Group B *Streptococcus* and *E. coli* LPS-induced NO-dependent hyporesponsiveness to noradrenaline in isolated intrapulmonary arteries of neonatal piglets

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- 1 The effects of endotoxin (*E. coli* lipopolysaccharide, LPS) and heat inactivated group B *Streptococcus* (GBS) were studied on the contractile responses to noradrenaline (NA) in isolated pulmonary arteries and on the activity of the constitutive and inducible nitric oxide synthase (NOS) in lung fragments of neonatal piglets.
- 2 Short-term (≤ 5 h) incubation with LPS ($1 \mu\text{g ml}^{-1}$) or GBS (3×10^7 colonies forming units ml^{-1}) did not modify the vascular responsiveness to NA (10^{-8} M– 10^{-4} M) in isolated intrapulmonary arteries. However, long-term incubation (20 h) with LPS or GBS produced a significant reduction in the maximal contractile responses and shifted the concentration-response curve for NA downwards.
- 3 Endothelium removal or the cyclo-oxygenase inhibitor meclofenamate (10^{-5} M) did not affect the GBS- and LPS-induced hyporesponsiveness to NA.
- 4 The presence of the nitric oxide (NO) precursor, L-arginine (10^{-5} M), 30 min prior to the contractility challenge increased the LPS- and GBS-induced pulmonary vascular hyporesponsiveness to NA. In contrast, the addition, prior to the challenge with NA, of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) or coincubation with dexamethasone (3×10^{-6} M), a potent inhibitor of the induction of NOS, or with the protein synthesis inhibitor cycloheximide (10^{-5} M) completely restored the reactivity to NA in LPS- and GBS-treated pulmonary arteries.
- 5 The incubation for 20 h of lung fragments with LPS and GBS produced a significant increase in the Ca²⁺-independent (inducible) NOS activity determined by the conversion of radiolabelled L-arginine to citrulline, but did not modify the constitutive NOS activity. This NOS induction was abolished by coincubation with dexamethasone (3×10^{-6} M).
- 6 These results demonstrated that prolonged incubation with GBS and LPS causes an induction of NOS activity which results in a reduced vascular responsiveness to NA in pulmonary arteries of neonatal piglets. Thus, induction of NOS seems to be responsible for the delayed pulmonary vascular hyporesponsiveness induced by GBS (a Gram-positive) and *E. coli* (a Gram-negative), the most common causal agents of neonatal sepsis.

Keywords: Group B *Streptococcus*; lipopolysaccharide; nitric oxide synthase; pulmonary artery of piglet

Introduction

E. coli, a Gram-negative bacterium, and group B *Streptococcus* (GBS), a Gram-positive bacterium, are the most common causal agents of neonatal sepsis (Anthony, 1985; Guerina, 1991). In general, sepsis is characterized by systemic arterial hypotension, inadequate tissue perfusion and decreased responses of vascular smooth muscle to exogenous vasoconstrictors (Groeneveld *et al.*, 1988). The pulmonary system also demonstrates important abnormalities that include changes in pulmonary haemodynamics and lung mechanics, increased vascular permeability and hypoxemia, and more subtle changes in responses of both airway and the pulmonary circulation to constrictor stimuli (Brigham & Meyrick, 1986). Lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, is the endotoxin presumed to cause injury to the lungs as well as to other organs (Thiemermann, 1994). Gram-positive bacteria do not contain LPS and a toxin common to all Gram-positive organisms has not been identified. However, intravenous infusions of GBS and other Gram-positive bacteria cause pulmonary haemodynamic and gas exchange abnormalities similar to those observed in LPS-injected animals (Rojas *et al.*, 1983; Schreiber *et al.*, 1992) suggesting a common pathway leading to these abnormalities (Auguet *et al.*, 1992).

