Review Article

# Infection-Related Perinatal Brain Injury: The Pathogenic Role of Impaired Fetal Cardiovascular Control

Yves Garnier, MD, PhD, Audrey B. C. Coumans, MD, PhD, Arne Jensen, MD, Tom H. M. Hasaart, MD, PhD, and Richard Berger, MD

*There is a growing body of evidence from clinical and epidemiologic studies that in utero exposure to infection plays an important role in the genesis of fetal or neonatal injury leading to cerebral palsy and chronic lung disease. Thus, after chorioamnionitis the incidence of immature neonates with periventricular white matter damage and periventricular or intraventricular hemorrhage is significantly elevated. Recent clinical and experimental data support the hypothesis that a fetal inflammatory response links antenatal infection with brain white matter damage and subsequent motor handicap. A variety of studies support the view that cytokines released during intrauterine infection directly cause injury to the immature brain. In this review, we provide evidence that in utero exposure to bacterial infection can severely alter fetal cardiovascular function, resulting in dysregulation of cerebral blood flow and subsequent hypoxic-ischemic brain injury. (J Soc Gynecol Investig 2003;10:450–9)* *Copyright © 2003 by the Society for Gynecologic Investigation.*

Despite improvements in perinatal medicine, the prevalence of cerebral palsy has increased over the last 2 decades,[1] and the etiology of cerebral palsy remains poorly understood. As demonstrated by a variety of recent clinical and epidemiologic studies, inflammatory reactions not only aggravate secondary neuronal damage after cerebral ischemia but might also affect the immature brain directly. Thus, after chorioamnionitis the incidence of periventricular leukomalacia and periventricular or intraventricular hemorrhage in immature newborn infants is significantly increased.[2–7] Recent studies provide evidence that chorioamnionitis instigates a fetal inflammatory response and that this inflammation contributes to neonatal brain injury and subsequent cerebral palsy.[2,6,8,9] Reports of elevated cytokine levels in both neonatal blood[10,11] and amniotic fluid[12,13] in children with cerebral palsy support the notion that cerebral palsy is preceded by perinatal inflammatory disease. However, the pathogenesis of infection-related neuronal and oligodendroglial cell damage remains unclear. Various experimental studies provide evidence that impaired fetal cardiovascular control during endotoxemia, resulting in sustained hypotension and loss of cerebral autoregulation, precedes hypoxic-ischemic brain injury. In this review, we first summarize the current studies on direct cytotoxic effects of endotoxins and proinflammatory cytokines on cerebral tissue. In the second part, we review the experimental data available on endotoxemia-induced alterations of the fetal cardiovascular function and outline their significance in the pathogenesis of infection-related perinatal brain injury.

## CLINICAL DATA AND DEFINITIONS

Clinical chorioamnionitis is an infection of the uterus and its contents during pregnancy. Its diagnosis is based on the presence of fever (> 38C) plus two or more of the following conditions: maternal tachycardia, fetal tachycardia, uterine tenderness, foul smelling amniotic fluid, or maternal leukocytosis.[14] The incidence varies between 10% and 20%.[15]

Histologically, chorioamnionitis is defined by the presence of polymorphonuclear infiltrates in the placenta and its membranes. It affects 20% of term pregnancies and up to 60% of preterm pregnancies and is often an occult finding.[16] Recently, Dashe and coworkers[17] reported a simultaneous diagnosis of histologic chorioamnionitis in mothers with clinical chorioamnionitis in 80% of cases, whereas 20% had no histologic evidence of infection. Furthermore, Grether and Nelson[5] observed that both clinical and histopathologic evidence of placental infection were associated with an increased risk of unexplained cerebral palsy (CP) (odds ratio [OR] 9.3, 95% confidence interval [CI] 2.7, 31 for clinical chorioamnionitis [CA]; OR 8.9, 95% CI 1.9, 40 for histologic CA]). Recently, a meta-analysis evaluated the potential association between CA and CP in both full-term and preterm infants.[18] The authors reported an association between clinical CA and both CP (relative risk [RR] 1.9; 95% CI 1.5, 2.5) and cystic periventricular leukomalacia (cPVL) (RR 2.6; 95% CI 1.7, 3.9).

The overall mortality rate of neonates with congenital neonatal sepsis ranges from 25% to 90% (for review see [19]). This wide range of results may reflect the effect of gestational age on

From the Department of Obstetrics and Gynecology, University of Bochum, Bochum, Germany; and Department of Obstetrics and Gynecology, University of Maastricht, Maastricht, The Netherlands.

Address correspondence and reprint requests to: Professor Dr. Richard Berger, Universitätsfrauenklinik Bochum, Knappschaftskrankenhaus, In der Schornau 23-25, 44982 Bochum. E-mail: richard.berger@ruhr-uni-bochum.de



1071-5576/03/$30.00
doi:10.1016/S1071-5576(03)00150-3

the likelihood of survival. One study, which focused on infants born before 33 weeks' gestation, found that the mortality rate was 33% for infected and 17% for uninfected fetuses.[20] The rate of fetal or early-onset neonatal bacteremia ranged from 10% to 33%, if amniotic fluid cultures were positive but was only 4% in negative cultures.[21,22] Thus, subclinical fetal infection is far more common than traditionally recognized.

## INFECTION-RELATED PERINATAL BRAIN DAMAGE: AN OVERVIEW

A wealth of experimental studies has emerged describing the pathophysiologic mechanisms implicated in perinatal brain injury in response to hypoxia-ischemia. These mechanisms involve the acute breakdown of cerebral energy metabolism leading to the release of excitatory amino acids such as glutamate. Glutamate binds to postsynaptically located glutamate receptors that regulate calcium channels.[23] The resulting calcium influx, so-called calcium overload, activates proteases, lipases, and endonucleases which destroy the cellular skeleton.[24] A second wave of neuronal cell damage occurs during the reperfusion phase. This cell damage is thought to be caused by the postischemic release of oxygen radicals, synthesis of nitric oxide, inflammatory reactions, and an imbalance between the excitatory and inhibitory neurotransmitter systems (for review see [25]).

### Direct Neurotoxic Effects of Endotoxins and Cytokines

An increasing body of evidence shows that endotoxins and released proinflammatory cytokines may also damage the fetal brain directly, especially the periventricular white matter. Thus, animal studies provide evidence that administration of endotoxin induces increased cytokine expression in adult rat brains[26–28] and in fetal hippocampal tissue.[29] An increase in the release of tumor necrosis factor (TNF)-α, in particular, has been thought to be associated with brain injury.[30] In addition, increased expression of interleukin (IL)-1β and TNF-α mRNA has been shown in the brain of fetal rats after intraperitoneal application of lipopolysaccharide (LPS) to the dam. This was followed by minimal white matter injury.[31] Similar observations have been described in immature rabbits after uterine infection with bacteria[32] and in newborn kittens after intraperitoneal injection of LPS.[33]

Because periventricular leukomalacia (PVL), the common type of fetal brain injury associated with ascending intrauterine infection, occurs well before the onset of active myelination, it has been suggested that cytokines could damage the progenitors of oligodendrocytes directly.[4,34] In fact, the inhibitory effects of TNF-α on the proliferation of enriched oligodendrocyte progenitors and their subsequent differentiation into mature myelinating oligodendrocytes have been reported.[35] Moreover, it has been shown that TNF-α could compromise the growth of oligodendrocytes and the expression of mRNA for myelin basic protein (MBP) in cultures of mixed glial cells from rodent brains and could be cytotoxic at high concentrations.[36–38] The combined application of TNF-α and interferon (IFN)-γ severely reduced survival and inhibited differentiation of oligodendrocyte progenitors in a primary culture prepared from neonatal rats (Feldhaus B, Dietzel ID, Berger R. TNF-α induces cell death in primary cultures of oligodendrocyte progenitors [Abstract]. J Soc Gynecol Investig 2001;8:248A.). It is obvious that loss or functional alteration of these oligodendrocyte progenitors may underlie the disrupted myelination that characterizes PVL.

