Endotoxin-Induced Circulatory Changes in the Newborn Brain: A Review

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Abstract
Endotoxin induces complex changes in the newborn cerebral circulation, including vasoconstriction by down-regulating endothelial nitric oxide synthase and stimulating the production of endothelin and cyclooxygenase-2 derived vasoconstrictors including thromboxane A2, while also promoting vasodilatation by increasing the expression of inducible nitric oxide synthase and cyclooxygenase-2 derived vasodilators. Together, these changes represent a complex dysregulation of the newborn cerebral circulation. This review considers the effects of neonatal endotoxaemia on cerebral circulatory changes and endothelium-derived factors, and their implications for cerebral circulatory control.

Key words
Cerebral blood flow; Endothelium; Endotoxin; Newborn

Introduction
Due to deficiencies in the host defense systems, neonates are particularly vulnerable to bacterial infections. Neonatal infection has been reported to affect 6.6 per 1000 live births in Australia. It has become an increasing problem in neonatal intensive care units with an overall rate of 21.3 infected infants or 29.9 infections per 100 ventilated admissions since the late 1990s. Endotoxaemia, a systemic inflammatory response to endotoxin is one of the major causes of morbidity and mortality in the newborn.

Endotoxin is the lipopolysaccharide (LPS) outer coat of all Gram-negative bacteria. It consists of three main parts: an outer branched chain polysaccharide portion (the O antigen), a mid portion R antigen polysaccharide core; and an inner toxic lipid A portion which accounts for the majority of the toxicity of endotoxin. Normally, only small amounts of naturally occurring endotoxin are released systemically, and these play an important role in the development of host immune responses. Excessive amounts of LPS are released when the bacterial cell wall breaks down in conditions such as infection, immune disturbances resulting in the ‘overgrowth’ of pathogenic bacteria, and when bacteria are killed by bactericidal antibiotics.

Among endotoxin-induced haemodynamic changes, blood pressure instability is a common feature observed in septic neonates in neonatal intensive care units. A reduced responsiveness to vasoconstrictor stimuli associated with decreased systemic vascular resistance and mean blood pressure had been observed in humans following Gram-negative bacteraemia. Furthermore, endotoxin-induced septic shock might not be associated with a positive blood culture. Endotoxin can cause not only systemic hypotension, but also multiple organ dysfunction in the newborn due to dysregulation of the systemic circulation, such as myocardial, respiratory and renal failure and coagulopathy.
**Effect of Endotoxins on the Systemic Endothelium and Circulation**

The endothelium plays a critical role in blood pressure and blood flow regulation by releasing vasodilators such as nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) derived vasodilator factors, as well as vasoconstrictors, such as endothelin (ET) and COX-2 derived vasoconstrictors including thromboxane A₂ (TXA₂). During infection, endotoxin stimulates widespread endothelial damage by interacting with soluble CD14, a surface receptor of mononuclear cells. The LPS-binding protein complex together with CD14 promote mononuclear phagocytes to produce reactive oxygen molecules, cytokines (such as tumour necrosis factor-α and interleukins-1) and prostaglandin metabolites. The complex also induces endothelial cell apoptosis. Following LPS administration, histological examinations show significant endothelial cell disruption, such as areas of denuded endothelium, cytoplasmic swelling and fragmentation. These anatomical characteristics of endothelial cell dysfunction are in keeping with functional impairment which have been demonstrated in patients with sepsis, and in animal models of endotoxin-induced septic shock. Functional impairments such as endothelium-dependent vasodilator responses to LPS vary in different organs. For example, they are unchanged in rat renal arteries but increased in rat aorta. In the brain, vascular resistance is increased by a combination of vasoconstriction as well as vasodilatation (see below).

