

Intravenous Endotoxin Triggers Pulmonary Vasoconstriction and Pulmonary Hypertension in Broiler Chickens

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ABSTRACT Bacterial endotoxins stimulate endothelin-mediated, thromboxane-dependent increases in pulmonary vascular resistance in mammals, and thromboxane has been shown to cause an immediate but transient pulmonary vasoconstriction in broiler chickens. In the present study, i.v. injections of 1 mg endotoxin into anesthetized male broilers caused a pulmonary vasoconstrictive response that was delayed in onset by 15 min and that elevated the pulmonary arterial pressure by 10 mm Hg within 25 min postinjection. Thereafter, pulmonary hemodynamic variables gradually (≥ 15 min) returned toward pre-injection levels, and supplemental injections of 4 mg endotoxin during this recovery period failed to reinitiate pulmonary hypertension. In contrast, injecting the thromboxane A₂ mimetic U44069 during the endotoxin recovery period triggered pulmonary vasoconstriction and pulmonary hypertension similar in magnitude to the responses triggered by U44069 before endotoxin had been administered. The time course and magnitude of the pulmonary hemodynamic responses to endotoxin were highly variable among individual broilers, whereas

the individual responses to U44069 were more consistent. Unanesthetized broilers resembled anesthetized broilers in the time course, magnitude, and variability of their pulmonary hemodynamic responses to endotoxin. Overall, these observations are consistent with the hypothesis that endotoxin initiates a biochemical cascade, culminating in the delayed onset of pulmonary vasoconstriction and pulmonary hypertension within 20 min postinjection. Subsequently, the pulmonary vasculature remains responsive to large bolus injections of exogenous thromboxane mimetic; however depletion of endogenous vasoconstrictive components of the endotoxin-mediated cascade, a compensatory increase in endogenous vasodilators, or the induction of a transient cellular tolerance to endotoxin prevented fourfold higher doses of endotoxin from reversing the return toward a normal pulmonary vascular tone. Individual differences among broilers in their susceptibility to pulmonary hypertension syndrome (ascites) may be related to innate or acquired variability in their pulmonary vascular responsiveness to vasoactive mediators.

(Key words: broilers, endotoxin, lipopolysaccharide, pulmonary hypertension, ascites)

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INTRODUCTION

Worldwide, approximately 4% of all broilers die from pulmonary hypertension syndrome (PHS, ascites), amounting to a loss estimated at \$1 billion annually (Maxwell and Robertson, 1997). Broilers are susceptible to PHS whenever their pulmonary vascular capacity is inadequate to accept and oxygenate, at a sufficiently low pulmonary arterial pressure, the cardiac output required to support tissue metabolism. The pulmonary vascular capacity is defined broadly to encompass anatomical constraints related to the compliance and volume of the blood vessels, as well as metabolic limitations related to the tone (state of contracture) maintained by the resistance vessels. Genetic

selection experiments have confirmed that susceptibility to PHS depends substantially on an inherent inability of the pulmonary vasculature to accommodate the requisite cardiac output (Wideman and French, 1999, 2000). All factors contributing to an increase in cardiac output or an overall reduction in the pulmonary vascular capacity theoretically can accelerate the pathogenesis of PHS if the right ventricle of the heart must increase the pulmonary arterial pressure to propel blood flow through the lungs (Wideman and Bottje, 1993, Wideman, 1997, 2000; Wideman and Tackett, 2000; Wideman et al., 2000).

Thromboxane is a potent vasoconstrictor derived from circulating thrombocytes or lung and vascular tissues. Intravenous injections of the potent thromboxane A₂ mimetic U44069 directly increase the pulmonary vascular resistance and the pulmonary arterial pressure in broilers. Thromboxane also may mediate the pulmonary vasoconstriction triggered by acidosis in broilers (Wideman et al., 1998, 1999).

Abbreviation Key: LPS = lipopolysaccharide; PHS = pulmonary hypertension syndrome.

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The pulmonary vasoconstriction and pulmonary hypertension triggered by thromboxane have a functional significance related to the presumed contribution of poor air quality and respiratory disease to the ascites syndrome. Accordingly, it has been proposed that inhaled dust and pathogens may cause focal damage and pulmonary vasoconstriction by increasing the intrapulmonary production of reactive oxygen species and thromboxane, coupled with reduced synthesis of the vasodilator prostacyclin (Bottje and Wideman, 1995; Wideman et al., 1999). Indeed, various particulates and bacterial endotoxins stimulate endothelin-mediated thromboxane-dependent increases in pulmonary vascular resistance and pulmonary arterial pressure in mammals (Morel et al., 1989; Longworth et al., 1994; Staub, 1994; Faltin et al., 1996). During endotoxin infusion in mammals, both the vasoconstrictor thromboxane and the vasodilator prostacyclin normally are produced and can exert dynamically antagonistic influences on pulmonary vascular resistance, depending on the experimental protocol (Conary et al., 1994; Longworth et al., 1994; Frank et al., 1996). Prostacyclin also attenuates the pulmonary vasoconstriction associated with pulmonary hypertension in human patients, as well as the pulmonary hypertension initiated by experimental exposure to hypoxia or metabolic acidosis (Farrukh et al., 1989; Yamaguchi et al., 1989; Christman et al., 1992; Tod et al., 1992; Frank et al., 1996). Similar interactions between locally induced pulmonary vasoconstrictors and vasodilators may be involved in the pathogenesis of ascites triggered in broilers by intratracheal inoculation with *Escherichia coli* and infectious bronchitis virus (Tottori et al., 1997) or by pulmonary aspergillosis (Julian and Goryo, 1990). The variable efficacy of aspirin on the incidence of ascites induced by cool temperature exposure or hypobaric hypoxia may reflect aspirin's non-specific nullification of the protective role of prostacyclin as well as the detrimental impact of thromboxane on pulmonary vascular resistance (Shlosberg et al., 1996; Balog et al., 2000). The present study was conducted to evaluate the pulmonary and systemic vascular responses of broilers to endotoxin. We evaluated the hypothesis that intravenous endotoxin injections should initiate a biochemical cascade culminating in pulmonary vasoconstriction and pulmonary hypertension if vasoconstrictive components of the cascade overwhelmed vasodilatory components.