At present several mechanisms are known to be involved in cytokine-induced cell damage. TNF-α signals apoptosis through two membrane receptors (p60 and p80) with different cytoplasmic domains.[39] Activated receptors bind to a family of proteins (TRAF1-6) and to another protein (TRADD) through the death domain, which then recruits the proapoptotic caspases 8/9 to activate caspase-3 (CPP32) and possibly caspase 7 to deliver the death signal.[40] Furthermore, a sphingomyelin cycle has been identified in which the action of cytokines, such as TNF-α, results in the activation of one or more sphingomyelinases, cleavage of membrane sphingomyelin, formation of ceramide, and activation of multiple cellular and biochemical targets leading to apoptosis.[41,42]

### LPS Sensitizes the Fetal Brain to Subsequent Hypoxic-Ischemic Insults

Recently, Eklind and coworkers[43] sensitized the 7-day-old neonatal rat brain to hypoxic-ischemic insults by intraperitoneal injection of LPS. Moreover, it was found in neonatal rat pups that LPS applied intracisternally enhanced susceptibility to subsequent hypoxic-ischemic brain damage.[44] In adult rats brain injury was reduced when intravenous LPS was applied several days before middle cerebral artery occlusion.[45] However, the interval between the primary and secondary insult seems to be the most important variable for determining whether an alleviation or an aggravation of the final outcome occurs. Thus, sensitization of the immature fetal sheep brain to injury has been demonstrated after repeated brief episodes of cerebral hypoxia-ischemia.[46] A similar relationship may exist between LPS and hypoxia-ischemia.

## IMPAIRED FETAL CARDIOVASCULAR CONTROL DURING ENDOTOXEMIA

### Fetal Circulatory Redistribution Induced by Endotoxin

In addition to direct cytotoxic effects of cytokines on cerebral tissue, impaired fetal cardiovascular control during endotoxemia-bacteremia resulting in a loss of cerebral autoregulation may contribute to fetal brain injury (Figure 1). LPS, an endotoxin extracted from the cell wall of Gram-negative bacteria, seems to have a major effect on the pathophysiology of infection-related perinatal brain injury. The effects of systemically administered LPS on fetal cardiovascular function have been studied in various animal models. In chronically instrumented fetal sheep (0.7 gestation) intravenous injection of LPS (*Escherichia coli*; O127:B8, Sigma-Aldrich, Deisenhofen, Germany; 53 ± 3 μg per kg fetal body weight; bolus injection over 2

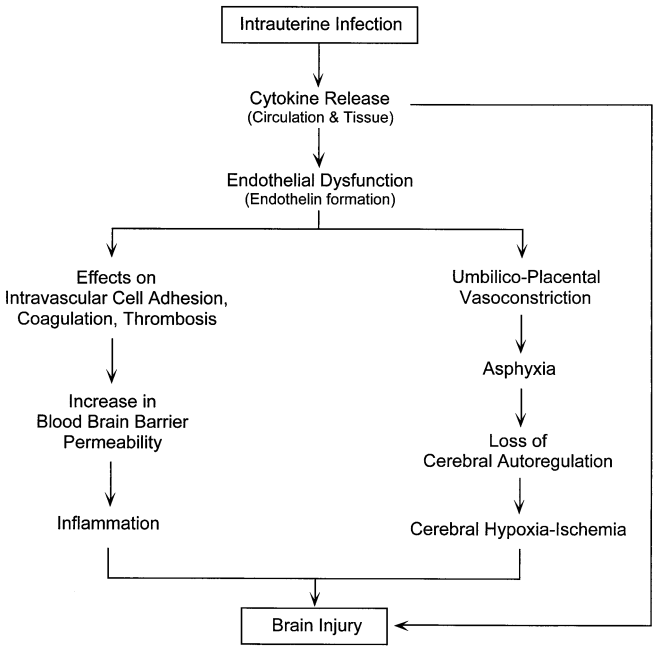


**Figure 1.** Potential mechanisms linking intrauterine infection and brain injury.

minutes) severely decreased placental blood flow within 1 hour, whereas blood flow to the peripheral organs, eg, to the carcass, increased.[47] During a short period of superimposed asphyxia in utero, there was clear evidence of circulatory decentralization; ie, both placental blood flow and cerebral oxygen delivery were nearly arrested, whereas hyperperfusion of peripheral organs, eg, liver, lungs, gastrointestinal tract, and carcass, occurred (Figure 2). Because the umbilical and placental vessels lack autonomic innervation,[48] the regulation of umbilical and placental blood flow must depend on circulating or locally released vasoactive substances (for review see [49,50]). In fetal sheep the placental microcirculation is remarkably unresponsive to many vasoconstrictors, including norepinephrine and angiotensin II, whereas the umbilical artery and vein are more responsive to vasoactive substances.[51,52] The decrease in placental blood flow was accompanied by sustained hypotension, hypoxemia, and mixed acidosis causing dysregulation of cerebral blood flow.

During endotoxemia there was no increase in blood flow to the brain, although oxygen saturation decreased by 50%.[47] This finding is surprising, because an inverse relationship between cerebral blood flow and arterial oxygen content has been described in term as well as in preterm fetal sheep.[53,54] One possible explanation for this phenomenon may be that the physiologic mechanisms mediating the response of cerebral blood flow to hypoxia are altered by LPS, leading to a loss of cerebral autoregulation. Furthermore, LPS-treated fetuses suffered from severe hypotension during the recovery period after hypoxia, which was accompanied by a 50% reduction in brain blood flow compared with control fetuses.[47] At mean arterial blood pressures below 25–30 mmHg there is a reduction in cerebral blood flow because of an increasing loss of cerebral autoregulation. This reduction affects the parasagittal region of the cerebrum and the white matter in particular.

It is well-established that hypoxic-ischemic brain injury occurs under such circumstances. Thus, Rees and coworkers[55] studied the effects of hypoxemia caused by restriction of umbilicoplacental blood flow on various brain structures in midgestation fetal sheep. A reduction of fetal arterial oxygen content by approximately 50% for 6 or 12 hours resulted in prominent white matter injury as well as subcortical gray matter damage to the striatum, thalamus, and hippocampus. Moreover, the same group investigated the effects of reduced uterine blood flow on neuropathologic outcome in near-midgestation fetal sheep. Lowering fetal arterial oxygen saturation by 50–60% for 12 hours caused white matter injury and reduced neuronal cell count in the hippocampus, cerebellum, and cerebral cortex. They also found an increased number of astrocytes in the ventral hippocampi 35 days later.[56] Similar findings were described after sustained maternal hypoxemia.[57] In addition, hemorrhagic hypotension in 113-day-old fetal sheep was reported to induce PVL-like lesions with areas of coagulation necrosis and accumulation of activated microglia.[58] A recent study showed that a severe hypoxic period of 25 minutes, brought about by occlusion of the umbilical cord, is sufficient to cause white matter damage in fetal sheep at 90–93 days' gestation. Thus, there is convincing evidence that hypoxic insults, as observed after bolus injection of LPS in preterm fetal sheep,[47] may result in brain damage.