There is evidence that enhanced release of NO plays an important role in the loss of systemic (McKenna, 1990; Julou-Schaffer *et al.*, 1990; Moncada *et al.*, 1991; for a review see Thiemermann, 1994) and pulmonary (Szabó *et al.*, 1993; Zelenkov *et al.*, 1993) vascular responsiveness that occurs in sepsis. There are at least two distinct isoforms of NO synthase (NOS) which catalyzes the formation of NO from L-arginine (Moncada *et al.*, 1991; Stuehr & Griffith, 1992). A constitutive, Ca²⁺-dependent isoform (cNOS), is present in vascular endothelial cells. In addition, LPS and cytokines induce a Ca²⁺-independent, isoform (iNOS) in the lung (Knowles *et al.*, 1990), macrophages (Marletta *et al.*, 1988), endothelial cells (Radomski *et al.*, 1990) and vascular smooth muscle (Busse & Mülsch, 1990). Induction of iNOS has been found in rat pulmonary arteries incubated with LPS (Zelenkov *et al.*, 1993) and in rat lung after exposure to endotoxin (Salter *et al.*, 1991; Szabó *et al.*, 1993; Thiemermann, 1994). Unfortunately, the effects of GBS on vascular reactivity and on cNOS and iNOS are still unknown. Therefore, the aim of this work was: (1) to study and compare the effects of GBS with those of LPS on the contractile responses to noradrenaline (NA) in intact and endothelium-denuded pul-

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monary arteries of neonatal piglets, and (2) to determine whether GBS- and LPS-mediated pulmonary vascular hyporesponsiveness could be related to the activation of cNOS and/or iNOS.

Methods

Tissue preparation and incubation

Male neonatal piglets (10–17 days of age, 4277 ± 343 g) obtained from the local abattoir were used in this study. Piglets were killed by exsanguination and the lungs were rapidly immersed in cold (4°C) Krebs solution containing ampicillin ($10 \mu\text{g ml}^{-1}$) and gentamicin ($10 \mu\text{g ml}^{-1}$) and transported immediately to the laboratory. The third branch of the pulmonary arteries (internal diameter 1–2 mm) was carefully dissected free of parenchyma and connective tissue and cut into rings of 2–3 mm of length. Pulmonary rings were then incubated in Krebs solution (composition in mM: NaCl 118, KCl 4.75, NaHCO_3 25, MgSO_4 1.2, CaCl_2 2.0, KH_2PO_4 1.2 and glucose 11) containing ampicillin ($10 \mu\text{g ml}^{-1}$) and gentamicin ($10 \mu\text{g ml}^{-1}$). The solution was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C.

The pulmonary artery rings were initially incubated in Krebs solution in the presence of vehicle, LPS ($1 \mu\text{g ml}^{-1}$) or heat inactivated GBS (3×10^7 colony forming units, c.f.u. ml^{-1}) for 1, 5 or 20 h. To confirm that LPS and GBS induced iNOS, in some experiments pulmonary rings were cocubated for 20 h in Krebs solution containing dexamethasone (3×10^{-6} M), an inhibitor of NOS induction, (Radomski *et al.*, 1990) or cycloheximide (10^{-5} M, an inhibitor of protein synthesis). After incubation, two L-shaped stainless-steel wires were inserted into the arterial lumen and the rings were introduced in Allin organ chambers filled with Krebs solution. One wire was attached to the chamber and the other to an isometric force-displacement transducer (Grass FT07) and connected to a polygraph (Grass, model 7) as previously described (Pérez-Vizcaino *et al.*, 1993). The rings were stretched to a resting tension of 0.5 g and allowed to equilibrate for 60–90 min. During this period tissues were restretched and washed every 30 min with warm Krebs solution. In some experiments the endothelium was removed by gently rubbing the intimal surface of the rings with a metal rod. The presence of functional endothelium was verified by addition of acetylcholine (ACh, 10^{-6} M) in arteries precontracted with NA. The ability of ACh to induce relaxation of unrubbed rings was taken as an indicator of the presence of functional endothelium.

Experimental protocol

In pulmonary rings previously incubated with vehicle, LPS or GBS for 1, 5 or 20 h, concentration-response curves to NA (10^{-8} M to 10^{-4} M) were constructed by increasing the organ chamber concentration by cumulative increments after a steady state response had been reached with each increment. In three groups of intact pulmonary arteries, following the incubation period of 20 h, cumulative concentration-response curves to NA were performed in the presence of N^G -nitro-L-arginine-methyl ester (L-NAME, 10^{-4} M, an inhibitor of both cNOS and iNOS, Sakuma *et al.*, 1988), L-arginine (10^{-5} M, the precursor of NO, Palmer *et al.*, 1988) or meclofenamate (10^{-5} M, an inhibitor of cyclo-oxygenase) which were added 30 min before the concentration-response curve to NA was obtained.