MATERIALS AND METHODS

Male broilers from a commercial hatchery² were transported on the day of hatch (Day 1; August 2, 1999; October 15, 1999; and January 7, 2000, for Experiments 1, 2 and 3, respectively) to the Poultry Environmental Research Lab at the University of Arkansas Poultry Research Farm,

where they were wing-banded and placed on fresh wood shavings litter in environmental chambers (8 m² floor space). Chicks were brooded at 33 C on Days 1 to 5, 29 C on Days 6 to 10, and 21 C thereafter. They were fed a 23% CP corn-soybean meal-based broiler ration formulated to meet or exceed the minimum NRC (1984) standards for all ingredients. Feed and water were provided ad libitum. Lights were on for 24 h/d through Day 5 and for 23 h/d thereafter.

Experiment 1

Seven birds (30 to 32 d old, 1,482 ± 207 g, mean ± SEM) were prepared for surgery as described previously (Wideman et al., 1996, 1998, 1999). All birds had full access to feed and water until they were anesthetized. A surgical plane of anesthesia was induced with intramuscular injections of allobarbital (5,5-diallyl-barbituric acid,³ 25 mg/kg/BW). The birds were fastened in dorsal recumbency on a surgical board thermostatically regulated to maintain a surface temperature of 30 C. An incision was made to open the thoracic inlet, and the left and right pulmonary arteries were located. A Transonic ultrasonic flowprobe⁴ was positioned on the right pulmonary artery, and the probe was connected to a Transonic T206 blood flow meter⁴ to confirm signal acquisition. A 27-GA × 1-inch needle, bent to a 90° angle mid-way along its length, was scored and snapped off adjacent to the hub. The resulting blunt end was pressure-fit into 30 cm of Silastic® Tubing (0.012 inch I.D., 0.037 inch O.D.)⁵ filled with heparinized saline. The point of the needle was inserted into the left pulmonary artery, a 4-cm loop of the Silastic® tubing was coiled inside the thoracic inlet to provide strain relief during respiratory motion, and the thoracic inlet was sealed with stainless steel wound clips. The distal end of the Silastic® tubing was attached to a blood pressure transducer interfaced through a Transbridge™ preamplifier⁶ to a Biopac MP 100 data acquisition system⁷ using ACQKnowledge software.⁷ The left basilica vein (wing vein) was cannulated with PE-50 polyethylene tubing for i.v. injections. Ongoing intravenous infusions were not administered. The left brachial artery was cannulated with PE-50 polyethylene tubing filled with heparinized saline, the cannula was advanced toward the descending aorta and was attached to a blood pressure transducer for continuous monitoring of systemic arterial pressure.

After surgical preparations were complete and a stabilization period of 10 min had elapsed, control data were recorded for 20 min. A 23-GA needle attached to a 1-mL syringe was used to withdraw 0.8 mL of blood from the wing vein cannula, then 0.2 mL of PBS was drawn into the same syringe and mixed with the blood. This mixture was re-injected through the wing vein cannula as a volume control, and volume control data were recorded for 20 min. Bacterial endotoxin (lipopolysaccharide, LPS, from *Salmonella typhimurium*)³ was dissolved at 5 or 20 mg/mL in PBS, stored at -80 C in sealed vials containing 1-mL aliquots, and thawed immediately prior to use. Blood (0.8 mL) was withdrawn from the wing vein cannula and mixed with 0.2 mL of the 5 mg/mL endotoxin solution.

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³Sigma Chemical Co., St. Louis, MO 63178-9916.

⁴Transonic Systems Inc., Ithaca, NY 14850.

⁵Konigsberg Instruments, Inc., Pasadena, CA 91107-3294.

⁶World Precision Instruments, Sarasota, FL 34230.

⁷Biopac Systems, Inc., Santa Barbara, CA 93117.

This mixture containing 1 mg endotoxin was re-injected, and data were recorded for 30 min. Blood (0.8 mL) was withdrawn from the wing vein cannula and mixed with 0.2 mL of the 20 mg/mL endotoxin solution. This mixture containing 4 mg endotoxin was re-injected, data were recorded for 30 min., then the experiment was terminated with a 10-mL i.v. injection of 0.1 M KCl. The 1- and 4-mg doses of endotoxin (approximately 0.5 and 2.75 mg/kg BW, respectively) were chosen to be intermediate between the much lower levels ($\leq 15 \mu\text{g/kg BW}$) required to prime leukocytes without directly altering systemic or pulmonary hemodynamics (Weidner and Lancaster, 1999), and higher levels (5.0 mg/kg BW) capable of triggering febrile and stress responses, and endotoxin shock associated with systemic vasodilation and hypotension (Butler et al., 1977; Merrill et al., 1981; Xie et al., 2000).

Experiment 2

Eight birds (37 to 38 d old, $1,804 \pm 48$ g, mean \pm SEM) were anesthetized and prepared as described above. When surgical preparations were complete and a stabilization period of 10 min had elapsed, control data were recorded for 10 min, then 0.5 mL of 2.5% mannitol (25 g mannitol/L of water) was injected, and volume control data were recorded for 10 min. The thromboxane A₂ mimetic U44069³ was diluted to 1 mmol/mL in 2.5% mannitol (1 mM; 0.3505 mg U44069 per mL of 2.5% mannitol), stored at -80°C in sealed vials containing 0.5-mL aliquots, and thawed immediately prior to use. Characteristics of, and physiological responses to, U44069 were described previously (Rose et al., 1976; Wideman et al., 1999). Birds were injected with 0.3 mL of the 1 mM U44069, and data were recorded for 10 min. Blood (0.8 mL) was drawn from the wing vein cannula into a 1-mL syringe and mixed with 0.2 mL of the 5 mg/mL endotoxin solution (*vide supra*). This mixture containing 1 mg endotoxin was re-injected, and data were recorded for 30 min. Finally, a second 0.3-mL injection of the 1 mM U44069 was administered, data were recorded for 15 min, then the experiment was terminated with a 10-mL i.v. injection of 0.1 M KCl.

