1–1.5</td>
<td>17.3</td>
<td>6.0</td>
</tr>
<tr>
<td>2–2.5</td>
<td>13.9</td>
<td>2.6</td>
</tr>
<tr>
<td>4–4.5</td>
<td>7.3</td>
<td>4.6</td>
</tr>
<tr>
<td>8–8.5</td>
<td>11.4</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*Time spent active (sum of the time spent eating and moving) during 30-min sampling periods.

As a first attempt to systematically study sickness in pigs, we injected LPS. Although this model makes it impossible to elucidate the effect(s) of a specific cytokine, it is more likely to be representative of the pig's response to infection. In Exp. 1, barrows responded to LPS by reducing feed intake, reducing activity, and becoming febrile. Both the intensity and duration of these effects were dose-dependent. Following treatment with LPS, barrows also were lethargic and somnolent (casual observation). These data are consistent with previous reports (Hart, 1988; Kent et al., 1992b) and suggest that pigs activate behavioral and physiological systems to maintain homeostasis during infection.

Anorexia, lethargy, somnolence, and fever also occur in food animals injected peripherally with recombinant cytokines (Kelley et al., 1993). Because cytokines such as interleukin-1 and tumor necrosis factor-α can cause all these symptoms, it has been proposed that cytokines act as hormones to inform the central nervous system that a foreign agent has gained access to the body (Kent et al., 1992b). The mechanisms at work in this neuroimmune axis are for the most part unknown. For example, it is uncertain whether peripherally produced interleukin-1 can cross the blood-brain barrier (Fontana et al., 1984; Cocceani et al., 1988; Banks et al., 1989). Nonetheless, there are interleukin-1 receptors both inside and outside the central nervous system (Haour et al., 1990; Takao et al., 1990; Parnet et al., 1993), and transcripts for interleukin-1 are produced in discrete brain areas following peripheral injection of LPS (Ban et al., 1992).

A working hypothesis regarding communication in the neuroimmune axis suggests that cytokines interact with astrocytes at circumventricular organs to stimulate the metabolism of arachidonic acid to PG (Katsuura et al., 1989). Prostaglandins then diffuse into adjacent brain areas and cause sickness behavior and fever. In laboratory animals, the reduction in feed motivation and social exploration (Crestani et al., 1991), anorexia (Uehara et al., 1989), and fever caused by interleukin-1 are inhibited by pretreatment with IND (Stitt and Bernheim, 1985; Hashimoto et al., 1988).

To determine whether the behavioral and physiological effects of LPS are mediated by a PG-dependent mechanism in pigs, a second experiment was conducted in which barrows were injected with IND, and their behavior and T_B following a challenge dose of LPS were assessed. Consistent with what has been reported in other food animals (Johnson et al., 1993b), pretreatment with IND inhibited the anorexia and inactivity caused by LPS. Because IND is a specific inhibitor of the cyclooxygenase pathway (Shen and Winter, 1977), these data are interpreted to suggest that anorexia and inactivity caused by LPS involve activation of a PG system.

Indomethacin, alone, increased feed intake at 1 to 2 h. For example, IND/SAL barrows consumed 374 g, whereas, VEH/SAL barrows consumed 247 g (see Table 2). In addition to being antipyretic and anti-inflammatory, IND also is analgesic, which may, in part, explain the increase in feed intake (Morley et al., 1983). Therefore, the ability of IND to reverse LPS-induced behavior may have been the result of both cyclooxygenase inhibition and analgesia. However, the best available information on this subject suggests that prostaglandin synthesis is important. Administering PG into the central nervous system of rats induces anorexia (Levine and Morley, 1981) and sleep (Hayashi, 1988). In chickens, IND inhibited the fever caused by injecting O somatic antigen from Shigella dysenteriae, but not that caused by injecting PGE₂ (IND inhibits cyclooxygenase but not the activity of PG) (Nistico and Rotiroti, 1978). Thus, at least in the case of fever, the effects of IND seem to be PG-dependent.

Unfortunately, we were unable to determine the involvement of PG in the pig's febrile response to LPS because the VEH/LPS-treated barrows failed to develop a significant increase in T_B. There is good
evidence, however, that the expression of sickness behavior is not necessarily dependent on fever (see Kluger, 1991). In a recent study, Kent et al. (1992a) used a specific antagonist of the type I interleukin-1 receptor to show that induction of fever does not share the same receptor mechanism as that responsible for the decrease in feed motivation and social exploration.

### Implications

The results of this study indicate that pigs respond to a challenge dose of lipopolysaccharide by activating both behavioral and physiological systems. The fact that we were able to inhibit the reduced feed intake and inactivity caused by lipopolysaccharide by pretreatment with indomethacin suggests that the effects of lipopolysaccharide are mediated by a prostaglandin-dependent mechanism. Therefore, it seems reasonable to postulate that by altering the activity of cyclooxygenase, behavioral symptoms of sickness can be enhanced or inhibited to either prevent disease or enhance recovery in pigs.

### Literature Cited


