Three Different Strains of Heat-Killed Group B β -Hemolytic Streptococcus Cause Different Pulmonary and Systemic Hemodynamic Responses in Conscious Neonatal Lambs

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ABSTRACT. Although group B β -hemolytic streptococcus (GBS) causes pathologic hemodynamic alterations in both human neonates and neonatal animal models of sepsis, little is known about strain-dependent differences in hemodynamic responses to GBS. This study compared pulmonary and systemic hemodynamic dose-response profiles in conscious neonatal lambs with three different strains of heat-killed GBS originally isolated from infected human neonates (group 1: serotype Ib, early-onset sepsis; group 2: serotype Ib, necrotizing enterocolitis; and group 3: serotype III, meningitis). Regression models of hemodynamic responses were characterized after lambs were injected with heat-killed GBS (dose range $0.1\text{--}6.0 \times 10^9$ colony-forming units, i.v.). All three GBS strains caused dose-dependent increases in mean pulmonary arterial pressure and pulmonary and systemic vascular resistances and decreases in cardiac output and heart rate. The GBS strain used in group 1 caused a greater effect on mean pulmonary arterial pressure and systemic vascular resistance than those used in groups 2 and 3 and was the only strain to cause an increase in mean systemic arterial pressure. The GBS strains used in groups 1 and 2 had a greater effect on pulmonary vascular resistance than that used in group 3. No group differences were observed in cardiac output and heart rate responses, which were, however, influenced by age, gender, and duration of postoperative recovery of the lambs. No attenuation or augmentation of hemodynamic effect was observed after sequential doses of 10^9 colony-forming units of GBS given in a single day. This study demonstrates strain-dependent quantitative differences in pulmonary vascular response and qualitative differences in systemic vascular response to heat-killed GBS. (*Pediatr Res* 33: 373–379, 1993)

Abbreviations

GBS, group B β -hemolytic streptococcus
cfu, colony-forming unit
 P_{pa} , mean pulmonary arterial pressure
 R_p , pulmonary vascular resistance
 P_{sa} , mean systemic arterial pressure
 R_s , systemic vascular resistance
 Q_{pa} , cardiac output

HR, heart rate
 PaO_2 , arterial oxygen tension
 $PaCO_2$, arterial carbon dioxide tension
 So_2 , arterial oxyhemoglobin saturation
ANOVA, analysis of variance

GBS is the leading cause of bacterial pneumonia and sepsis in the human neonate and remains an important cause of shock, respiratory failure, persistent pulmonary hypertension, and death in the neonatal period (1–7).

Infusion into animals of live GBS bacteria isolated from infected human neonates produces cardiac depression (8–12), splanchnic hypoperfusion (12, 13), and pulmonary hypertension (8–22) in several neonatal animal models. In addition, a pulmonary pressor response to infusion of heat-killed GBS has been described in conscious piglets (23), in conscious yearling sheep (14), and in neonatal lambs (24, 25). Although previous studies have used various serotypes of GBS, the similarities or differences between different GBS strains on pulmonary and systemic hemodynamic responses in animal models have not been adequately studied.

Thus, we designed this study to compare and contrast pulmonary and systemic hemodynamic dose-response profiles in conscious neonatal lambs with three different GBS strains, to evaluate other experimental conditions that may influence the hemodynamic responses to GBS, and to evaluate whether sequential doses of heat-killed GBS in a single day would lead to attenuation or augmentation of hemodynamic effects.

MATERIALS AND METHODS

Animal care and procedures were approved by the Institutional Animal Care and Use Committee of Michael Reese Hospital and Medical Center.

Chronic Surgical Preparation. Thirteen mixed-breed newborn lambs (eight female) underwent instrumentation at age 1–16 d (mean 5 ± 1 d) while anesthetized with 0.5–1.5% isoflurane delivered by a small-animal ventilator; the details of instrumentation and recovery have been previously described (24–26).

Briefly, polyvinyl vascular catheters were advanced into the descending aorta and inferior vena cava through vessels exposed by an incision in the lower hindlimb. A left-sided thoracotomy and pericardiectomy were performed to ligate the ductus arteriosus and to place a blood flow transducer around the main pulmonary artery. In the first nine lambs, this transducer was electromagnetic (Carolina Medical Electronics, King, NC); in

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four later lambs, this transducer was ultrasonic (Transonics Systems, Inc., Ithaca, NY). Baseline values for pulmonary blood flow volume for these two measurement systems were not different in the lambs. In addition, vascular catheters were placed into the internal mammary vein (advanced into the right atrium) and artery, the pulmonary artery, and the left atrium for the measurement of vascular pressures and arterial blood pH and gas tensions. The flow transducer cable and catheters were exteriorized and secured to the lamb's flank. After recovery, lambs were returned to their ewes to recover for several days before study. Lambs were given procaine penicillin G 400 000 units, streptomycin 500 mg, and gentamicin 10 mg intramuscularly daily for 5 d beginning the day of surgery and were also given the penicillin and streptomycin after any GBS study. Vascular catheters were cleared and refilled with heparin regularly. Satisfactory catheter position was confirmed at postmortem examination when studies were complete.