Experiment 3

Five birds (42 to 43 d old, $2,412 \pm 57$ g, mean \pm SEM) were restrained in dorsal recumbency without general anesthesia. A 2% (wt/vol) Lidocaine solution was infiltrated intracutaneously along the left basilica vein, which then was cannulated with Silastic® Tubing (0.012 inch I.D., 0.037 inch O.D.) filled with heparinized saline. The distal end of the tubing was attached to a blood pressure transducer interfaced to the Biopac MP 100 data acquisition system, and the cannula was advanced into the pulmonary artery as judged by characteristic pressure tracings (Guthrie et al., 1987; Owen et al., 1995). The left brachial artery was cannulated with PE-50 polyethylene tubing filled with heparinized saline, and the cannula was attached to a blood pressure transducer for continuous monitoring of systemic arterial pressure. A 10-min stabilization period elapsed

following insertion of the cannulae, then control data were recorded for 10 min. Blood (0.6 mL) was drawn from the wing vein cannula into a tuberculin syringe and mixed with 0.4 mL of the 5 mg/mL endotoxin solution (*vide supra*). This mixture containing 2 mg LPS was re-injected, data were recorded for 40 min, then the experiment was terminated with a 10-mL i.v. injection of 0.1 M KCl.

Data Analysis

The primary channels recorded by the Biopac MP 100 data acquisition system included systemic arterial pressure (mm Hg), pulmonary arterial pressure (mm Hg), and blood flow through the right pulmonary artery (mL/min). Heart rate (beats/min) was obtained by counting systolic peaks over time in the pulmonary arterial pressure recording. These primary data were averaged electronically during representative sample intervals at the start of data collection (start), at 5-min intervals throughout the initial control period (C5, C10, etc.), within 30 s after injecting a volume control (VC), at 5 min intervals throughout the volume control period (VC5, VC10, etc.), within 30 s after injecting endotoxin (E inj), at 5-min intervals after endotoxin injection (E5, E10, etc.), during the maximum pulmonary arterial pressure response to endotoxin (E Pk), during the maximum pulmonary arterial pressure response within 90 s after injecting the thromboxane mimetic U44069 (TX Pk), at 5-min intervals after injecting U44069 (TX5, TX10), and at the end of the experiment (final). The protocol used for data averaging accommodates the influences of pulse pressure and respiratory cycles on pulmonary and systemic arterial pressures (Wideman et al., 1996). The primary data were used to calculate cardiac output, stroke volume, pulmonary vascular resistance, and total peripheral resistance. Based on the assumption that cardiac output (mL/min) normally is divided approximately equally between the lungs, cardiac output was calculated as $2 \times$ blood flow. The cardiac output is the product of heart rate \times stroke volume (mL/beat), consequently stroke volume was calculated as cardiac output divided by heart rate. Assuming the pressure gradients across the pulmonary and systemic circulations are essentially equal to pulmonary arterial pressure and systemic arterial pressure, respectively (Wideman et al., 1996, 1998), then the relationships between pressure gradients, flow rates, and resistances are summarized by the respective equations: pulmonary arterial pressure = cardiac output \times pulmonary vascular resistance, and mean systemic arterial pressure = cardiac output \times total peripheral resistance. Thus, pulmonary vascular resistance was calculated in relative resistance units as pulmonary arterial pressure (mm Hg) divided by cardiac output (mL/min), and total peripheral resistance was calculated in relative resistance units as mean systemic arterial pressure (mm Hg) divided by cardiac output (mL/min) (Besch and Kadono, 1978; Sturkie, 1986; Wideman et al., 1996, 1998). Due to the direct proportionality between cardiac output and body weight (Wideman, 1999), all data derived from blood flow measurements were normalized for body weight on an individual

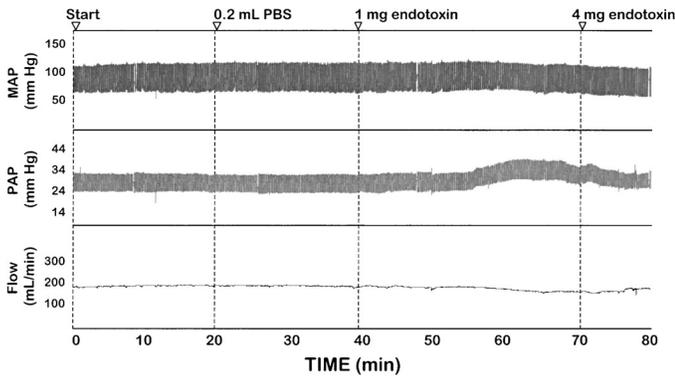


FIGURE 1. Physiograph recording from an individual male broiler in Experiment 1, showing continuous values for mean systemic arterial pressure (MAP), pulmonary arterial pressure (PAP), and blood flow through the right pulmonary artery (Flow) during an initial 20-min control period, after an i.v. injection of PBS as a volume control, and after i.v. injections of 1 and 4 mg endotoxin.

bird basis, prior to calculating group means. Respiratory rate (breaths/min) was not measured. Data were analyzed over time (across sample intervals) using the SigmaStat® repeated measures analysis of variance procedure, and means were differentiated by the Student-Newman-Keuls method (Jandel Scientific, 1994). Body weight normalization of the flow-related data neither created nor eliminated any instance in which the repeated measures analysis of variance detected a significant ($P \leq 0.05$) change over time, when compared with repeated measures analysis of the absolute data (not shown).