**Endothelial NO Synthase (eNOS)**

eNOS is stimulated via the shear stress in the endothelial cells produced by blood flow and by various receptor agonists. Under normal basal conditions, eNOS synthesises endothelial NO, which plays important roles in endogenous vasodilatation counterbalancing the vasoconstriction produced by the sympathetic nervous system and the renin-angiotensin system, and in anti-thrombosis by inhibiting platelet aggregation and adhesion to the vascular wall. During sepsis, the constitutive isoforms of NOS including eNOS and neuronal NO synthase (nNOS) are downregulated or even inactivated. Inducible NO Synthase (iNOS) In sepsis, the constitutive isoforms of NOS including eNOS and neuronal NO synthase (nNOS) are downregulated or even inactivated.

**Inducible NO Synthase (iNOS)**

iNOS has the ability to synthesise NO, but it is not present in significant amounts under normal conditions. iNOS is induced by a wide range of factors, including cellular products of endotoxin, Gram-positive bacteria and proinflammatory cytokines in a variety of cell types including macrophages, vascular smooth muscle cells, astrocytes, and neurons. Induction of iNOS reduces NO-mediated vasodilatation initiated by the other two major NOS, i.e. eNOS and nNOS, and NO inhibitors were able to reverse LPS-induced hypotension in animals, so that over-production of NO by iNOS for prolonged period of time appears primarily responsible for endotoxin-induced hypotension.

**Endothelin (ET)**

ET is the most potent, long-acting vasoconstrictor secreted from endothelial cells upon mechanical stimuli. There are three isopeptides of ET: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). ET-1 is normally produced by endothelium. Because of ET-1’s affinity for endothelin-A (ETₐ) receptors, its vasoconstriction is eight times stronger than ET-2, and 1000 times stronger than ET-3. ET-1 is believed to play a key role in vasoconstriction during sepsis, increasing at 4-12 hours and returning to normal at 24-48 hours. ET-1 may be elevated as early as 15 minutes after peripheral injection of tumour necrosis factor-α (TNF-α). The significantly increased plasma ET-1 levels in sepsis may explain the increased vascular resistance and decreased organ blood flow under such conditions. On the other hand, the activation of ETₐ receptors by increasing ET-1 concentration also enhances vasodilatation via stimulated iNOS expression and NO production in systemic vessels, contributing to LPS-induced hypotension in both human and animal studies. Furthermore, elevated plasma ET-1 is related to the severity of clinical manifestations, especially oliguria, acidosis and systemic hypotension in neonatal sepsis.

**Cyclooxygenase-2 (COX-2) and Prostaglandins**

Cyclooxygenase is an enzyme involved in the endothelial production of prostaglandins, which is present in constitutive cyclooxygenase-1 and inducible COX-2 isoforms. COX-2 though present in resting human endothelial cells, it does not appear to regulate basal systemic vascular tone. Following an inflammatory response induced by LPS, reactive oxygen species, some cytokines, and growth factors, COX-2 expression is up-regulated. Functionally, infusion of COX-2 altered regional blood flow. The production of prostaglandins increases after COX-2 is expressed. The main prostaglandins produced during inflammation are the vasodilators PGE₂, PGI₂ and PGD₂. These are potent...
dilators whereas other prostaglandins, such as PGF\textsubscript{2\alpha} and TXA\textsubscript{2} act as vasoconstrictors.\textsuperscript{47} TXA\textsubscript{2} can also cause platelet aggregation.

Apart from systemic effects, LPS can also induce subsequent hypoxic ischaemic damage in the neonatal rat brain.\textsuperscript{48} The exact mechanisms regarding the changes in cerebral blood flow (CBF) and cerebral vascular resistance (CVR) remain unclear.

**Effects of Endotoxins on the Cerebral Endothelium and Circulation**

Brain infection has the potential to substantially modify the expression of genes that have crucial roles in cerebral vascular regulation. By injecting group B streptococcus (GBS), a Gram-positive bacteria, into the cerebral ventricle of newborn piglets, the upper limit of cerebral vascular autoregulation is impaired within the first 2 hours post-injection. This autoregulatory change correlates well with the induction of NOS gene expression.\textsuperscript{49} Both eNOS and COX-2 were down-regulated post-GBS treatment in the brain of newborn piglets.\textsuperscript{50}