Measurements and Calculations. Vascular pressures, including pulmonary and systemic arterial and left and right atrial, were measured with Gould-Statham P23ID transducers (Gould Inc., Cleveland, OH). Pulmonary artery blood flow was measured with Carolina FM501 flowmeters or Transonics T201 flowmeters as appropriate. A Gould 3800 direct-writing pen recorder was interfaced with these instruments and continuously recorded vascular pressures and pulmonary blood flow. HR was determined from the phasic arterial tracing. Vascular pressure transducers and amplifiers were calibrated before each study and flow transducers were precalibrated before implantation. Zero references for physiologic variables were checked regularly throughout a study. Arterial blood gas tensions and pH (Radiometer ABL30, Copenhagen, Denmark) and Hb concentration and oxygen saturation (Radiometer OSM-3) were measured at points defined in the protocol, after being drawn from the descending aorta. Blood gas tensions and pH were corrected to 39.5°C, the lambs' estimated temperature.

Q_{pa} was measured as pulmonary blood flow and indexed to animal weight and reported as $\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. Peak R_p and R_s were calculated with peak P_{pa} and P_{sa} and simultaneous atrial pressures and Q_{pa} as follows: $R_p = (P_{pa} - P_{la})/Q_{pa}$ and $R_s = (P_{sa} - P_{ra})/Q_{pa}$, where P_{la} and P_{ra} are mean left atrial pressure and mean right atrial pressure, respectively. Stroke volume was calculated with peak Q_{pa} and simultaneous HR in $\text{mL} \cdot \text{kg}^{-1} \cdot \text{beat}^{-1}$ as Q_{pa}/HR .

GBS Clinical Syndromes and Serotypes. Each of the three different strains of GBS used in this study was isolated from the blood of an infected human neonate. Lambs treated in group 1 were infused with GBS serotype Ib isolated from a very-low-birth-weight preterm neonate with fatal early-onset sepsis, pneumonia, shock, meningitis, and severe hypoxemia; this strain has been the standard GBS used in previous studies from our laboratory (24, 25) and in previous studies of our colleagues (10–13, 20). Lambs treated in group 2 were infused with a different GBS serotype Ib isolated from a 32-wk gestation preterm neonate during an episode of necrotizing enterocolitis in the 3rd week of life that was associated with neutropenia but not pneumonia, meningitis, or cardiovascular collapse. Lambs treated in group 3 were infused with GBS serotype III, previously designated M-732 (strain 31475, ATCC catalogue, Rockville, MD) isolated from an infant with meningitis.

Analysis of serotypes of all three strains of GBS was performed by immunofluorescence with rabbit antisera in the laboratory of Kenneth M. Boyer, M.D., at Rush-Presbyterian-St. Luke's Medical Center in Chicago, IL.

GBS Preparation. Live GBS strains were prepared by previously described techniques (10–13). In summary, GBS strains were grown in 250 mL of Todd-Hewitt broth to late-log phase (10^9 cfu $\cdot \text{mL}^{-1}$). The bacteria were then centrifuged, the supernatant decanted, and the organisms resuspended to their original concentration in sterile normal saline.

In addition, bacteria were then killed by heating the suspension

to 60°C for 60 min as previously described (24, 25); death of the bacteria was subsequently verified by failure to isolate bacterial growth from a sample of the heated suspension on blood agar. Suspended, heat-killed GBS was stored in aliquots at -70°C until the study day. Growth and preparation of each of the three different strains of GBS, from recovery to killing to storage, were identical.

Experimental Protocols. Lambs were studied while awake, breathing room air, resting quietly upright in a sling, and attached appropriately to all instrumentation.

Protocol 1. At the time of study, lambs in this protocol were 4–35 d (mean 14 ± 2 d) of age and had recovered 3–27 d (mean 9 ± 2 d) from thoracotomy; gender, postnatal age at the time of study, and duration of recovery from thoracotomy were similar between the three treatment groups.