RESULTS

Experiment 1

Pilot studies indicated the peak pulmonary hypertensive response to 1 mg endotoxin could not be surpassed in amplitude or duration by injecting doses of up to 10 mg endotoxin (data not shown). Data for an individual broiler are shown in Figure 1. The initial pulmonary arterial pressure averaged approximately 29 mm Hg throughout the 20-min control period. Injecting 0.2 mL PBS as a volume control had no impact on pulmonary arterial pressure, which averaged 28 mm Hg during the subsequent 20-min period. Injecting 1 mg endotoxin into the wing vein did not elicit an immediate response; however, the pulmonary arterial pressure began to increase within 15 min and reached a peak value of 39 mm Hg at 20 min postinjection. The pulmonary arterial pressure then subsided to 34 mm Hg, and injecting 4 mg endotoxin did not prevent the ongoing decline in pulmonary arterial pressure to a value that averaged 31 mm Hg by the end of the experiment. Mean systemic arterial pressure remained stable at approximately 83 mm Hg until 15 min after the 1-mg endotoxin injection, then the systemic arterial pressure gradually declined to a final value of 73 mm Hg. Blood flow through the right pulmonary artery gradually declined following the first endotoxin injection (Figure 1).

The pulmonary hemodynamic responses to endotoxin were widely varied among individual birds, as reflected by the pulmonary arterial pressure data presented in Figure 2. Pulmonary arterial pressures ranged between 20 and 30 mm Hg during control sample intervals. In response to the initial 1-mg endotoxin injection, the pulmonary arterial pressure in three birds increased by ≥ 20 mm Hg, then remained elevated in one bird, and returned toward the baseline in the others. Several birds exhibited more modest pulmonary hypertensive responses to the initial 1-mg endotoxin injection, and their responses were not further amplified by the subsequent 4-mg endotoxin injection. Similarly patterns of variability were evident in plots of the pulmonary vascular resistance data for individual birds (not shown).

Average responses to endotoxin by the group as a whole are shown in Figures 3 to 5. The pulmonary arterial pressure averaged 25 mm Hg during the control period, was unaffected by the volume control, and increased to a peak value of 35 mm Hg within 20 min after the 1-mg endotoxin injection. The 4-mg endotoxin injection did not reinitiate an increase in the pulmonary arterial pressure, which instead declined to approximately 29 mm Hg by the end of the experiment (Figure 3). The pulmonary hypertensive response to 1 mg endotoxin was caused by pulmonary vasoconstriction, as reflected by a contemporaneous increase in the pulmonary vascular resistance without an increase pulmonary blood flow (Figure 3). Cardiac output, stroke volume, and heart rate remained unchanged throughout the experiment (Figure 4). The reduction in the mean systemic arterial pressure following the 1-mg endotoxin injection apparently reflects trends toward gradual reductions in cardiac output and total peripheral resistance (Figure 5).

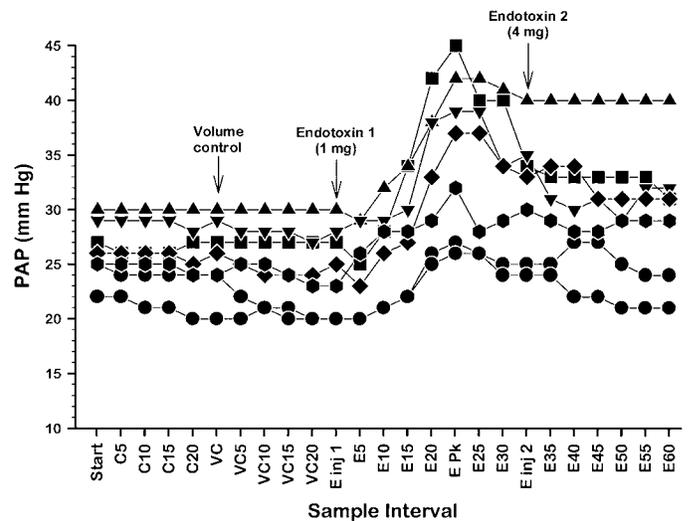


FIGURE 2. Individual pulmonary arterial pressure (PAP) values for seven male broilers in Experiment 1, at the start of data collection (Start), at 5-min intervals during the control period (C5 to C20), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 to VC20), within 30 s after the 1-mg endotoxin injection (E inj 1), at 5-min intervals after the 1 mg endotoxin injection (E5 to E30), during the maximum PAP response to 1 mg endotoxin (E Pk), within 30 s after the 4-mg endotoxin injection (E inj 2), and at 5-min intervals thereafter (E35 to E60).

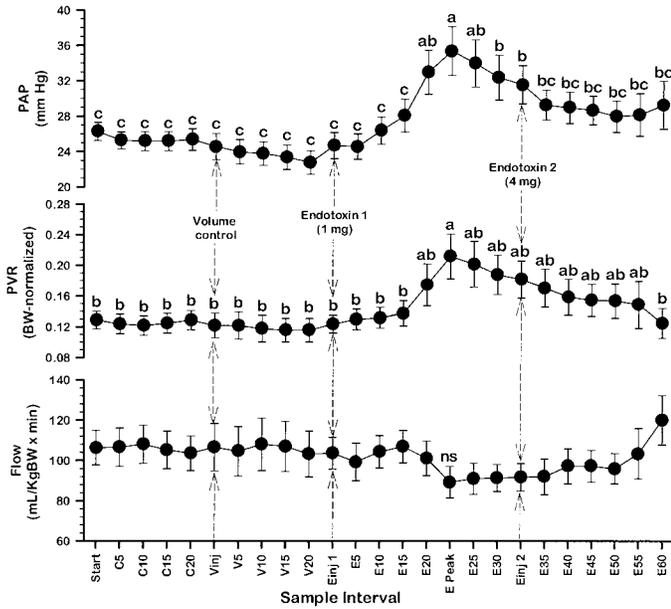


FIGURE 3. Pulmonary arterial pressure (PAP, upper panel), pulmonary vascular resistance (PVR, middle panel), and blood flow through the right pulmonary artery (Flow, lower panel) for male broilers (mean \pm SEM, n = 7) in Experiment 1, at the start of data collection (Start), at 5-min intervals during the control period (C5 to C20), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 to VC20), within 30 s after the 1-mg endotoxin injection (E inj 1), at 5-min intervals after the 1-mg endotoxin injection (E5 to E30), during the maximum PAP response to 1 mg endotoxin (E Peak), within 30 s after the 4-mg endotoxin injection (E inj 2), and at 5-min intervals thereafter (E35 to E60). Different letters (a,b,c) designate differences between means over time ($P \leq 0.05$); ns = not significant ($P > 0.05$).