Assay of NOS

Lung fragments (weight 100–150 mg) were incubated in the same conditions as described for the arterial rings. After incubation, tissues were immediately stored at -80°C until studied. The frozen tissues were homogenized at $0-4^\circ\text{C}$ in 5

vols of a buffer containing 320 mM sucrose, 50 mM Tris, 1 mM EDTA, 1 mM DL-dithiothreitol, $100 \mu\text{g ml}^{-1}$ phenylmethylsulfonyl fluoride, $10 \mu\text{g ml}^{-1}$ leupeptin, $100 \mu\text{g ml}^{-1}$ soybean trypsin inhibitor and $2 \mu\text{g ml}^{-1}$ aprotinin brought to pH 7.0 at 20°C with HCl. The homogenates were then centrifuged at 4°C at $12000 g$ for 20 min. The pellets were discarded and the supernatants were placed on ice until incubation the same day. NOS activity was determined by measuring in duplicate the conversion of L-[U- ^{14}C]-arginine to L-[U- ^{14}C]-citrulline by 10 min incubation at 37°C as described in detail by Salter *et al.* (1991). The incubation buffer contained 50 mM L-valine to minimize any interference from arginase. Ca^{2+} -dependent NOS (cNOS) activity was calculated from the difference between the L-[U- ^{14}C]-citrulline produced from control samples containing incubation buffer and samples containing buffer plus 1 mM EGTA. The activity of the Ca^{2+} -independent NOS (iNOS) was determined from the difference between samples containing 1 mM EGTA in the incubation buffer and samples containing 1 mM EGTA plus 2 mM N^G -monomethyl-L-arginine (a competitive inhibitor of NOS, Palmer *et al.*, 1988).

Drugs and heat-killed GBS preparation

The following drugs were used: (–)-noradrenaline bitartrate, acetylcholine chloride, lipopolysaccharide from *E. coli* (serotype 055:B5), dexamethasone, cycloheximide, L-NAME, L-arginine (Sigma Chemical Co., London) and meclofenamate (Warner Lambert Co., U.S.A.). Meclofenamate was dissolved in absolute ethanol. The concentrations are expressed as final molar concentration in the tissue chamber. L-[U- ^{14}C]-arginine was obtained from Amersham International (U.K.).

GBS type III was isolated from the blood of a neonate who developed early-onset sepsis. Bacteria were grown in Todd-Hewitt broth for 18–36 h at 37°C to late log phase and harvested by centrifugation at 5000 r.p.m. for 15 min. Bacteria were resuspended in sterile isotonic saline to a concentration determined by serial viable counts to be 1×10^9 c.f.u. ml^{-1} . Heat-killed bacteria were obtained by heating bacteria to 60°C for 60 min. GBS killing was confirmed by no growth on blood agar. Endotoxin levels in the heat-killed GBS preparation were undetectable as assayed by a standard Limulus assay kit (Sigma). Aliquots of heat-killed GBS were stored at -80°C until the study day.

Statistical analysis

Results are expressed as means $\pm$ s.e. mean of measurements in n arteries. Individual cumulative concentration-response curves to NA were fitted to a logistic equation. In most of the experiments the point corresponding to 10^{-4} M NA was below the maximal tension and was therefore excluded from the fitting. The drug concentration exhibiting 50% of the maximal contraction to NA was calculated for each ring and expressed as negative log molar (pD_2). The E_{max} was defined as the maximal tension induced by NA in each ring. Statistically significant differences were calculated by means of an ANOVA analysis followed by a Newman Keuls means comparison testing. $P < 0.05$ was considered statistically significant.

Results

Effects of LPS and GBS in the presence or absence of endothelium

Short-term incubation of intact pulmonary arteries with LPS ($1 \mu\text{g ml}^{-1}$) or GBS (3×10^7 c.f.u. ml^{-1}) for 1 or 5 h produced no significant effects on the concentration-response curve to NA (Table 1). However, as shown in Figure 1a, when the endothelium-intact arteries were incubated for a

longer period (20 h) with LPS ($1 \mu\text{g ml}^{-1}$) or GBS (3×10^7 c.f.u. ml^{-1}) and then transferred to the organ bath in the absence of bacterial products, the maximal contractile response to NA was reduced and thus, a downward shift of the