Lambs in each of the three treatment groups received i.v. bolus injections of varying amounts of heat-killed GBS in random order in a single day; the possible doses included 0.10, 0.25, 0.50, 1.0, 3.0, and 6.0×10^9 cfu (maximum volume 6 mL, $n = 80$ total doses). Injections were given over <10 s. Twelve lambs were treated in group 1 (serotype Ib, early-onset sepsis) and received from one to six injections a day ($n = 32$ total doses). Four lambs were treated in group 2 (serotype Ib, sepsis with necrotizing enterocolitis) and each received six injections per day ($n = 24$ doses). Four lambs were treated in group 3 (serotype III, meningitis) and also received six injections per day ($n = 24$ doses). Inclusion of an individual lamb into more than one treatment group was infrequent; no single lamb accounted for more than seven of the 80 total injections.

Baseline values consisted of hemodynamic measurements and arterial blood gas samples obtained during quiet rest and after a stabilization period of hemodynamic measurements. After each injection of GBS, hemodynamic measurements were continuously monitored and arterial blood gas samples were obtained at 5 and 30 min after injection. Peak responses of measured P_{pa} , P_{sa} , mean left atrial pressure, mean right atrial pressure, and Q_{pa} were assessed from the continuous recording; peak responses of calculated R_p , R_s , and stroke volume were determined as previously described. HR was assessed at 5 min after injection. Hemodynamic responses were permitted to stabilize for at least 30 min before a subsequent injection.

Protocol 2. This protocol was conducted specifically to examine whether hemodynamic responses to heat-killed GBS injection were augmented or attenuated by previous doses in a day. At the time of study, lambs in this protocol had a similar postnatal age, duration of postthoracotomy recovery, and gender distribution as those in protocol 1.

Twelve lambs in group 1 had sequential injections of 1.0×10^9 cfu of heat-killed GBS ranging between one and four doses in a single day ($n = 24$ total doses); four lambs in group 1 and four lambs in group 2 each had four sequential i.v. injections of 1.0×10^9 cfu heat-killed GBS in a single day ($n = 16$ doses in each group). Thus, 56 total 1.0×10^9 -cfu doses were given. The 1.0×10^9 -cfu dose was chosen because pilot data had indicated that it would elevate mean P_{pa} by approximately 100% in the average-sized lamb weighing 4–5 kg. At least 30 min elapsed between doses, and hemodynamic responses were permitted to stabilize before subsequent doses as in protocol 1. Hemodynamic measurements and arterial blood gas samples were obtained in the same time sequences as in protocol 1.

Statistical Analysis. All data analysis was performed using the microcomputer version of Statistical Analysis Systems (SAS, SAS Institute, Cary, NC).

The stepwise multiple regression technique (27) was used to develop models that would describe the variability of the response of six hemodynamic variables (P_{pa} , R_p , P_{sa} , R_s , Q_{pa} , and HR) to injections of GBS. Responses considered in the models included the absolute change and the percentage of change of the response variable from baseline. Independent variables and their values considered in the models included dose of GBS (absolute and

log, corrected for weight of the lamb), the three treatment groups coded as two dichotomous variables, two interaction terms of dose and treatment groups, gender (GENDER: male = 1, female = 2), postnatal age in days (AGE), and duration of postoperative recovery in days (POD). The codes for the three treatment groups were as follows: the value for the variable GP1 = 1 when the treatment was group 1, otherwise GP1 = 0; the value for the variable GP2 = 1 when the treatment was group 2, otherwise GP2 = 0. As a result, when the treatment was group 3, GP1 and GP2 were 0. Variables not reaching significance were then removed and the regression analysis was repeated to achieve the best model. Plots of the residual *versus* predicted values for all variables revealed random patterns and no important outliers were detected. A *p* value <0.05 was considered a statistically significant relationship; the best-fit model was then described by the appropriate regression equation.

To detect any confounding change in other relevant variables, a two-factor ANOVA for repeated measures was used to test the response of mean atrial pressures, stroke volume, PaO_2 , PaCO_2 , SaO_2 , and pH. A similar one-factor ANOVA was used to detect whether changes in baseline values of the variables analyzed in the regression analysis occurred with the multiple injections throughout a day. A similar two-factor ANOVA evaluated the peak response of the multiple 10^9 -cfu injections in a day in protocol 2 to assess augmentation or attenuation of response with repeated doses. When an ANOVA indicated statistical significance at the *p* < 0.05 level, follow-up group comparisons were performed with the Student-Newman-Keuls test. All variance was reported as SEM.

RESULTS

Protocol 1. In the analysis of each variable, the log of the GBS dose (log dose) and the percentage of change of the variable from the baseline value provided a better prediction of response than absolute dose and absolute change, respectively. Responses of the cardiovascular variables to injection of heat-killed GBS were immediate in onset, peak response typically occurred within 30 to 90 s, and variables had returned to baseline values by 10 min, as previously reported (24, 25).