Experiment 2

The pulmonary arterial pressure responses of individual broilers are shown in Figure 6. Pulmonary arterial pressures ranged between 17 and 35 mm Hg during control sample intervals. The volume control injection had no effect, whereas the first injection of thromboxane analog consistently triggered immediate but transient pulmonary hypertension in every bird. Endotoxin triggered pulmonary hypertensive responses that varied widely in time of onset, amplitude, and duration. Superimposed upon the variable response to endotoxin, the second injection of thromboxane analog consistently triggered pulmonary hypertension.

Average responses by the group as a whole are shown in Figures 7 to 9. The volume control injection did not influence any of the parameters. Within 15 s postinjection, the first injection of thromboxane analog increased the pulmonary vascular resistance and pulmonary arterial pressure and marginally, but not significantly ($P = 0.085$), reduced the blood flow through the right pulmonary artery (Figure 7). All values returned to baseline within 5 min postinjection. The endotoxin injection triggered a delayed-onset pulmonary vasoconstriction and pulmonary hypertension that peaked approximately 25 min postinjection. Superimposed upon the endotoxin response, the second injection of thromboxane analog triggered peaks in pulmo-

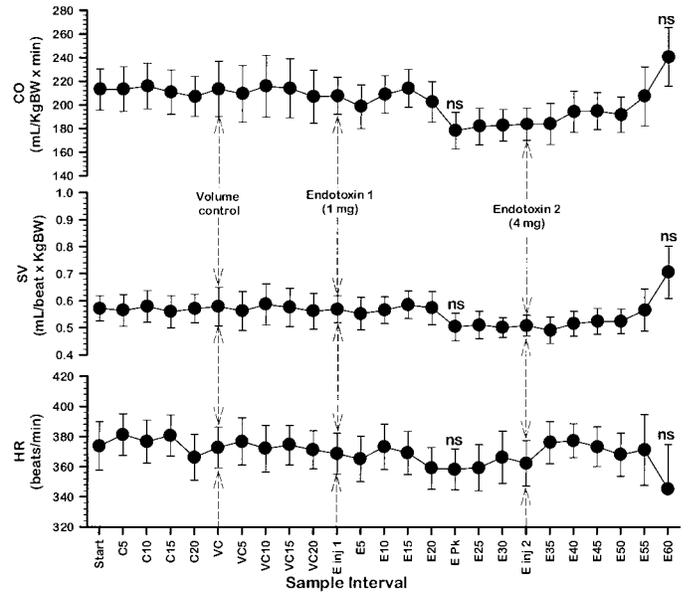


FIGURE 4. Cardiac output (CO, upper panel), stroke volume (SV, middle panel), and heart rate (HR, lower panel) for male broilers (mean \pm SEM, n = 7) in Experiment 1, at the start of data collection (Start), at 5-min intervals during the control period (C5 to C20), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 to VC20), within 30 s after the 1-mg endotoxin injection (E inj 1), at 5-min intervals after the 1-mg endotoxin injection (E5 to E30), during the maximum pulmonary arterial pressure response to 1 mg endotoxin (E Peak), within 30 s after the 4-mg endotoxin injection (E inj 2), and at 5-min intervals thereafter (E35 to E60).

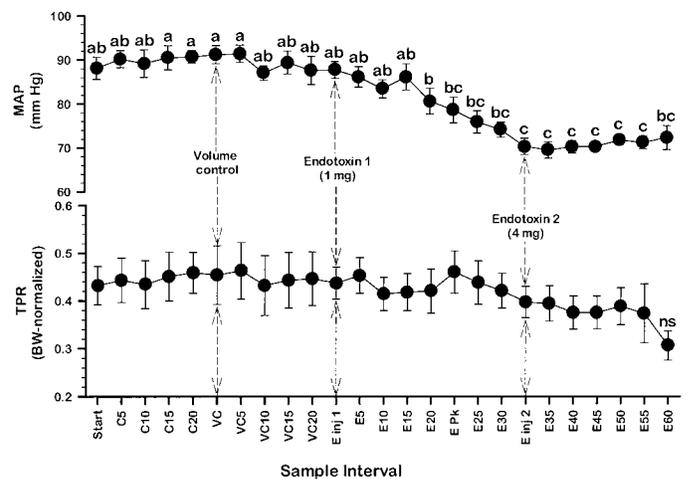


FIGURE 5. Mean systemic arterial pressure (MAP, upper panel) and total peripheral resistance (TPR, lower panel) for male broilers (mean \pm SEM, n = 7) in Experiment 1, at the start of data collection (Start), at 5-min intervals during the control period (C5 to C20), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 to VC20), within 30 s after the 1-mg endotoxin injection (E inj 1), at 5-min intervals after the 1-mg endotoxin injection (E5 to E30), during the maximum pulmonary arterial pressure response to 1 mg endotoxin (E Peak), within 30 s after the 4-mg endotoxin injection (E inj 2), and at 5-min intervals thereafter (E35 to E60). Different letters (a,b,c) designate differences between means over time ($P \leq 0.05$); ns = not significant ($P > 0.05$).