Figure 1 Concentration-response curve to noradrenaline (NA, 10^{-8} – 10^{-4} M) in intrapulmonary arterial rings (a) with or (b) without endothelium, preincubated for 20 h in Krebs solution in the presence of vehicle (control, ○), *E. coli* lipopolysaccharide (LPS, $1 \mu\text{g ml}^{-1}$, ■) or heat inactivated group B *Streptococcus* (GBS, 3×10^7 c.f.u. ml^{-1} , ▲). The concentration-response curve to NA was carried out in the absence of bacterial products. Data are expressed as means $\pm$ s.e. mean of 7–22 observations. * $P < 0.05$ and ** $P < 0.01$ represent significant differences with respect to control group.

concentration-response curve to NA was observed. This was accompanied by a small, but significant, decrease of the pD_2 values (Table 1). These results indicate that pulmonary vascular hyporesponsiveness to NA is a delayed process and suggested the induction of a biological activity. Endothelium-denuded arteries showed significantly greater maximal responses to NA as compared to endothelium-intact arteries ($P < 0.01$) incubated with vehicle, LPS or GBS (Table 1). Nevertheless, mechanical removal of endothelial cells did not affect the hyporeactivity to NA observed after long-term incubation (20 h) with LPS or GBS (Figure 1b).

Effects of L-arginine, L-NAME and meclofenamate

In order to assess the role of NO production in the hyporesponsiveness to NA, a concentration-response curve to NA was performed in control, LPS- and GBS-treated arteries acutely treated with either the NO-precursor, L-arginine (10^{-5} M), or the NOS inhibitor, L-NAME (10^{-4} M). As shown in Figure 2a and Table 1, in control arteries treated with L-arginine the concentration-response curve to NA was not modified compared to that obtained in arteries in the absence of L-arginine. In contrast, in arteries incubated with LPS or GBS for 20 h, the presence of L-arginine potentiated ($P < 0.01$) the hyporesponsiveness to NA. Addition of 10^{-4} M L-NAME to resting arteries produced a small contractile effect averaging 45.7 ± 6.6 mg, 44.3 ± 9.6 mg and 43.1 ± 5.9 mg, in untreated and in arteries treated with LPS and GBS, respectively. Thereafter, L-NAME induced an upward shift of the concentration-response curve to NA in the LPS- and GBS-treated arteries resulting in no differences between control, LPS- or GBS-treated arteries (Figure 2b). Therefore, L-NAME reversed the hyporesponsiveness to NA induced by LPS and GBS.

The possible role of vasodilator prostaglandins in the hyporeactivity to NA was assessed by acute treatment of the arteries with the cyclo-oxygenase inhibitor, meclofenamate. The presence of 10^{-5} M meclofenamate did not affect the hyporesponsiveness to NA in LPS- and GBS-treated arteries (Table 1).

Effect of dexamethasone and cycloheximide

In order to assess the induction of NOS by LPS and GBS, intact pulmonary arteries were simultaneously incubated with LPS and GBS and 3×10^{-6} M dexamethasone or 10^{-6} M cycloheximide for 20 h. As shown in Figure 3, both dexamethasone and cycloheximide completely restored the reactivity to NA in LPS- and GBS-treated arteries. Moreover, dexamethasone (but not cycloheximide)-treated arteries showed greater pD_2 values in control arteries (Table 1).

Table 1 Effects of *E. coli* lipopolysaccharide (LPS, $10 \mu\text{g ml}^{-1}$) and group B *Streptococcus* (GBS, 3×10^7 c.f.u. ml^{-1}) on the parameters (E_{max} and pD_2) of the concentration-response curve to noradrenaline (NA) in the absence and presence of various inhibitors