Baseline values. Repeated baseline values for all hemodynamic and respiratory variables were not different either across the several injections performed in 1 d or between treatment groups (ANOVA, *p* > 0.05); therefore, baseline values for these variables were cumulated for convenience. Baseline values for mean atrial pressures, stroke volume, PaO_2 , PaCO_2 , pH, and SaO_2 are found in Table 1. Baseline values for variables used in the regression analysis were (mean $\pm$ SEM): P_{pa} 16.6 $\pm$ 0.5 mm Hg, R_p 0.0580 $\pm$ 0.0027 mm Hg $\cdot$ (mL $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$) $^{-1}$, P_{sa} 73.6 $\pm$ 0.9 mm Hg,

R_s 0.2712 $\pm$ 0.0797 mm Hg $\cdot$ (mL $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$) $^{-1}$, Q_{pa} 294 $\pm$ 9 mL $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$, and HR 206 $\pm$ 4 beats $\cdot$ min $^{-1}$.

Pulmonary circulation. GBS injection of all three types caused a dose-dependent increase in mean P_{pa} and R_p (Fig. 1). The increase in P_{pa} was positively influenced not only by the dose of GBS but was additionally influenced by the type of GBS and by the duration of postoperative recovery; the type of GBS used in group 1 resulted in a greater increase in P_{pa} than the types used in groups 2 and 3. The regression equation was as follows: % change P_{pa} = 123.8 + 91.2 (log dose) + 45.7 (GP1) + 3.1 (POD) (*p* = 0.0001, *r* = 0.80). Using this regression equation, a lamb 3 d postoperative given a GBS dose of 1.5 mL/kg would be predicted to have a peak P_{pa} response to the different types of GBS as follows: group 1 % increase = 123.8 + 91.2 (0.176) + 45.7 (1) + 3.1 (3) = 195%; groups 2 and 3 % increase = 123.8 + 91.2 (0.176) + 45.7 (0) + 3.1 (3) = 149%.

The response of R_p was also influenced by the dose of GBS, the type of GBS, and the interaction term of dose of group 2 (Fig. 1); the types of GBS used in groups 1 and 2 resulted in a greater increase in R_p than the type used in group 3. The regression equation was as follows: % change R_p = 130.1 + 117.4 (log dose) + 92.0 (GP1) + 133.5 (GP2) + 62.0 (log dose * GP2) (*p* = 0.0001, *r* = 0.75).

Systemic circulation. Only factors related to the type of GBS used in group 1 produced a clear dose-dependent effect on the response of P_{sa} (Fig. 2); P_{sa} was not influenced by dose of all groups or by factors related to the other treatment groups. The regression equation for all injections was as follows: % change P_{sa} = 6.4 + 25.1 (GP1) + 19.8 (log dose * GP1) (*p* = 0.0001, *r* = 0.53). Because only factors related to group 1 influenced the overall response of P_{sa} , indicating a qualitative difference in response to the three treatment groups, regression models were developed for the response to individual treatment groups. The regression equation for group 1 was as follows: % change P_{sa} = 31.5 + 19.8 (log dose) (*p* = 0.0001, *r* = 0.55). Group 2 had no dose-dependent effect on P_{sa} (*p* > 0.05, power = 0.99) and group 3 had a marginal effect that was not apparent in the overall model: % change P_{sa} = 13.6 + 7.3 (log dose) (*p* = 0.05, *r* = 0.48).

The response of R_s was positively influenced by the dose of GBS and the type of GBS; the GBS used in group 1 resulted in a greater increase in R_s than the types used in groups 2 and 3 (Fig. 2). The regression equation was as follows: % change R_s = 78.3 + 28.8 (log dose) + 32.7 (GP1) (*p* = 0.0002, *r* = 0.41).

Q_{pa} and HR. The response of Q_{pa} to GBS injection was negatively influenced by the dose of GBS (Fig. 3) and duration of postoperative recovery and positively influenced by postnatal age of the lamb; that is, longer duration of recovery enhanced the depression of Q_{pa} and advanced postnatal age protected against this depression. The equation was as follows: % change

Table 1. Respiratory gas exchange, atrial pressures, and stroke volume data before and after injection of heat-killed GBS in conscious lambs*