In contrast to the findings in immature fetal sheep, mature fetuses appear to be less sensitive to endotoxin administered systemically. It was demonstrated that intravenous LPS (*E coli*; 50 μg/kg fetal body weight; bolus injection over 2 minutes) treatment compromised fetal cardiovascular control during and shortly after in utero asphyxia.[59] After injection of endotoxin there were increases in arterial blood pressure, as well as in concentrations of hemoglobin, glucose, lactate, catecholamines, vasopressin, and angiotensin II and a decrease in base excess and granulocyte counts; however, both arterial oxygen saturation and oxygen tension remained unchanged. Blood flow to the brain, placenta, and carcass decreased, whereas that to the lungs, heart, pituitary gland, gastrointestinal tract, pancreas, and liver increased. During asphyxia, blood flow to the brain did not increase, thus circulatory centralization was impaired. However, in contrast to immature fetuses, all these changes occurred transiently and recovered partially within 1 hour.[59]

## Chronic LPS Exposure

Recently, the effects of low-dose LPS-treatment on cardiovascular control were studied in 0.7 gestation fetal sheep. Intravenous application of LPS to the fetus (*E coli*; O127:B8, Sigma-Aldrich; 100 ng/kg fetal body weight; bolus injection over 2 minutes) caused a substantial and long-lasting decrease in umbilical blood flow resulting in sustained fetal hypoxemia without acidemia (Garnier Y, Coumans ABC, Vaihinger HM, et al. Low dose endotoxin (LPS) results in substantial umbilicoplacental vasoconstriction and discrete neuropathological

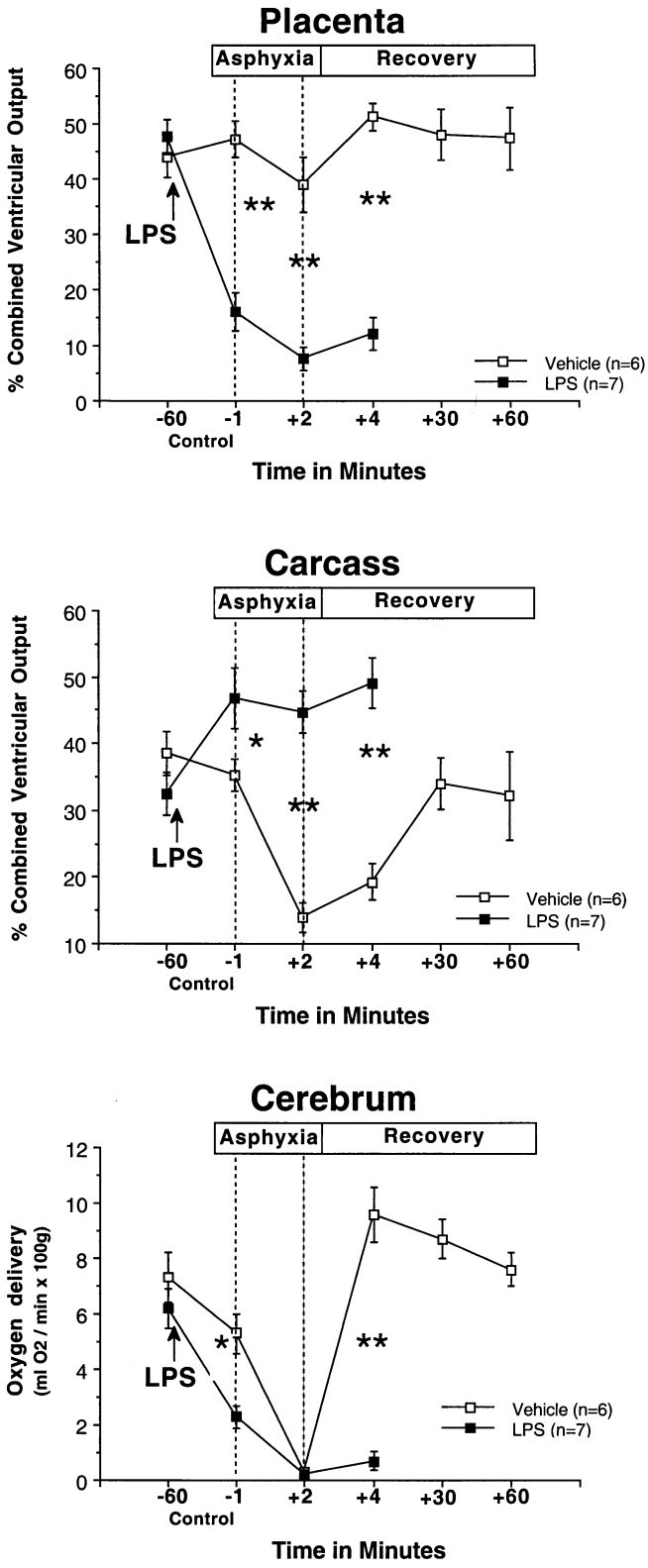


**Figure 2.** Endotoxin-induced fetal circulatory redistribution. Combined ventricular output directed to the placenta and carcass in control ($n = 6$) and LPS-treated ($n = 7$) chronically catheterized immature fetal sheep before, during, and after arrest of uterine blood flow for 2 minutes.[47] In relation to vehicle-treated controls, intravenous administration of LPS (*E coli*; 53 μg/kg) severely decreased the percentage of combined ventricular output directed to the placenta but significantly increased that directed to the carcass. During arrest of uterine blood flow, the portion distributed to the carcass remained elevated in fetuses of the study group. Moreover, autoregulation of cerebral blood flow was severely impaired causing an arrest of cerebral oxygen delivery. Within 60 minutes of induction of asphyxia, five of seven LPS-treated fetuses died, whereas control fetuses completely recovered during this period. Values are given as means ± standard error of the mean. The data were analyzed within and between groups using a two-way analysis of variance followed by Games-Howell post-hoc test (*$P < .05$, **$P < .01$).

changes in preterm sheep [Abstract]. J Soc Gynecol Investig 2002;9:72A–73A). Placental blood flow began to decrease 1 hour after LPS injection and was lowest (−40%) at 4–5 hours after LPS, whereas placental vascular resistance increased by 75% during this period. Thereafter, placental blood flow slowly returned to control values at 12–16 hours after LPS application. Both fetal heart rate and mean arterial blood pressure increased at 4–5 hours after LPS infusion and remained elevated for the following 12 hours. Histopathologic examination revealed an increase in the periventricular white matter cell count, accompanied by intense and uniform nuclear staining and chromatin condensation with karyorrhexis in response to systemically administered LPS. Electron micrographs showed characteristic chromatin condensation and segregation, extracellular apoptotic bodies, and cell fragments phagocytosed in macrophages in the periventricular white matter.