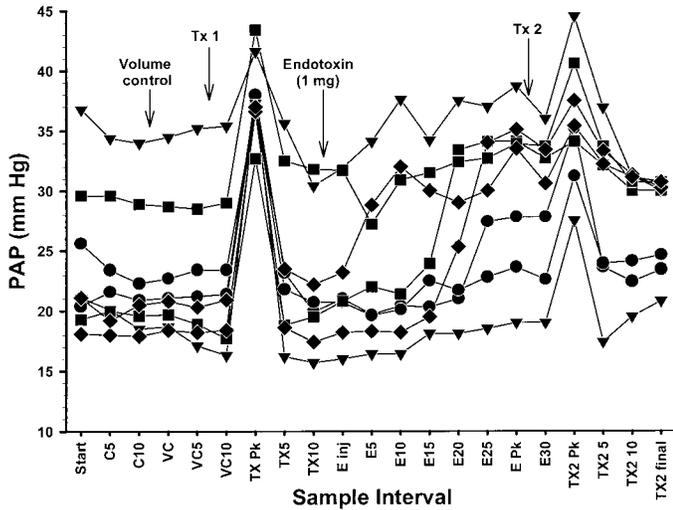


FIGURE 6. Individual pulmonary arterial pressure (PAP) values for eight individual male broilers in Experiment 2, at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting the volume control injection (VC), at 5-min intervals during the volume control period (VC5 and VC10), during the maximum PAP response within 90 s after the first injection of the thromboxane A₂ mimetic U44069 (TX Pk), at 5-min intervals after the first thromboxane mimetic injection (TX5 and TX10), within 30 s after endotoxin injection (E inj), at 5-min intervals after endotoxin injection (E5 to E30), during the maximum PAP response to endotoxin (E Pk), during the maximum PAP response within 90 s after the second injection of thromboxane mimetic (TX2 Pk), and at 5-min intervals after the second thromboxane mimetic injection (TX2 5 to TX2 final).

nary arterial pressure and pulmonary vascular resistance and reductions in pulmonary arterial blood flow that, in absolute amplitude, were similar to or greater than the responses elicited by the first injection of thromboxane analog (Figure 7). Cardiac output and stroke volume remained unchanged over the course of the experiment and heart rate declined 20 min following the endotoxin injection (Figure 8). The mean systemic arterial pressure gradually declined over the course of the experiment, apparently reflecting trends toward gradual reductions in cardiac output and total peripheral resistance. The first thromboxane analog injection increased the total peripheral resistance and transiently increased the mean arterial pressure (Figure 9).

Experiment 3

Unanesthetized broilers exhibited individual pulmonary hypertensive responses that varied widely in time of onset, amplitude, and duration following endotoxin injection (Figure 10). The peak response to endotoxin occurred 20 to 25 min postinjection. The mean systemic arterial pressure gradually declined over the course of the experiment (Figure 11).

DISCUSSION

Endotoxin stimulates endothelin-mediated thromboxane-dependent pulmonary vasoconstriction leading to pulmonary hypertension in mammals (see Introduction). In

a variety of mammalian species, increases in thromboxane production and a transient pulmonary vasoconstriction occur within 1 min following i.v. endotoxin infusion (Staub, 1994). Bacterial endotoxins are ubiquitous in the environment of broilers, and any factor capable of increasing the pulmonary vascular resistance potentially can accelerate the pathogenesis of PHS. Accordingly, three independent experiments were conducted to evaluate the pulmonary hemodynamic responses of anesthetized and unanesthetized male broilers to endotoxin. Intravenously administered endotoxin triggered pulmonary hypertension without increasing the cardiac output, providing conclusive evidence that the increase in pulmonary arterial pressure was caused by pulmonary vasoconstriction. In most birds, the pulmonary vascular resistance did not increase until approximately 15 min following the endotoxin injection, and peak response levels were not attained until 20 to 25 min postinjection. Injecting fourfold higher doses of endotoxin following the peak response to the first endotoxin injection failed to reestablish pulmonary vasocon-

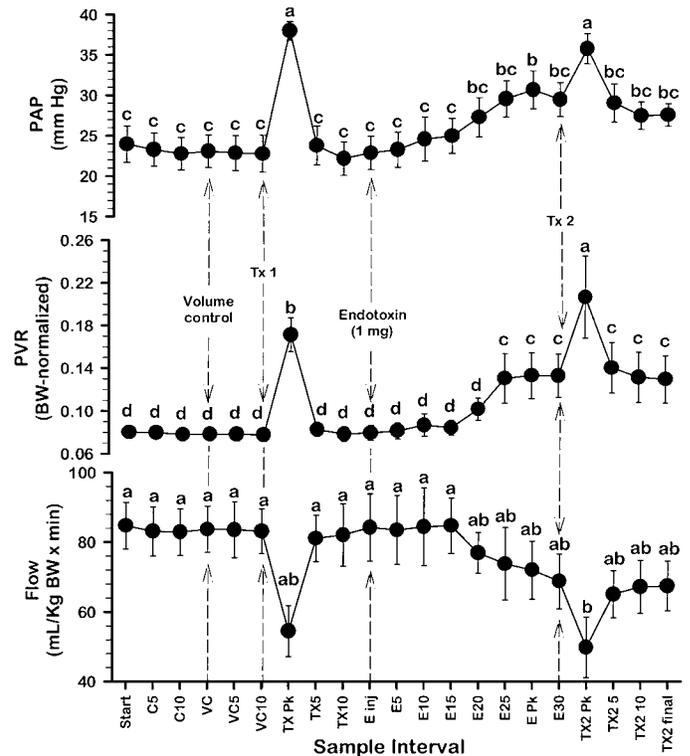


FIGURE 7. Pulmonary arterial pressure (PAP, upper panel), pulmonary vascular resistance (PVR, middle panel), and blood flow through the right pulmonary artery (Flow, lower panel) for male broilers (mean \pm SEM, $n = 8$) in Experiment 2, at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 and VC10), during the maximum PAP response within 90 s after the first injection of the thromboxane A₂ mimetic U44069 (TX Pk), at 5-min intervals after the first thromboxane mimetic injection (TX5 and TX10), within 30 s after endotoxin injection (E inj), at 5-min intervals after endotoxin injection (E5 to E30), during the maximum PAP response to endotoxin (E Pk), during the maximum PAP response within 90 s after the second injection of thromboxane mimetic (TX2 Pk), and at 5-min intervals after the second thromboxane mimetic injection (TX2 5 to TX2 final). Different letters (a,b,c) designate differences between means over time ($P \leq 0.05$).