Variable/time	GBS dose (mL)†						
	Baseline‡ (n = 80)	0.1 (n = 12)	0.25 (n = 13)	0.50 (n = 13)	1.0 (n = 20)	3.0 (n = 12)	6.0 (n = 10)
PaO_2 (kPa)	11.2 (0.2)	11.5 (0.4)	11.1 (0.3)	11.5 (0.3)	10.7 (0.4)	10.1 (0.3)	10.7 (0.3)
SaO_2 (%)	95 (0.5)	95 (1.2)	95 (0.9)	96 (1.0)	95 (1.0)	95 (1.0)	94 (0.9)
PaCO_2 (kPa)	5.5 (0.1)	5.3 (0.2)	5.3 (0.2)	5.6 (0.2)	5.7 (0.2)	5.6 (0.2)	5.7 (0.2)
pH	7.39 (0.01)	7.39 (0.01)	7.39 (0.01)	7.38 (0.01)	7.38 (0.01)	7.39 (0.02)	7.40 (0.01)
P_{la} (mm Hg)	0.5 (0.2)	0.7 (0.4)	0.5 (0.6)	0.8 (0.4)	2.2 (0.5)	1.3 (0.7)	2.0 (1.0)
P_{ra} (mm Hg)	-0.6 (0.2)	-0.6 (0.5)	-0.5 (0.7)	0.3 (0.5)	1.7 (0.6)	0.6 (0.8)	1.7 (0.9)
Stroke volume (mL $\cdot$ kg $^{-1}$ $\cdot$ beat $^{-1}$)	1.5 (0.1)	1.5 (0.1)	1.5 (0.1)	1.4 (0.1)	1.5 (0.1)	1.2 (0.1)	1.4 (0.1)

* Data are mean (SEM); 1 kPa = 0.1333 mm Hg; n = 80 total injections. P_{la} , mean left atrial pressure; P_{ra} , mean right atrial pressure. No differences between baseline and posttreatment values were observed for any variable (ANOVA, *p* > 0.05).

† All preinjection baseline and postinjection dose-group values were cumulated because no differences were detected across dose or treatment groups (ANOVA, *p* > 0.05).

‡ Refers to values 5 min after (for pH and gas tensions) and peak response (for P_{la} , P_{ra} , and stroke volume) after each dose of heat-killed GBS (10^9 cfu $\cdot$ mL $^{-1}$).

Fig. 1. Pulmonary vascular response of conscious lambs to three strains of heat-killed GBS, dose range $0.1\text{--}6.0 \times 10^9 \text{ cfu} \cdot \text{mL}^{-1}$, i.v. $n = 32$ injections in treatment group 1 (type Ib), 24 injections in group 2 (type Ib), and 24 injections in group 3 (type III). Numbers in inset represent treatment groups; y axis represents % change from baseline. GBS injection of all three strains caused dose-dependent increases in P_{pa} and R_{pa} ; group 1 bacteria had a more potent effect on P_{pa} , whereas groups 1 and 2 had additional influence on R_{pa} .

$Q_{pa} = -32.4 - 13.6 (\log \text{dose}) - 2.4 (\text{POD}) + 2.3 (\text{AGE})$ ($p = 0.0001$, $r = 0.57$). The response of HR was influenced negatively by the dose of GBS (Fig. 3) and positively by age and gender; that is, advanced postnatal age and female gender protected against the depression of HR. The regression equation was as follows: % change HR = $-42.3 - 10.1 (\log \text{dose}) + 5.5 (\text{GENDER}) + 0.6 (\text{AGE})$ ($p = 0.0001$, $r = 0.58$).

Atrial pressures and stroke volume. Mean atrial pressures and stroke volume did not differ between preinjection baseline and the postinjection peak response over the range of doses studied and responses did not differ between treatment groups; therefore, values for these variables were cumulated (ANOVA, all $p > 0.05$, Table 1).

Arterial blood gas tensions, pH, Sao_2 . PaO_2 , Sao_2 , Paco_2 , and pH did not differ between preinjection baseline and the 5-min postinjection periods over the range of doses studied and responses did not differ between treatment groups; therefore, values for these variables were cumulated (ANOVA, all $p > 0.05$, Table 1).

Initial depressor response. Although the most important response to injection of heat-killed GBS in these studies was a pressor response of both pulmonary and systemic circulations, an initial depressor response that was immediate, brief, and small in magnitude was commonly observed.