Harding and coworkers studied the effects of sublethal doses of LPS on both cerebral and placental blood flow in the chronically prepared preterm ovine fetus (Cock ML, Dalitz PA, Harding R. Placental blood flow following endotoxin administration in the preterm ovine fetus [Abstract]. J Soc Gynecol Investig 2002;9:191A) (Dalitz PA, Cock ML, Rees S, Harding R. Cerebral blood flow and oxygen delivery following endotoxin exposure in the preterm ovine fetus [Abstract]. J Soc Gynecol Investig 2002;9:120A). Endotoxemia impaired fetal oxygenation transiently and caused acidemia by reducing umbilicoplacental blood flow. In the presence of hypoxemia, a prolonged decrease in oxygen delivery to the brain of approximately 40% was observed. Thus, mechanisms that normally increase fetal cerebral blood flow in the presence of hypoxemia are apparently impaired after systemic LPS exposure.

Furthermore, they investigated the effects of systemically applied endotoxin on the perinatal brain in the preterm ovine fetus. In response to LPS injection, the fetuses suffered from hypoxemia, acidemia, and hypotension for several hours.[60] With increasing numbers of LPS injections, the changes in hemodynamic variables became less and less pronounced, whereas white matter injury was present 10–11 days after the initial LPS injection. Injury was most prominent in the cerebral white matter and ranged from diffuse subcortical damage

to focal periventricular leukomalacia, which is typical of white matter injury in preterm fetuses. Most recently, Mallard and coworkers[61] compared neuropathologic findings in response to intrauterine exposure to asphyxia or endotoxemia in midgestation fetal sheep. Both experimental paradigms resulted in white matter damage and inflammation. However, whereas LPS treatment resulted in selective white matter injury and regional infiltrates of inflammatory cells, injury after severe asphyxia involved cortical gray matter and was associated with microglial activation.

## PATHOGENESIS OF IMPAIRED FETAL CARDIOVASCULAR CONTROL DURING ENDOTOXEMIA

A hallmark of endotoxemia and sepsis is the heterogeneous pattern of vasoconstriction and vasodilation in different organs, culminating in a decrease in total peripheral vascular resistance concomitant with regional maldistribution of blood flow. These changes in the distribution of cardiac output are most likely caused by vasoactive substances, eg, nitric oxide (NO), prostacyclin, angiotensin-converting enzyme activity, endothelin, and adrenomedullin, which are released from the endothelium under experimental septic conditions. In this context we will discuss the role of two important vasoactive agents, NO and endothelin, in the endotoxin-mediated alterations of fetal cardiovascular control.

### The Pivotal Role of Nitric Oxide in Fetal Cardiovascular Control During Normoxia and Hypoxia

Nitric oxide is known to be a potent mediator in the regulation of resting tone in cerebral, renal, mesenteric, and hindquarter vascular beds and hence in blood pressure homeostasis in adults.[62] It has also been suggested that NO contributes to cardiovascular control during hypoxia in adults and fetal sheep.[62–65] There is also evidence that NO might, in part, mediate the fetal circulatory centralization that occurs during partial cord occlusion.[66] This cardiovascular response to reduced oxygen supply is a crucial mechanism that protects the fetal brain from neuronal injury by increasing cerebral blood flow during hypoxia. Furthermore, NO is an important vasodilator in the cerebral circulation, and developmental changes in nitric oxide synthase (NOS) production may contribute to developmental changes in hypoxic cerebral vasodilation.[64] In fetal sheep, endothelial NOS is already present in blood vessels by 0.4 gestation.[67] Moreover, cortical NOS catalytic activity increases threefold between 0.6 and 0.9 gestation.[68]

Green and coworkers[63] elegantly demonstrated the central role of NO in maintaining fetal cardiovascular function during normoxia and hypoxia. In term fetal sheep, intravenous application of the NOS inhibitor $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) resulted in bradycardia and decreased carotid blood flow. This decreased flow was accompanied by transient hypertension and an increase in both carotid and femoral vascular resistance. During intrauterine exposure to hypoxia the magnitude of the subsequent chemoreflex bradycardia was reduced after L-NAME treatment, and the well-known rebound tachycardia during recovery was absent with NOS inhibition.[63] Moreover, NOS inhibition blunted the increase in carotid blood flow and the decrease in carotid vascular resistance during hypoxia. Because there were no significant differences in mean arterial pressure between control and L-NAME-infused groups during hypoxia, it is thought that NO mediates vasodilation of the carotid vascular bed during hypoxia in the fetus.

Smolich[69] investigated the effects of NO on the redistribution of fetal blood flow between body and placenta, as well as oxygen extraction and oxygen consumption. In term fetal sheep, NOS inhibition redistributed systemic blood flow toward the placenta and increased fetal body oxygen extraction. The latter initially increased whole-body oxygen consumption and maintained it near baseline levels after a decrease in placental perfusion. The effects of NOS inhibition on blood flow distribution were further investigated during hypoxia in the chronically prepared preterm ovine fetus.[70] (J Soc Gynecol Investig 2001;8:105A). Under normoxic conditions, L-NAME infusion decreased blood flow to the fetal body and to the placenta by more than 60%. During a short period of superimposed acute hypoxia, L-NAME did not change the redistribution of cardiac output toward the central organs. However, the control of fetal heart rate and blood pressure was altered in L-NAME-treated animals. After hypoxia L-NAME delayed the recovery of cardiac output and blunted the increase in blood flow to the brain and heart. Thus, NO plays an essential role in fetal cardiovascular control during normoxia and acute hypoxia.

### Nitric Oxide as a Mediator of Fetal Circulatory Shock

During endotoxemia, enhanced formation of NO contributes to the acute and delayed therapy-resistant decrease in blood pressure and the vascular hyporeactivity that develops in response to endogenous and exogenous catecholamines in adults. For instance, in anesthetized adult rats, LPS administration triggers the release of NO, resulting in a decrease in blood pressure and reduced responsiveness to intravenously applied vasonconstrictors.[71,72] Enhanced formation of NO after activation of the constitutive isoform of NOS present in endothelial cells mediates the immediate release of NO in response to LPS. Longer periods of endotoxemia are associated with the formation of cytokine-inducible NOS in many cells and organs, including the vessel wall. The enhanced generation of NO resulting from cytokine- or endotoxin-related induction of inducible NOS contributes to inappropriate vasodilation, delayed hyporeactivity to adrenergic agonists, and peripheral vascular failure associated with endotoxic shock.[71–73]

Glucocorticoids, which are potent inhibitors of the induction of NOS as well as of cyclo-oxygenase-2, protect against cardiovascular failure in septic and hemorrhagic shock.[74] Adrenalectomized animals, lacking endogenous glucocorticoids, develop a more severe form of circulatory shock in response to LPS, which can be prevented by pretreatment with exogenous