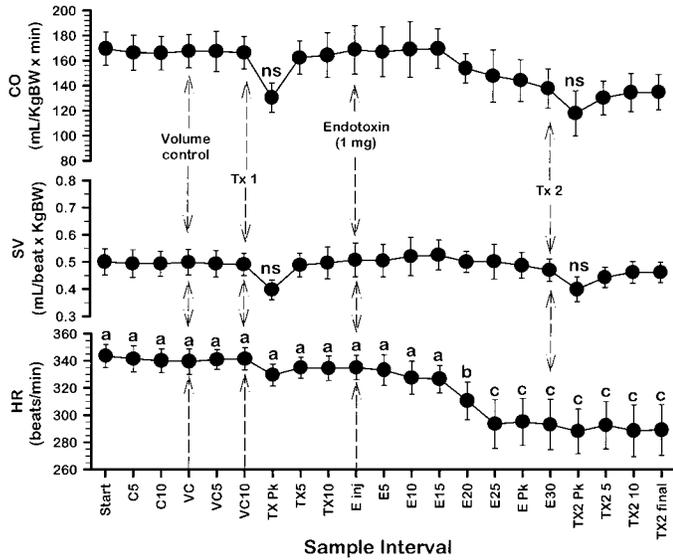


FIGURE 8. Cardiac output (CO, upper panel), stroke volume (SV, middle panel), and heart rate (HR, lower panel) for male broilers (mean \pm SEM, $n = 8$) in Experiment 2, at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 and VC10), during the maximum PAP response within 90 s after the first injection of the thromboxane A₂ mimetic U44069 (TX Pk), at 5-min intervals after the first thromboxane mimetic injection (TX5 and TX10), within 30 s after endotoxin injection (E inj), at 5-min intervals after endotoxin injection (E5 to E30), during the maximum PAP response to endotoxin (E Pk), during the maximum PAP response within 90 s after the second injection of thromboxane mimetic (TX2 Pk), and at 5-min intervals after the second thromboxane mimetic injection (TX2 5 to TX2 final). Different letters (a,b,c) designate differences between means over time ($P \leq 0.05$); ns = not significant ($P > 0.05$).

striction within 30 min, whereas the thromboxane mimetic U44069 was an equally effective pulmonary vasoconstrictor when injected before or after endotoxin. These observations are consistent with the hypothesis that endotoxin initiates a biochemical cascade requiring 15 min to culminate in pulmonary vasoconstriction. Thereafter, a compensatory increase in endogenous vasodilators (prostacyclin, nitric oxide) or depletion of endogenous vasoconstrictive precursors in the cascade might have prevented fourfold higher doses of endotoxin from reversing the return toward a normal pulmonary vascular tone (Julou-Schaeffer et al., 1990; Christman et al., 1992; Tod et al., 1992; Frank et al., 1996). An increase in vasodilators appears less likely than depletion of endogenous precursors, as the pulmonary vasculature remained responsive to large bolus injections of thromboxane mimetic during the recovery period following the first endotoxin injection. Alternatively, prior exposure of leukocytes to endotoxin can acutely inhibit the endotoxin signaling cascade and reduce the production of key cytokines, thereby transiently desensitizing leukocytes to subsequent endotoxin exposure (Medvedev et al., 2000). An acute desensitization of the cells involved in the pulmonary hypertensive response to endotoxin is a likely explanation for the failure of the 4-mg endotoxin injection to initiate a secondary pulmonary hemodynamic response in Experiment 1. The roles of endothelin, thromboxane,

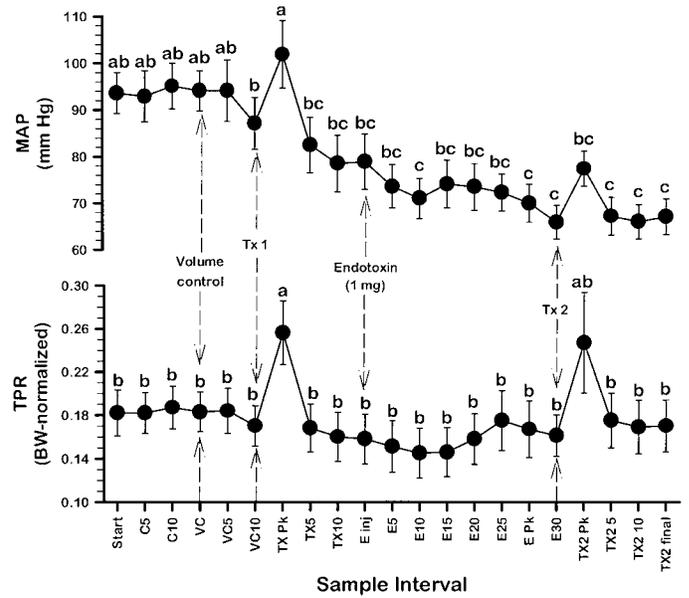


FIGURE 9. Mean systemic arterial pressure (MAP, upper panel) and total peripheral resistance (TPR, lower panel) for male broilers (mean \pm SEM, $n = 8$) in Experiment 2, at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 and VC10), during the maximum PAP response within 90 s after the first injection of the thromboxane A₂ mimetic U44069 (TX Pk), at 5-min intervals after the first thromboxane mimetic injection (TX5 and TX10), within 30 s after endotoxin injection (E inj), at 5-min intervals after endotoxin injection (E5 to E30), during the maximum PAP response to endotoxin (E Pk), during the maximum PAP response within 90 s after the second injection of thromboxane mimetic (TX2 Pk), and at 5-min intervals after the second thromboxane mimetic injection (TX2 5 to TX2 final). Different letters (a,b,c) designate differences between means over time ($P \leq 0.05$).

prostacyclin, nitric oxide, and leukocyte modulation in the pulmonary hypertensive response to endotoxin remain to be determined.