Drug	t (h)	Control				LPS				GBS			
		E_{max} (mg)	pD_2	n		E_{max} (mg)	pD_2	n		E_{max} (mg)	pD_2	n	
None	+E	1	1198 ± 141	6.39 ± 0.15	11	$1212 \pm 153^{**}$	6.47 ± 0.43	9	$1157 \pm 245^{**}$	6.31 ± 0.21		8	
None	+E	5	1192 ± 212	6.37 ± 0.08	9	$1111 \pm 127^{**}$	$6.66 \pm 0.15^*$	8	876 ± 160	6.51 ± 0.19		9	
None	+E	20	923 ± 80	6.52 ± 0.07	17	$492 \pm 47^{**}$	$6.25 \pm 0.10^*$	13	$613 \pm 52^{**}$	$6.17 \pm 0.08^{**}$		22	
None	-E	20	$1676 \pm 208^{**}$	6.46 ± 0.08	7	$946 \pm 140^{***}$	$6.17 \pm 0.08^*$	7	$877 \pm 68^{***}$	$5.98 \pm 0.10^{**}$		8	
Meclofenamate	+E	20	1026 ± 98	6.49 ± 0.08	11	$585 \pm 55^{**}$	6.44 ± 0.06	11	$450 \pm 77^{**}$	6.10 ± 0.04		11	
L-Arginine	+E	20	1032 ± 124	6.39 ± 0.09	8	$299 \pm 50^{***}$	6.07 ± 0.08	11	$341 \pm 77^{***}$	6.22 ± 0.13		9	
L-NAME	+E	20	1047 ± 120	6.72 ± 0.11	10	$1023 \pm 99^{**}$	6.49 ± 0.09	14	$1164 \pm 55^{**}$	$6.66 \pm 0.08^{**}$		12	
Dexamethasone	+E	20	876 ± 63	$6.89 \pm 0.06^*$	14	$923 \pm 62^{**}$	$6.72 \pm 0.08^*$	11	$831 \pm 64^*$	$6.82 \pm 0.06^{**}$		11	
Cycloheximide	+E	20	879 ± 95	6.64 ± 0.07	7	$1107 \pm 103^{**}$	$6.71 \pm 0.09^*$	7	911 ± 82	6.66 ± 0.11		8	

t = incubation period. +E = endothelium intact, -E = endothelium denuded. Meclofenamate (10^{-5} M), L-arginine (10^{-5} M) or N^G -nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) were added to the organ bath solution 30 min before the addition of NA. Dexamethasone (3×10^{-6} M) or cycloheximide (10^{-5} M) were included during the 20 h incubation period.

* $P < 0.05$ and ** $P < 0.01$ GBS- or LPS-treated vs control arteries. * $P < 0.05$ and ** $P < 0.01$ vs arteries +E, 20 h, no drug. The drug concentration exhibiting 50% of the maximal contraction to NA was calculated for each ring and expressed as negative log molar (pD_2). The E_{max} was defined as the maximal tension induced by NA in each ring.

Figure 2 Effects of (a) L-arginine (10^{-5} M) or (b) N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) on *E. coli* lipopolysaccharide (LPS)- and group B *Streptococcus* (GBS)-induced hyporeactivity to noradrenaline (NA) in intrapulmonary artery rings. Concentration-response curves to noradrenaline (NA, 10^{-8} – 10^{-4} M) were performed in intrapulmonary arterial rings previously incubated for 20 h in Krebs solution in the presence of vehicle (control, ○), LPS ($1 \mu\text{g ml}^{-1}$, ■) or heat-inactivated GBS (3×10^7 c.f.u. ml^{-1} , ▲). L-Arginine (10^{-5} M) or L-NAME (10^{-4} M) was added 30 min before addition of NA. The curve was obtained in the absence of bacterial products. Data are expressed as means $\pm$ s.e.mean of 8–14 observations. * $P < 0.05$ and ** $P < 0.01$ represent significant differences with respect to control group.

Effects of LPS and GBS on NOS activity

NOS activity was measured by the conversion of radio-labelled L-arginine to citrulline (Salter *et al.*, 1991). Using this method, NOS activity was almost undetectable in pulmonary arteries. Therefore, the cNOS (Ca^{2+} -dependent) and iNOS (Ca^{2+} -independent) activities were determined in lung fragments. Control values for cNOS and iNOS activities were 61.5 ± 10.7 and 36.0 ± 6.4 pmol $\text{min}^{-1} \text{mg}^{-1}$ of tissue, respectively. As shown in Figure 4, no change in cNOS activity was detected after incubation with $1 \mu\text{g ml}^{-1}$ LPS or 3×10^7 c.f.u. ml^{-1} GBS for 20 h. In contrast, LPS and GBS significantly increased the iNOS activity ($P < 0.01$). Dexamethasone (3×10^{-6} M) produced no change in cNOS activity but completely abolished the LPS- and GBS-induced increase in iNOS activity (Figure 4).