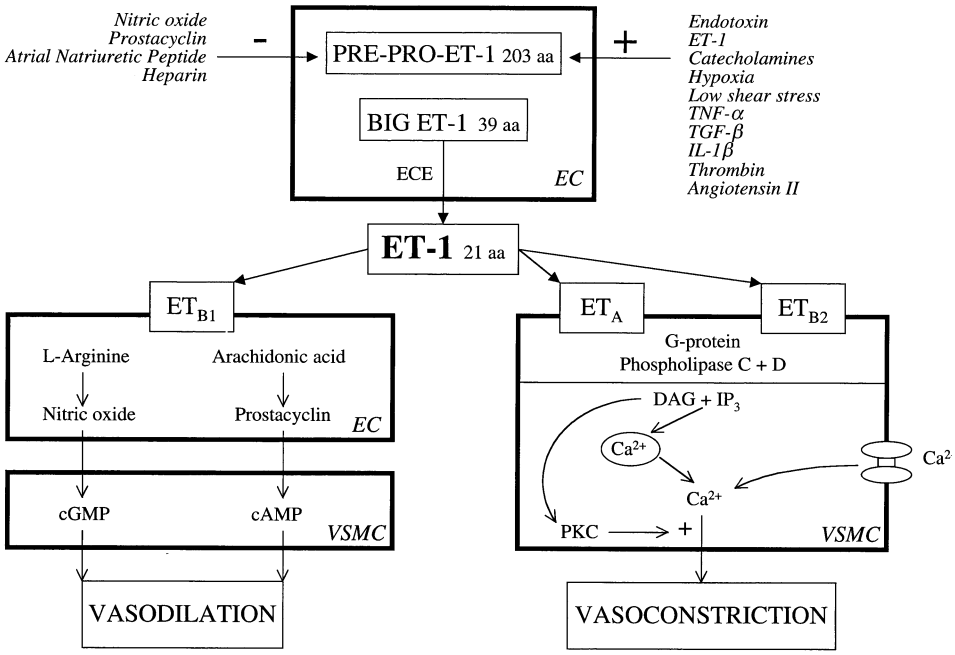


**Figure 3.** Brief schematic overview of the endothelin system. ET-1 is produced from a prepropeptide, which is proteolytically cleaved to big ET-1. Big ET-1 is subsequently converted to ET-1 by the metalloprotease ECE-1, which is essential for its biologic activity. ET-1 is released in response to several stimuli, including hypoxia, endotoxemia, cytokines, increased pressure, and shear stress. In response to these stimuli, ET-1 has vasodilating and vasoconstricting effects, which are mediated through two receptor subtypes, $ET_A$ and $ET_B$. $ET_A$ receptors and a small subpopulation of $ET_{B2}$ receptors of vascular smooth muscle cells mediate vasoconstriction. A larger subpopulation of $ET_{B1}$ receptors is located on vascular endothelial cells and is responsible for the vasodilating effects, mediated by nitric oxide-production. aa = amino acid; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; DAG = diacylglycerol; EC = endothelial cell; ECE = endothelin-converting enzyme; $IP_3$ = inositol triphosphate; PKC = protein kinase C; VSMC = vascular smooth muscle cell.

glucocorticosteroids. Attenuation of the induction of NOS by endogenous glucocorticosteroids accounts for endotoxin tolerance.[75] However, most of these studies were performed in adult animals, and their significance for the fetal circulation has not yet been established.

## The Role of Endothelin-1 During Endotoxemia

In addition to the relaxing factors prostacyclin and NO, the vascular endothelium synthesizes the 21-amino-acid peptide, endothelin-1 (ET-1). ET-1 is produced from a prepropeptide, which is proteolytically cleaved to big ET-1[76] (Figure 3). Big ET-1 is subsequently converted to ET-1 by the metalloprotease, endothelin-converting enzyme-1 (ECE-1).[77] The conversion of big ET-1 to ET-1 is essential for its biological activity, because the pressor action of big ET-1 is almost completely abolished by inhibition of ECE-1.[78] ET-1 is the most active pressor substance yet discovered, with a potency some ten times that of angiotensin II.[79]

ET-1 is released in response to several stimuli, including hypoxia,[80,81] endotoxemia,[82,83] increased pressure,[84] and shear stress.[85] In response to these stimuli, ET-1 causes both acute responses, such as vasodilation and vasoconstriction, as well as chronic changes, such as smooth muscle proliferation. The vascular responses to ET-1 vary during development in fetal and neonatal sheep, and these effects are vascular-bed specific. Thus, ET-1-induced vasoconstriction increases with gestational age in femoral, middle cerebral, and renal arteries but not in adrenal arteries.[86] These hemodynamic effects are mediated through two receptor subtypes, $ET_A$ and $ET_B$. $ET_A$ receptors and a small subpopulation of $ET_B$ receptors of vascular smooth muscle cells mediate vasoconstriction. A larger subpopulation of $ET_B$ receptors is located on vascular endothelial cells and are responsible for the vasodilating effects, mediated by NO-production.[87]

Among the pathophysiologic conditions known to involve the endothelin system, sepsis produces the highest plasma levels of ET-1.[88] The possible involvement of the endothelin system in human septic shock is further supported by a correlation between endothelin plasma levels and morbidity and mortality in septic patients.[89–91] Experimentally, endotoxin induces the expression of prepro ET-1 mRNA in various organs,[49] and elevated plasma ET-1 levels are seen in various species during endotoxemia.[92,93] Infusion of ET-1 to humans causes cardiovascular changes resembling, in part, those during sepsis, ie, decreased cardiac output and vasoconstriction in the pulmonary, splanchnic, and renal circulation.[94,95] Bacterial endotoxin and septic conditions increase ET-1 concentrations in fetal umbilical arterial plasma more than fivefold in both pigs and humans, reaching levels close to threshold vasoconstriction.[96] Other proinflammatory agents, such as IL-1, TNF-α, and transforming growth factor-β, which are released during

endotoxemia, also increase the production of ET-1 from endothelial cells. In fetal sheep ET-1 causes both constriction of the fetoplacental microcirculation and a decrease in fetal oxygen consumption.[97] Prolonged alterations of placental gas exchange are known to induce fetal cardiovascular dysfunction with subsequent arterial hypotension and cerebral hypoperfusion. It is therefore quite conceivable that ET-1-induced effects on the placental vascular bed could finally result in hypoxic-ischemic fetal brain damage.

Another mechanism by which endothelin could affect fetal cardiovascular control during endotoxemia could be through its pronounced effects on pulmonary circulation.[83] The pathophysiology includes a characteristic biphasic increase in mean pulmonary artery pressure and the pulmonary vascular resistance index and is thought to involve different mediators, including cytokines, which lead to increased expression of adhesion molecules, leukocyte activation, and endothelial damage resulting in endothelial edema, vascular obliteration, and vasoconstriction.[98] The involvement of both the cyclooxygenase pathway in the early phase and the endothelin system in the late phase of endotoxin-induced pulmonary hypertension has been shown, and unselective as well as selective endothelin $ET_A$ receptor antagonism can counteract the late changes in the experimental setting.[99,100] In contrast, intrauterine exposure to systemically applied endotoxin decreased pulmonary vascular resistance and increased lung perfusion in fetal sheep.[47] Although ET-1 produces systemic vasoconstriction, its effects on the pulmonary circulation vary with age and vascular tone.[101–103] In the lung of the fetal lamb, the $ET_B$ receptor is highly expressed. ET-1 activates the $ET_B$ receptor, which stimulates endothelial NOS activity and NO production, mediating vascular smooth muscle relaxation and pulmonary vasodilation.[104] In fact, pulmonary vasodilation has been described after exposure to NO, a substance that is produced in large amounts after LPS application. These observations warrant further experiments investigating the effects of LPS on pulmonary vascular control.