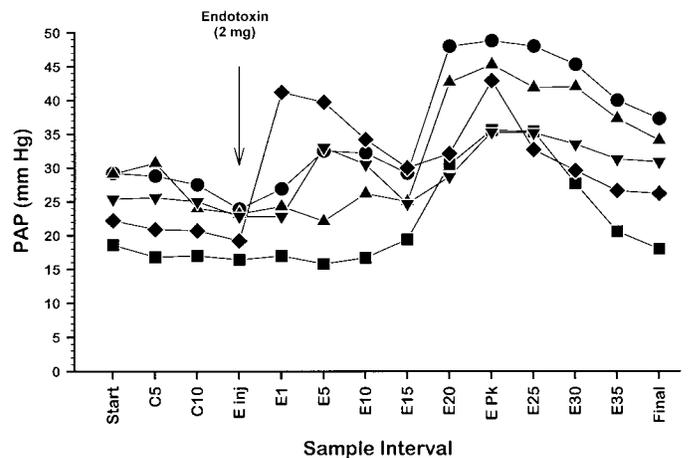


FIGURE 10. Individual pulmonary arterial pressure (PAP) values for five unanesthetized male broilers in Experiment 3, at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting endotoxin (E inj), at 1-min (E1) and then 5-min intervals after the endotoxin injection (E5 to Final), and during the maximum PAP response to endotoxin (E Pk).

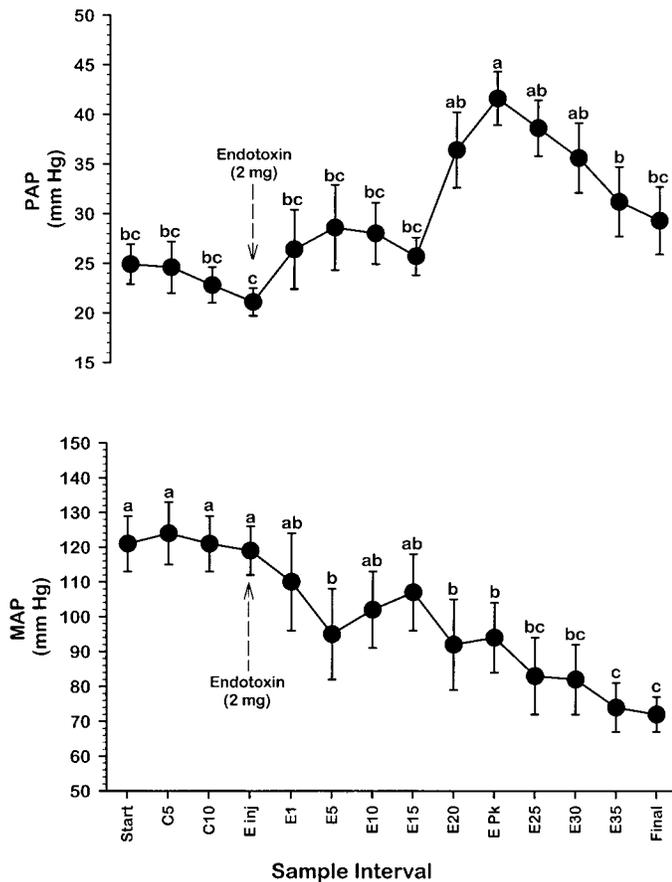


FIGURE 11. Pulmonary arterial pressure (PAP, upper panel) and mean systemic arterial pressure (MAP, lower panel) for anesthetized male broilers (mean \pm SEM, $n = 5$) in Experiment 3, at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting endotoxin (E inj), at 1-min (E1) and then 5-min intervals after the endotoxin injection (E5 to Final), and during the maximum PAP response to endotoxin (E Pk). Different letters (a,b,c) designate differences between means over time ($P \leq 0.05$).

The 1-mg i.v. injection of endotoxin used in the present study apparently was sufficient to elicit a maximal pulmonary hemodynamic response, based on the failure of higher initial endotoxin doses (up to 10 mg) to further amplify the pulmonary hypertensive response and the failure of a subsequent 4-mg endotoxin injection to reinitiate pulmonary vasoconstriction in Experiment 1. Nevertheless, wide ranges of variability in the time of onset, amplitude, and duration of the pulmonary hemodynamic responses to endotoxin were evident among broilers that had been hatched and reared together within each experiment, regardless of the presence or absence of general anesthesia or invasive instrumentation across all three experiments. A similar degree of variability previously was observed in the pulmonary hemodynamic responses of individual broilers to bolus acid (HCl) injections (Wideman et al., 1998). In mammals, the pulmonary vasoconstrictive responses to bolus injections of acid and endothelin-1 are associated with the rate-dependent production of thromboxane A_2 (Orr et al., 1990; Faltin et al., 1996). In the present study, individual broilers were much more consistent in their responses to the thromboxane mimetic U44069 than

to endotoxin, suggesting that some component of the endotoxin cascade upstream from thromboxane-initiated mechanisms may be responsible for the variability in individual responses. The widely varying responses to endotoxin among individual broilers may be innate, perhaps reflecting a genetic component of susceptibility to pulmonary hypertension (Conary et al., 1994; DeLong et al., 1999; Wideman and French, 1999, 2000). Alternatively, variability in prior exposure to pathogens or endotoxins might have led to acquired sensitization of the pulmonary hemodynamic responsiveness to endotoxin. For example, pretreatment of White Leghorn roosters with endotoxin apparently activated circulating leukocytes and enabled the microparticulate tracer Monastral blue to trigger a profound pulmonary hypertension that could not be elicited with injections of endotoxin (15 $\mu\text{g}/\text{kg}$ BW) or Monastral blue alone (Weidner and Lancaster, 1999). Changes induced by prior exposure to endotoxin not only may enhance the ability of the pulmonary vasculature and leukocytes to remove blood-borne pathogens (Weidner and Lancaster, 1999) but also may amplify the capacity for a cascading inflammatory response leading to pulmonary hypertension (Bottje and Wideman, 1995; DeLong et al., 1999; Wideman et al., 1999). Understanding the mechanisms underlying the individually variable pulmonary hypertensive responsiveness to endotoxin will likely contribute to our understanding of the multifactorial pathogenesis of PHS in broilers.

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