Discussion

The present results demonstrated that only after prolonged (> 5 h) incubation, did LPS and GBS reduce vascular res-

Figure 3 Effects of (a) dexamethasone (3×10^{-6} M) or (b) cycloheximide (10^{-5} M) when coincubated with *E. coli* lipopolysaccharide (LPS) and group B *Streptococcus* (GBS). Concentration-response curves to noradrenaline (NA, 10^{-8} – 10^{-4} M) were performed in intrapulmonary arterial rings previously incubated for 20 h in Krebs solution in the presence of vehicle (○), LPS ($1 \mu\text{g ml}^{-1}$, ■) or heat-inactivated GBS (3×10^7 c.f.u. ml^{-1} , ▲). The curve was obtained in the absence of bacterial products. Data are expressed as means $\pm$ s.e.mean of 7–14 observations. * $P < 0.05$ and ** $P < 0.01$ represent significant differences when compared to control group.

ponsiveness to NA in isolated pulmonary artery rings of neonatal pigs. After incubation, the experiments were performed in the absence of LPS or GBS which indicated that the hyporesponsiveness was a delayed process that was not due to a direct action of bacterial toxins but more likely to the induction of biological activity. The decreased response did not require the presence of an intact endothelium and was not mediated by products of cyclo-oxygenase activity since it was unaffected by endothelium removal or the cyclo-oxygenase inhibitor, meclofenamate, respectively. Our results strongly suggested that induction of iNOS is implicated in the GBS- and LPS-induced pulmonary vascular hyporesponsiveness to NA. To our knowledge this is the first paper showing that GBS produces hyporesponsiveness to NA and induction of iNOS activity.

There is a growing consensus that NO plays a major role in the control of pulmonary vascular smooth muscle tone (Moncada *et al.*, 1991; Dinh-Xuan, 1992; Stamler *et al.*, 1994). Thus, an impaired release of NO has been associated with an increased pulmonary vascular reactivity to constrictor stimuli and may contribute to the pathogenesis of pulmonary hypertension (Moncada *et al.*, 1991; Dinh-Xuan, 1992). In the present experiments, L-arginine potentiated the reduced vascular reactivity to NA in pulmonary arteries of

Figure 4 Ca^{2+} -dependent (constitutive, open columns) and Ca^{2+} -independent (inducible, solid columns) nitric oxide synthase (NOS) activities in lung fragments incubated for 20 h in 37°C oxygenated Krebs solution in the presence of vehicle (control), *E. coli* lipopolysaccharide (LPS, $1 \mu\text{g ml}^{-1}$) or group B *Streptococcus* (GBS, $3 \times 10^7 \text{ c.f.u. ml}^{-1}$) and in the absence or presence of dexamethasone ($3 \times 10^{-6} \text{ M}$). Data are expressed as percentage of the control Ca^{2+} -dependent and Ca^{2+} -independent activities, respectively (means $\pm$ s.e.mean of 5–7 observations). Control values for cNOS and iNOS activities were 61.5 ± 10.7 and $36.0 \pm 6.4 \text{ pmol min}^{-1} \text{ mg}^{-1}$ of tissue, respectively. ** $P < 0.01$ represent significant differences when compared to control group.

newborn piglets incubated with LPS and GBS, while L-NAME completely reversed it. Moreover, dexamethasone, which inhibits the induction of NOS (Radomski *et al.*, 1990) and cycloheximide, an inhibitor of protein synthesis when coincubated with LPS or GBS for 20 h, completely reversed the reduced response to NA. All these results suggest that GBS- and LPS-induced pulmonary vascular hyporesponsiveness to NA is associated with enhanced formation of NO via the iNOS and requires the synthesis *de novo* of the enzyme in the pulmonary vasculature. However, in pulmonary arteries the NOS activity was below the limit of detection. This can be related to the fact that cNOS and iNOS activities in vascular smooth muscle are 5–50 times lower than in lung (Salter *et al.*, 1991; Mitchell *et al.*, 1993). Thus, we assessed the effects of LPS and GBS on both cNOS and iNOS in lung fragments. LPS and GBS had no effect on cNOS but induced a marked increase in iNOS activity which was abolished when lung fragments were incubated with dexamethasone. GBS- and LPS-induction of iNOS in the lung supported the functional evidence of induction of iNOS in pulmonary arteries. As previously reported in rat and rabbit lung (Salter *et al.*, 1991; Mitchell *et al.*, 1993; Szabo *et al.*, 1993), we found that iNOS activity was expressed basally in porcine lung tissue. Since basal iNOS activity was lowered by dexamethasone, it might indicate a certain endotoxin contamination of the incubation media.