Apart from its profound cardiovascular effects, there are several mechanisms by which ET-1 may contribute to the pathophysiology of sepsis, endotoxemia, and consequent perinatal brain injury. It was shown that ET-1 activates neutrophilic leukocytes and enhances expression of vascular cell adhesion molecule, thereby promoting adhesion of leukocytes to the vascular endothelium.[105] Furthermore, ET-1 has been shown to induce the production of reactive oxygen species, which are fundamental mediators in the development of endotoxic shock.[106] Moreover, ET-1 affects the endothelial barrier as it produces a dose-dependent increase in permeability to protein and extravasation of albumin with concomitant hemoconcentration.[104] Endothelins have also been shown to cause mitochondrial damage in several organ systems, a possible mechanism underlying the cytopathic hypoxia seen in sepsis.[107] ET-1 was shown to delay the clearance of *E coli* bacteria from the circulation and to increase the colonization of several vital organs, reflecting a reduction in bacterial killing function.[108] Therefore, the endothelin system may contribute to infection-associated perinatal brain injury not only by marked changes in organ perfusion but also by other deleterious mechanisms.

## CONCLUSION

It is obvious from recent clinical and epidemiologic studies that in utero exposure to bacterial infection increases the incidence of periventricular leukomalacia and periventricular or intraventricular hemorrhage in immature newborn infants. Recent data provide evidence that chorioamnionitis gives rise to a fetal inflammatory response and that this inflammation contributes to neonatal brain injury and subsequent cerebral palsy. Endotoxemia and systemic inflammation induce rapid and profound changes in endothelial function. From the experimental data available, it is evident that intrauterine exposure to infection severely alters fetal cardiovascular control, which contributes to hypoxic-ischemic brain injury, especially in the periventricular white matter. During endotoxemia, enhanced NO formation contributes to inappropriate vasodilation, delayed hyporeactivity to adrenergic agonists, and peripheral vascular failure associated with endotoxic shock. In addition to relaxing factors, the vascular endothelium releases ET-1 in response to endotoxemia and hypoxia. Among the pathophysiologic conditions known to involve the endothelin system, endotoxemia produces the highest plasma levels of ET-1.

## REFERENCES

1. Bhushan V, Paneth N, Kiely JL. Impact of improved survival of very low birth weight infants on recent secular trends in the prevalence of cerebral palsy. Pediatrics 1993;91:1094–100.
2. Adinolfi M. Infectious diseases in pregnancy, cytokines and neurological impairment: An hypothesis. Dev Med Child Neurol 1993;35:549–53.
3. Berger R, Bender S, Sefkow S, Klingmüller V, Künzel W, Jensen A. Peri/intraventricular haemorrhage: A cranial ultrasound study on 5286 neonates. Eur J Obstet Gynecol 1997;75: 191–203.
4. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. A review. Pedriatr Res 1997;42:1–8.
5. Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. JAMA 1997;278:207–11.
6. Perlman JM, Risser R, Broyles RS. Bilateral cystic periventricular leukomalacia in the premature infant: Associated risk factors. Pediatrics 1996;97:822–7.
7. Yoon BH, Romero R, Park JS, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol 2000;182: 675–81.
8. Dammann O, Leviton A. Infection remote from the brain, neonatal white matter damage, and cerebral palsy in the preterm infant. Semin Pediatr Neurol 1998;5:190–201.
9. Leviton A, Paneth N, Reuss L, et al. Maternal infection, fetal inflammatory response, and brain damage in very low birth weight infants. Pediatr Res 1999;46:566–75.
10. Nelson KB, Dambrosia JM, Grether JK. Neonatal cytokines and coagulation factors in children with cerebral palsy. Ann Neurol 1998;44:665–75.
11. Martinez E, Figueroa R, Garry D, et al. Elevated amniotic fluid interleukin-6 as a predictor of neonatal periventricular leu-

komalacia and intraventricular hemorrhage. J Matern Fetal Investig 1998;8:101–7.
12. Yoon BH, Romero R, Yang SH, et al. Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. Am J Obstet Gynecol 1996;174:1433–40.
13. Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1β, and tumor necrosis factor-α), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol 1997;177:19–26.
14. Gibbs RS, Blanco JD, St Clair PG, et al. Quantitative bacteriology of amniotic fluid from patients with clinical intraamniotic infection at term. J Infect Dis 1989;45:1–8.
15. Newton ER. Chorioamnionitis and intraamniotic infection. Clin Obstet Gynecol 1993;36:795–808.
16. Williams AB. Textbook of Obstetrics & Gynecology. London: Longmans, 1993.
17. Dashe JS, Rogers BB, McIntire DD, Leveno KJ. Epidural analgesia and intrapartum fever: Placental findings. Obstet Gynecol 1999;93:341–4.
18. Wu YW, Colford JM. Chorioamnionitis as a risk factor for cerebral palsy: A meta-analysis. JAMA 2000;284:1417–24.
19. Gonçalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disability Res Rev 2002;8:3–13.
20. Thompson PJ, Greenough A, Gamsu HR, Nicolaides KH, Philpott-Howard J. Congenital bacterial sepsis in very preterm infants. J Med Microbiol 1992;36:117–20.
21. Carroll SG, Papaioannou S, Ntumazah IL, Philpott-Howard J, Nicolaides KH. Lower genital tract swabs in the prediction of intrauterine infection in preterm prelabour rupture of the membranes. Br J Obstet Gynaecol 1996;103:54–9.
22. Sperling RS, Newton E, Gibbs RS. Intraamniotic infection in low-birth-weight infants. J Infect Dis 1988;157:113–7.
23. Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 1989;29:365–402.
24. Kristian T, Siesjo BK. Calcium in ischemic cell death. Stroke 1998;29:705–18.
25. Berger R, Garnier Y. Pathophysiology of perinatal brain damage. Brain Res Brain Res Rev 1999;30:107–34.
26. Gatti S, Bartfai T. Induction of tumor necrosis factor-alpha mRNA in the brain after peripheral endotoxin treatment: Comparison with interleukin-1 family and interleukin-6. Brain Res 1993;624:291–4.
27. Hillhouse EW, Mosley K. Peripheral endotoxin induces hypothalamic immunoreactive interleukin-1 beta in the rat. Br J Pharmacol 1993;109:289–90.
28. Van Dam AM, Bauer J, Tilders FJ, Berkenbosch F. Endotoxin-induced appearance of immunoreactive interleukin-1 beta in ramified microglia in rat brain: A light and electron microscopic study. Neuroscience 1995;65:815–26.
29. Berger R, Garnier Y, Pfeiffer D, Jensen A. Lipopolysaccharides do not alter energy metabolism and protein synthesis in an in vitro model of fetal cerebral ischemia. Pediatr Res 2000;48: 531–5.
30. Barone FC, Arvin B, White RF, et al. Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. Stroke 1997; 28:1233–44.
31. Cai Z, Pan Z, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res 2000; 47:64–72.
32. Yoon BH, Kim CJ, Romero R, et al. Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. Am J Obstet Gynecol 1997;177:406–11.
33. Gilles FH, Averill DR Jr, Kerr CS. Neonatal endotoxin encephalopathy. Ann Neurol 1977;2:49–56.
34. Back SA, Volpe JJ. Cellular and molecular pathogenesis of periventricular white matter damage. Ment Retard Dev 1997; 3:96–107.
35. Cammer W. Effects of TNFalpha on immature and mature oligodendrocytes and their progenitors in vitro. Brain Res 2000; 864:213–9.
36. Cammer W, Zhang H. Maturation of oligodendrocytes is more sensitive to TNF alpha than is survival of precursors and immature oligodendrocytes. J Neuroimmunol 1999;97:37–42.
37. Qi Y, Dal Canto MC. Effect of Theiler's murine encephalomyelitis virus and cytokines on cultured oligodendrocytes and astrocytes. J Neurosci Res 1996;45:364–74.
38. Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 1988;23: 339–46.
39. Van Antwerp DJ, Martin SJ, Verma IM, Green DR. Inhibition of TNF-induced apoptosis by NF-kappa B. Trends Cell Biol 1998;8:107–11.
40. Monney L, Olivier R, Otter I, Jansen B, Poirier GG, Borner C. Role of an acidic compartment in tumor-necrosis-factor-alpha-induced production of ceramide, activation of caspase-3 and apoptosis. Eur J Biochem 1998;251:295–303.
41. Kolesnick R, Golde DW. The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 1994;77: 325–8.
42. Scurlock B, Dawson G. Differential responses of oligodendrocytes to tumor necrosis factor and other pro-apoptotic agents: Role of ceramide in apoptosis. J Neurosci Res 1999;55:514–22.
43. Eklind S, Mallard C, Leverin A, et al. Bacterial endotoxin sensitizes the immature brain to hypoxic-ischaemic injury. Eur J Neurosci 2001;13:1101–6.
44. Coumans ABC, Middelanis J, Garnier Y, et al. Intracisternal application of endotoxin enhances the susceptibility to subsequent hypoxic-ischemic brain damage in neonatal rats. Pediatr Res. 2003;53:770–5.
45. Dawson DA, Furuya K, Gotoh J, Nakao Y, Hallenbeck JM. Cerebrovascular hemodynamics and ischemic tolerance: Lipopolysaccharide-induced resistance to focal cerebral ischemia is not due to changes in severity of the initial ischemic insult, but is associated with preservation of microvascular perfusion. J Cereb Blood Flow Metab 1999;19:616–23.
46. Mallard EC, Williams CE, Gunn AJ, Gunning MI, Gluckman PD. Frequent episodes of brief ischemia sensitize the fetal sheep brain to neuronal loss and induce striatal injury. Pediatr Res 1993;33:61–5.
47. Garnier Y, Coumans A, Jensen A, Berger R, Hasaart THM. Endotoxemia severely affects circulation during normoxia and asphyxia in immature fetal sheep. J Soc Gynecol Investig 2001; 8:134–42.
48. Reilly FD, Russel PT. Neurohistochemical evidence supporting an absence of adrenergic and cholinergic innervation in the human placenta and umbilical cord. Anat Rec 1977;188:277–86.
49. Hemsen A. Biochemical functional characterization of endothelin peptides with special reference to vascular resistance. Acta Physiol Scand 1991;602(Suppl):1–61.
50. Hemsen A, Gillis C, Larsson O, Haegerstrand A, Lundberg JM. Characterization, localization and actions of endothelins in umbilical vessels and placenta of man. Acta Physiol Scand 1991; 143:395–404.
51. van Huisseling H, Muijers GJJM, de Haan J, Hasaart THM. Fetal hypertension induced by norepinephrine infusion and umbilical artery flow velocity waveforms in fetal sheep. Am J Obstet Gynecol 1991;165:450–5.
52. Paulick RP, Meyers RL, Rudolph CD, Rudolph AM. Umbil-