A pulmonary depressor response was observed in 20 of the 80 total injections (25%) and ranged in magnitude from -1 to -6 mm Hg (mean -2.4 mm Hg) decrease from baseline; a systemic depressor response was observed in 14 of the 80 injections (18%) and ranged in magnitude from -3 to -15 mm Hg (mean -8.1

Fig. 2. Systemic vascular response of conscious lambs to three strains of heat-killed GBS, dose range $0.1\text{--}6.0 \times 10^9 \text{ cfu} \cdot \text{mL}^{-1}$, i.v. $n = 32$ injections in treatment group 1 (type Ib), 24 injections in group 2 (type Ib), and 24 injections in group 3 (type III). Numbers in inset represent treatment groups; y axis represents % change from baseline. GBS injection of only the strain used in group 1 caused a dose-dependent increase in P_{sa} ; GBS injection of all three strains caused a dose-dependent increase in R_{sa} ; group 1 bacteria had a more potent effect on R_{sa} than bacteria used in groups 2 and 3.

mm Hg). The duration until recovery of this response was less than 30 s. The depressor responses observed in the pulmonary and systemic circulations commonly occurred together; that is, the incidence of the systemic depressor response was greater when a similar pulmonary response also occurred compared with no such pulmonary response (40 versus 10%, $\chi^2 = 9.4$, $p = 0.002$).

The observation of the depressor responses was also strongly associated with the treatment group. A pulmonary depressor response was observed in 31% of injections in group 1, 42% in group 2, and was not observed at all in group 3 ($\chi^2 = 15.3$, $p < 0.0001$). Similarly, a systemic depressor response was observed in 13% of injections in group 1, 42% in group 2, and was not observed at all in group 3 ($\chi^2 = 12.2$, $p < 0.002$).

We used the same multiple regression techniques and variables that evaluated the pressor responses to generate models to best describe the magnitude of pulmonary and systemic depressor responses. The models demonstrated that the absolute magnitude of the pulmonary pressure drop was related to duration of postoperative recovery and the magnitude of the systemic pressure drop was related to gender; that is, less magnitude of a P_{pa} depressor response was observed with longer duration of post-thoracotomy recovery, whereas less P_{sa} response was observed with female gender. The magnitude of the depressor responses were not related to dose of GBS or postnatal age, were not different between treatment groups 1 and 2, and were not associated with decreases in atrial filling pressures or Q_{pa} ; in fact, Q_{pa} commonly increased with this response.

Fig. 3. Q_{pa} and HR responses in conscious lambs to three strains of heat-killed GBS, dose range $0.1\text{--}6.0 \times 10^9 \text{ cfu} \cdot \text{mL}^{-1}$, i.v. $n = 32$ injections in treatment group 1 (type Ib), 24 injections in group 2 (type Ib), and 24 injections in group 3 (type III). Numbers in inset represent treatment groups; y axis represents % change from baseline. GBS injection of all three strains caused dose-dependent decreases in Q_{pa} and HR without a treatment difference between the three strains.

Protocol 2. Up to four sequential 10^9 -cfu doses of heat-killed GBS did not produce attenuation or augmentation of cardiovascular responses. P_{pa} increased a mean $18 \pm 1 \text{ mm Hg}$ over baseline in response to all 10^9 -cfu injections (122% mean increase for group 1, 103% for group 2, and 93% for group 3) without a difference between sequential injections (ANOVA, $p > 0.05$). Similarly, P_{sa} increased a mean $8 \pm 1 \text{ mm Hg}$ (10%) in response to all sequential injections (mean $10 \pm 2 \text{ mm Hg}$, 13% increase for group 1) without a difference between injections (ANOVA, $p > 0.05$). Q_{pa} (mean $-17 \pm 6 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, -7%) and HR (mean $-10 \pm 3 \text{ beats} \cdot \text{min}^{-1}$, -5%) both decreased from baseline, although negligibly, in response to all sequential 10^9 -cfu injections without a difference between dose number (ANOVA, $p > 0.05$). No differences in response to sequential injections were found among treatment subgroups (ANOVA, $p > 0.05$). The magnitude of responses of each variable was influenced by the treatment group as described in the results of protocol 1. As in protocol 1, PaO_2 , SaO_2 , $Paco_2$, pH, atrial pressures, and stroke volume did not differ between preinjection baseline and the postinjection periods.

Recovery. With one exception, all lambs recovered uneventfully without distress and thrived after these studies of serial injections of heat-killed GBS in a day. The exception was a single 8-d-old, 5.5-kg female lamb studied in treatment group 2 who went on to receive additional larger doses of GBS not evaluated in these protocols, including a total for the day of 24.9 mL ($4.5 \times 10^9 \text{ cfu} \cdot \text{kg}^{-1}$) and a final dose of 9.0 mL ($1.6 \times 10^9 \text{ cfu} \cdot \text{kg}^{-1}$). This final high dose of heat-killed GBS caused a 21% decrease in Q_{pa} , which recovered within 5 min. This lamb developed

abdominal distension after enteral feeding and died several hours after the study; postmortem examination demonstrated distended bowel with necrotic areas.