GBS is able to produce sepsis only in the neonate and exceptionally in parturients and immunodepressive patients (Anthony, 1985). The mechanism of GBS-induced pulmonary injury in neonates is still a matter of controversy. Gram-positive bacteria do not contain LPS and a common toxin to all Gram-positive organisms has not been identified. It has been shown recently that lipoteichoic acid (LTA), a component of the peptidoglycan layer of the cell wall in most Gram-positive bacteria, decreased the responses to pressor agents and induced iNOS in cultured vascular smooth muscle cells, rat aorta (Auguet *et al.*, 1992) and in anaesthetized rats (De Kimpe *et al.*, 1994). Killed whole *S. aureus* also induced

NOS in macrophages (Cunha *et al.*, 1993). Clinical isolates of GSB, including type III, recovered from infants with sepsis, possessed significantly higher levels of LTA in their cell wall than those isolated from asymptomatic carriers (Nealon & Mattingly, 1983). The molecular analysis of GBS LTA demonstrated a close similarity with the Group A *Streptococcus* LTA (Maurer & Mattingly, 1991). Thus, the LTA component of GBS might be responsible for the effects on vascular reactivity and iNOS induction described in the present paper. In addition, two different GBS polysaccharide toxins producing pathophysiological changes mimicking those of GBS infection in neonates have been identified, a non specific mannan polysaccharide (Hellerqvist *et al.*, 1987) and the type III specific capsular polysaccharide (Hemming *et al.*, 1984). However, the type III specific capsular polysaccharide does not seem to be required for the acute phase but it may play a role in the late phase ($> 2 \text{ h}$) of GBS-induced pulmonary haemodynamic alterations in piglets (Gibson *et al.*, 1989). In the present experiments we used heat-inactivated GBS, i.e. an unfragmented capsule, isolated from the blood of a neonate who developed early-onset sepsis. Heat-inactivated GBS has been previously shown to cause similar haemodynamic effects as the live GBS (Schrieber *et al.*, 1992). Further studies would be necessary to elucidate the specific component of GBS responsible for NOS induction.

The lung is a major target organ in neonatal sepsis (Ablow *et al.*, 1976). In general, the lung plays a determinant role in the sepsis in some animal species that exhibit a pulmonary bacterial clearance (e.g. sheep, pig) but not in species (e.g. dog, rat, rabbit) in which bacteria localize predominantly in the liver and spleen (Winkler, 1988). The uptake of bacteria by pulmonary intravascular macrophages and the subsequent release of inflammatory mediators are central to the pathological changes produced in sepsis-induced adult respiratory distress syndrome in sheep (Warner *et al.*, 1987) and in GBS-induced pulmonary hypertension in newborn piglets (Bowdy *et al.*, 1990). In these models, as in man, sepsis produces a complex pulmonary response in which two different phases have been delimited (Brigham & Meyrick, 1986). Initially there is a marked increase in pulmonary artery pressure and hypoxemia, whereas the second phase is characterized by a returning of pulmonary pressure towards baseline and an increased lung vascular permeability. This latter phase is accompanied by marked increases in guanosine 3':5'-cyclic monophosphate (cyclic GMP, Snapper *et al.*, 1983). NO-mediated vasorelaxation has been correlated with the activation of soluble guanylate cyclase which in turn increases the intracellular concentrations of cyclic GMP (Ignarro *et al.*, 1987; Moncada *et al.*, 1991). Thus, the increased production of NO following the induction of iNOS in pulmonary arteries described here could be responsible for the previously reported increased cyclic GMP production which is a main feature of the latter phase of the pulmonary response to sepsis.

In conclusion, the present results demonstrated that prolonged incubation with GBS, as previously reported with LPS, causes an induction of iNOS which results in a loss of vascular responsiveness to NA in intrapulmonary arteries of neonatal piglets. Thus, the induction of iNOS seems to be a key mediator in the delayed pulmonary vascular hyporesponsiveness characteristic of the pulmonary injury induced by either Gram-positive or Gram-negative sepsis.

This work was supported by a CICYT (92/0157) and Fis (95/0308) Grant. E.V. is a recipient of the Asociación Española de Pediatría/Arbora S.A. Grant for Pediatric Research. We would like to thank Dr Elorza, Dr Romero and Mrs María Gómez for providing the heat-killed Group B *Streptococcus*.

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(Received September 26, 1994

Revised January 5, 1995

Accepted February 6, 1995)