ical and hepatic venous responses to circulating vasoconstrictive hormones in fetal lamb. Am J Physiol 1991;260:H1205–13.
53. Ashwal S, Dale PS, Longo LD. Regional cerebral blood flow: Studies in the fetal lamb during hypoxia, hypercapnia, acidosis, and hypotension. Pediatr Res 1984;18:1309–16.
54. Jensen A, Berger R. Fetal circulatory responses to oxygen lack. J Dev Physiol 1991;16:181–207.
55. Rees S, Stringer M, Just Y, Hooper SB, Harding R. The vulnerability of the fetal sheep brain to hypoxemia at mid-gestation. Brain Res Dev Brain Res 1997;103:103–18.
56. Rees S, Breen S, Loeliger M, McCrabb G, Harding R. Hypoxemia near mid-gestation has long term effects on fetal brain development. J Neuropathol Exp Neurol 1999;58:932–45.
57. Penning DH, Grafe MR, Hammond R, Matsuda Y, Patrick J, Richardson B. Neuropathology of the near-term and midgestation ovine fetal brain after sustained in utero hypoxemia. Am J Obstet Gynecol 1994;170:1425–32.
58. Matsuda T, Okuyama K, Cho K, et al. Induction of antenatal periventicular leukomalacia by hemorrhagic hypotension in the chronically instrumented fetal sheep. Am J Obstet Gynecol 1999;181:725–30.
59. Jensen A, Lang U, Braems G. Cardiovascular effects of endotoxin and asphyxia in fetal sheep near term. In: Künzel W, Kirschbaum M, eds. Oxygen: Basis of the regulation of vital functions in the fetus. New York, Berlin: Springer, 1992:156–7.
60. Duncan JR, Cock ML, Scheerlinck JP, et al. White matter injury after repeated endotoxin exposure in the preterm ovine fetus. Pediatr Res 2002;52:941–9.
61. Mallard C, Welin AK, Peebles D, Hagberg H, Kjellmer I. White matter injury following systemic endotoxemia or asphyxia in the fetal sheep. Neurochem Res 2003;28:215–23.
62. Gardiner SM, Compton AM, Bennet T, Palmer RMJ, Moncada S. Control of regional blood flow by endothelium derived nitric oxide. Hypertension 1990;15:486–92.
63. Green LR, Bennet L, Hanson MA. The role of nitric oxide synthesis in cardiovascular responses to acute hypoxia in the late gestation sheep fetus. J Physiol 1996;497:271–7.
64. Harris AP, Helou S, Gleason CA, Traystman RJ, Koehler RC. Fetal cerebral and peripheral circulatory responses to hypoxia after nitric oxide synthase inhibition. Am J Physiol Regul Integrative Comp Physiol 2001;281:R381–90.
65. Iadecola C, Pelligrino DA, Moscowitz MA, Lassen NA. Nitric oxide synthase inhibition and cerebrovascular regulation. J Cereb Blood Flow Metab 1994;14:175–92.
66. Santos AC, Yun EM, Bobby PD, Noble G, Arthur GR, Finster M. The effects of bupivacaine, L-nitro-L-arginine-methyl ester, and phenylephrine on cardiovascular adaptations to asphyxia in the preterm fetal lamb. Anesth Analg 1997;85:1299–306.
67. Northington FJ, Koehler RC, Traystman RJ, Martin LJ. Nitric oxide synthase 1 and nitric oxide synthase 3 protein expression is regionally and temporally regulated in fetal brain. Brain Res Dev Brain Res 1996;95:1–14.
68. Northington FJ, Tobin JR, Harris AP, Traystman RJ, Koehler RC. Developmental and regional differences in nitric oxide synthase activity and blood flow in the sheep brain. J Cereb Blood Flow Metab 1997;17:109–15.
69. Smolich JJ. NO modulates fetoplacental blood flow distribution and whole body oxygen extraction in fetal sheep. Am J Physiol 1998;274:R1331–7.
70. Coumans ABC, Garnier Y, Supcun S, Jensen A, Hasaart THM, Berger R. The role of nitric oxide on fetal cardiovascular control during normoxia and acute hypoxia in 0.75 gestation sheep. J Soc Gynecol Investig 2003;10:275–82.
71. Szabo C, Mitchell JA, Thiemermann C, Vane JR. Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. Br J Pharmacol 1993;108:786–92.
72. Thiemermann C, Vane J. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. Eur J Pharmacol 1990;182:591–5.
73. Hobbs AJ, Higgs A, Moncada S. Inhibition of nitric oxide synthase as a potential therapeutic target. Annu Rev Pharmacol Toxicol 1999;39:191–220.
74. Thiemermann C, Wu CC, Szabo C, Perretti M, Vane JR. Role of tumour necrosis factor in the induction of nitric oxide synthase in a rat model of endotoxin shock. Br J Pharmacol 1993;110:177–82.
75. Szabo C, Thiemermann C, Wu CC, Perretti M, Vane JR. Attenuation of the induction of nitric oxide synthase by endogenous glucocorticoids accounts for endotoxin tolerance in vivo. Proc Natl Acad Sci U S A 1994;91:271–5.
76. Kido T, Sawamura T, Hoshikawa H, et al. Processing of proendothelin-1 at the C-terminus of big endothelin-1 is essential for proteolysis by endothelin-converting enzyme-1 in vivo. Eur J Biochem 1997;244:520–6.
77. Xu D, Emoto N, Giaid A, et al. ECE-1: A membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. Cell 1994;78:473–85.
78. Matsumura Y, Hisaki K, Takaoka M, Morimoto S. Phosphoramidon, a metalloproteinase inhibitor, suppresses the hypertensive effect of big endothelin-1. Eur J Pharmacol 1990;185: 103–6.
79. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–5.
80. Elton TS, Oparil S, Taylor GR, et al. Normobaric hypoxia stimulates endothelin-1 gene expression in the rat. Am J Physiol Regul Integrative Comp Physiol 1992;263:R1260–4.
81. Li H, Chen SJ, Chen YF, et al. Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. J Appl Physiol 1994;77:1451–9.
82. Morel DR, Lacroix JS, Hemsen A, Steinig DA, Pittet JF, Lundberg JM. Increased plasma and pulmonary lymph levels of endothelin during endotoxin shock. Eur J Pharmacol 1989;167: 427–8.
83. Nakamura T, Kasai K, Sekiguchi Y, et al. Elevation of plasma endothelin concentrations during endotoxin shock in dogs. Eur J Pharmacol 1991;205:277–82.
84. Hishikawa K, Nakaki T, Marumo T, Suzuki H, Kato R, Saruta T. Pressure enhances endothelin-1 release from cultured human endothelial cells. Hypertension 1995;25:449–52.
85. Kuchan MJ, Frangos JA. Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. Am J Physiol Heart Circ Physiol 1993;264:H150–6.
86. Docherty CC, Kalmar-Nagy J, Engelen M, Nathanielsz PW. Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep. Am J Physiol Regul Integrative Comp Physiol 2001;280:R554–62.
87. Luscher TF, Yang Z, Tschudi M, et al. Interaction between endothelin-1 and endothelium derived relaxing factor in human arteries and veins. Circ Res 1990;66:1088–94.
88. Battistini B, Forget MA, Laight D. Potential roles for endothelins in systemic inflammatory response syndrome with a particular relationship to cytokines. Shock 1996;5:167–83.
89. Pittet JF, Morel DR, Hemsen A, et al. Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. Ann Surg 1991;213:261–4.
90. Volk T, Kox WJ. Endothelium function in sepsis. Inflamm Res 2000;49:185–98.
91. Wanecek M, Weitzberg E, Rudehill A, Oldner A. The endothelin system in septic and endotoxin shock. Eur J Pharmacol 2000;407:1–15.