DISCUSSION

Sepsis from group B beta-hemolytic streptococcus remains an important cause of neonatal morbidity and mortality. Previous studies of various serotypes of GBS isolated from infected human neonates and infused into animals have demonstrated that GBS causes important hemodynamic alterations. This study was conducted to further evaluate the influence of important experimental conditions on the hemodynamic response to GBS in our conscious lamb model. Specifically, the two main purposes were to examine whether GBS isolated from infected human neonates with different clinical syndromes and serotypes would cause different hemodynamic responses and whether other experimental factors may also influence these responses. We intentionally designed the study to evaluate the hemodynamic responses to doses of GBS that would not cause secondary and confounding changes in arterial pH and blood gas tensions, atrial pressures, or cardiac stroke volume and that would permit recovery of the chronically instrumented lamb.

The most reproducible hemodynamic effect of GBS is pulmonary hypertension. Pulmonary hypertension caused by GBS has been observed in human neonates (5, 7) and is a well-documented response in several animal models to live GBS (8–16, 19–22) or to heat-killed GBS (23–25, 28). In addition, we and others have shown that conscious animals may recover uneventfully from repeated injections of either heat-killed GBS (23–25) or type III GBS polysaccharide toxin (17, 18, 29–32), an observation that provides a methodologic advantage to sequential experimental studies. In yearling sheep, Rojas *et al.* (14) have demonstrated that the pulmonary hypertensive response is similar between live GBS, heat-killed GBS, and the polysaccharide toxin; in neonatal lambs, we (24) also demonstrated that the pulmonary hypertensive response is similar between live GBS and heat-killed GBS. In this study, we have reconfirmed the dose-dependent pulmonary hypertensive effect of heat-killed GBS in conscious lambs and have provided regression models that predict hemodynamic response to GBS; in addition, this study establishes the importance of other factors on the magnitude of the pulmonary hypertensive response.

These data indicate that the quantitative degree of pulmonary hypertension may be influenced by the strain of GBS studied; specifically, GBS strains used in treatment groups 1 (serotype Ib, early-onset sepsis) and 2 (Ib, necrotizing enterocolitis) caused more pulmonary vasoconstriction than group 3 (III, meningitis). To our knowledge, this differential influence of individual GBS strains has not been previously reported. Furthermore, the duration of recovery from surgery in our chronically instrumented lambs also directly influenced the magnitude of pulmonary hypertensive response to heat-killed GBS. Although hemodynamic responses in chronically instrumented lambs are generally considered to be influenced by duration of postoperative recovery (33), we have now quantified the magnitude of this influence for studies of heat-killed GBS in conscious lambs.

This study also demonstrates a qualitative, treatment group-dependent difference in the response of P_{sa} to GBS that has not been previously observed. Lambs receiving the GBS strain used in group 1 had a clear dose-dependent systemic pressor response to heat-killed GBS, whereas those receiving the GBS used in group 3 had a marginal effect; the GBS strain used in group 2 had no effect on P_{sa} . R_s was positively influenced by dose of GBS; once again, the strain used in group 1 produced more potent systemic vasoconstriction. Q_{pa} and HR were depressed by GBS in a dose-dependent manner and not influenced by treatment group. However, the dose-dependent depression of Q_{pa} produced by heat-killed GBS was protected by advanced postnatal age and accentuated by longer duration of postoperative

recovery; the apparent discrepant influences of advanced postnatal age and longer duration of postoperative recovery on Q_{pa} remain unexplained by these data. Similarly, the dose-dependent depression of HR produced by heat-killed GBS was protected by advanced postnatal age and female gender; to our knowledge, this observation and the female gender protection against the initial pulmonary depressor effect of heat-killed GBS are unique observations that gender may be important to cardiovascular responses in the neonatal period.

The third purpose of this study was to determine whether repetitive doses of heat-killed GBS in a single day would attenuate or augment subsequent hemodynamic responses to GBS. This study demonstrated that sequential doses of 0.2 to 0.3 mL $\cdot$ kg⁻¹ (10^9 cfu $\cdot$ mL⁻¹) of heat-killed GBS given i.v. to neonatal lambs in a single day could produce a potent and reproducible elevation of P_{pa} (mean 108% increase for all three groups) without attenuation or augmentation of response. This dose also provided negligible influence on other systemic variables and is an optimal dose in this model for primarily studying isolated pulmonary vascular effects of GBS. This observation of no attenuation or augmentation to serial doses is in contrast to the study of Philips *et al.* (23), in which a 2.5-fold potentiation of the pulmonary pressor response to heat-killed GBS in conscious piglets was observed with single daily doses of 1×10^8 cfu (10-fold less than doses evaluated in this study) over 2 wk. Hemming *et al.* (34), using a trichloroacetic acid extract of GBS, demonstrated that rechallenge 48 h after a first dose resulted in a 2-fold augmentation of the pulmonary pressure response in oophorectomized adult ewes. The failure to observe attenuation or augmentation of hemodynamic responses in a single day in our study provides the methodologic advantage of studying cardiovascular responses before and after experimental interventions in the same day (24, 25).

The initial depressor response of the pulmonary and systemic circulations to heat-killed GBS was an intriguing observation. This response was immediate, brief, isolated to treatment groups 1 and 2, and, although interrelated between the two circulations, was not observed with every dose. Interestingly, regression analysis indicated that the magnitude of this response was influenced by duration of postoperative recovery for P_{pa} and by gender for P_{sa} ; that is, a smaller depressor response was observed for longer duration of postoperative recovery and female gender for these two variables, respectively. This response was not related to changes in atrial filling pressures or to a decrease in Q_{pa} ; in fact, Q_{pa} typically increased with this response. To our knowledge, this depressor response and its related factors are unique observations with GBS injection.

However, a similar initial depressor response has been reported to be typical of the systemic but not pulmonary vascular response to leukotriene injection. Ahmed *et al.* (35) observed a similar initial systemic depressor response to injected leukotriene D_4 before an ultimate systemic pressor response in conscious adult sheep; indomethacin blocked the depressor response, leading the authors to conclude that this response was cyclooxygenase-mediated. A similar biphasic response of R_s to leukotriene D_4 injection in neonatal lambs was observed by both Yokochi *et al.* (36) and, inconsistently, by Schreiber *et al.* (37). In spontaneously hypertensive rats, very high dose leukotriene D_4 injection (20 μ g/kg intraarterially) has produced a similar systemic vasodepressor effect followed by a pressor response; however, this initial depressor effect was not observed in normotensive rats (38).

The mechanism of the dissimilar cardiovascular responses to different strains of heat-killed GBS was not investigated in this study; however, existing information suggests that organism-related factors are, in part, responsible. O'Brien *et al.* (39) and Hemming *et al.* (34) demonstrated that a trichloroacetic acid extract of type III GBS produces pulmonary hypertension in both lambs (39) and adult oophorectomized ewes (34); these authors observed qualitatively different P_{sa} responses in these two different age groups that were unexplained. Before this,

Hellerqvist *et al.* (31, 32) extensively fractionated type III GBS and isolated and studied a pure polysaccharide toxin capable of causing pulmonary hypertension when injected into yearling sheep; this toxin is different from either group B or type III polysaccharides.

More recently, Gibson *et al.* (40) studied the pulmonary vascular response to live isogenic mutants of type III GBS in neonatal piglets; these investigators demonstrated that GBS mutants devoid of either type III capsular polysaccharide or β -hemolysin remained capable of producing pulmonary hypertension equivalent to the parent type III GBS strain. These data led these authors to conclude that type III polysaccharide or β -hemolysin were not essential for type III GBS to induce the acute effects of GBS sepsis, including pulmonary hypertension and impaired respiratory gas exchange. Finally, Gibson *et al.* (41) have also reported that two additional gram-positive species (*Enterococcus* and *Staphylococcus epidermidis*) produce pulmonary hypertension and elevation of circulating thromboxane B_2 equivalent to those produced by live type III GBS in piglets; these data led the authors to suggest that components responsible for the hemodynamic effects of gram-positive bacteria in their model may be universally common or that the final common pathway may be stimulated by a number of nonspecific components. Whether a variable expression, concentration, or stimulatory potency of the physiologically active toxin or toxins occurs with various strains of GBS remains an unstudied possibility.

Finally, chronically instrumented lambs, with one exception, recovered uneventfully from these studies, providing a methodologic advantage to serial or paired studies, particularly in terms of studying responses before and after experimental interventions (24, 25). The finding that the single lamb that died developed an ischemic gastrointestinal lesion after an unusually large load of the GBS isolated from the blood of an infant with necrotizing enterocolitis is another intriguing observation that suggests the possibility of a strain-dependent gastrointestinal or mesenteric vascular virulence.

In summary, we have confirmed the pulmonary and systemic pressor effects of heat-killed GBS in lambs; in addition, we have demonstrated strain-dependent quantitative differences in pulmonary vascular response and qualitative differences in systemic vascular response, as well as several important experimental factors that may influence hemodynamic responses to GBS. These factors should be accounted for in studies investigating cardiovascular responses to GBS.

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