92. Kaszaki J, Wolfard A, Boros M, Baranyi L, Okada H, Nagy S. Effects of antiendothelin treatment on the early hemodynamic changes in hyperdynamic endotoxemia. Acta Chir Hung 1997; 36:152–3.
93. Pernow J, Hemsen A, Lundberg JM. Increased plasma levels of endothelin-like immunoreactivity during endotoxin administration in the pig. Acta Physiol Scand 1989;137:317–8.
94. Weitzberg E, Ahlborg G, Lundberg JM. Long-lasting vasoconstriction and efficient regional extraction of endothelin-1 in human splanchnic and renal tissues. Biochem Biophys Res Commun 1991;180:1298–303.
95. Weitzberg E, Ahlborg G, Lundberg JM. Differences in vascular effects and removal of endothelin-1 in human lung, brain, and skeletal muscle. Clin Physiol 1993;13:653–62.
96. Lundberg JM, Ahlborg G, Hemsen A, et al. Evidence for release of endothelin-1 in pigs and humans. J Cardiovasc Pharmacol 1991;17(Suppl):350–3.
97. Adamson SL, Whiteley KJ, Langille BL. Endothelin-1 constricts fetoplacental microcirculation and decreases fetal $O_2$ consumption in sheep. Am J Physiol 1996;270:H16–23.
98. Bigatello LM, Zapol WM. New approaches to acute lung injury. Br J Anaesth 1996;77:99–109.
99. Curzen NP, Mitchell JA, Jourdan KB, Griffiths MJ, Evans TW. Endothelin-1-induced contraction of pulmonary arteries from endotoxemic rats is attenuated by the endothelin-A receptor antagonist, BQ123. Crit Care Med 1996;24:2007–13.
100. Wanecek M, Oldner A, Rudehill A, Sollevi A, Alving K, Weitzberg E. Cardiopulmonary dysfunction during porcine endotoxin shock is effectively counteracted by the endothelin receptor antagonist bosentan. Shock 1997;7:364–70.
101. Black SM, Johengen MJ, Soiffer SJ. Coordinated regulation of genes of the nitric oxide and endothelin pathways during the development of pulmonary hypertension in fetal lambs. Pediatr Res 1988;44:821–30.
102. Wong J, Vanderford PA, Fineman JR, Chang R, Soiffer SJ. Endothelin-1 produces pulmonary vasodilation in the intact newborn lamb. Am J Physiol 1993;265:H1318–25.
103. Wong J, Vanderford PA, Fineman JR, Soiffer SJ. Developmental effects of endothelin-1 on the pulmonary circulation in the intact newborn lamb. Pediatr Res 1994;36:394–401.
104. Filep J. Endothelin peptides: Biological actions and pathophysiological significance in the lung. Life Sci 1993;52:119–33.
105. Helset E, Ytrehus K, Tveita T, Kjaeve J, Jorgensen L. Endothelin-1 causes accumulation of leukocytes in the pulmonary circulation. Circ Shock 1994;44:201–9.
106. Cheng TH, Shih NL, Chen SY, Wang DL, Chen JJ. Reactive oxygen species modulate endothelin-I-induced c-fos gene expression in cardiomyocytes. Cardiovasc Res 1999;41:654–62.
107. Fink M. Cytopathic hypoxia in sepsis. Acta Anaesthesiol Scand 1997;41:87–95.
108. Schmeck J, Heller A, Phan TL, Urbschek R, Koch T. Effects of endothelin-1 on bacterial clearance in rabbits. Eur J Anaesthesiol 1999;16:169–75.