Systemic injected LPS does not cross the capillary endothelium of the central nervous system, that is, the blood-brain barrier,\textsuperscript{51} but it increases blood-brain barrier permeation by binding to LPS receptors such as toll-like receptors (TLR) TLR-4 and TLR-2, as well as CD14 in the brain-endothelial cells.\textsuperscript{52} This activates a cascade of events involving proinflammatory genes such as IL-1\textbeta and iNOS genes, which, in turn, lead to inflammation\textsuperscript{51} and microglia activation in the brain.\textsuperscript{53} It also causes a capillary leak and diffusion of low molecular weight proteins from cerebral vessels into the cerebral spinal fluid, leading to cerebral oedema and increased intracranial pressure.\textsuperscript{51}

The endotoxin-induced alterations of the cerebral circulation vary in different studies, perhaps reflecting species differences. LPS dilates cerebral arterioles in rabbit,\textsuperscript{54} but vasoconstricts large arteries, such as the middle cerebral artery (MCA) in rats.\textsuperscript{47} CBF responses to LPS are complex. There have been reports of no change in sepsis-induced normotensive rats\textsuperscript{55} and sheep.\textsuperscript{56} In contrast, an increased regional CBF was recorded by laser-Doppler flowmetry without a fall in blood pressure within 4 hours of intracerebro-ventricular injection of LPS (1 mg/kg) in rats anaesthetised by halothane inhalation.\textsuperscript{52} Increased CBF has also been reported in ovine models of sepsis.\textsuperscript{57} On the other hand, by using an autoradiographic technique employing \textsuperscript{14}C-iodoantipyrine, Young et al showed endotoxin-induced hypotension with reduced rCBF in periventricular and occipital white matter in newborn dogs.\textsuperscript{58}

By applying an electromagnetic probe around the intact superior sagittal sinus or measuring directly from the cannulated confluence of the sagittal, straight and lateral sinuses, a reduction in CBF with increased CVR during the first hour of endotoxin shock were detected in anaesthetised dogs.\textsuperscript{59}

At Monash University, we have studied the cerebral haemodynamics and the neutrophil and platelet counts in conscious newborn lambs at 1-4 weeks of age following a single LPS infusion (2 \textmu g/kg). Endotoxaemia in the lambs resulted in a marked reduction of neutrophils and platelet counts by 73% and 19% from baseline respectively soon after LPS infusion (Figure 1). These results in lambs are compatible with the findings in LPS-treated neonatal calves.\textsuperscript{60}

![Figure 1](image_url)
We used a transonic flow probe placed over the sagittal sinus to measure CBF. This method not only allows continuous recording of the blood flow, but also provides a simple quantitative measurement of CBF that is linearly related to arterial inflow. The technique measures blood flow of the entire frontal lobe and the superior portion of the anterior parietal lobe, which represents 35% of the total brain mass of the lamb. We demonstrated that CBF was reduced by 33% and CVR was increased by 129% despite no reduction in blood pressure during the first 2-4 hour post-LPS (Figure 1).

**Cerebral Vasconstriction**

The possible mechanisms of endothelial-mediated, LPS-induced cerebral circulatory impairment are an alteration of vasoconstrictor and vasodilator factors:

1. **eNOS down-regulation.** LPS-induced cerebral endothelium impairment may lead to disruption of eNOS, impairment of cerebral vasodilatation, and reduction in CBF. Furthermore, induction of iNOS by LPS reduces NO-mediated vasodilatation initiated by eNOS in cerebral arteries and enhances the effect on cerebral vasoconstriction.

2. **ET-1 upregulation.** ET-1 mRNA can be detected in cerebral endothelium. Its production is increased by TNF-α, and vascular smooth muscle cells. It produces potent and long-lasting contraction of cerebral vessels, which is mediated by ETA receptors both in vivo and in vitro. ET-1 can also reduce cerebral artery sensitivity to NO by a protein kinase C-independent pathway, and therefore enhances cerebral vasoconstriction.

3. **COX-2 induction.** Intravenous LPS or proinflammatory cytokines increases expression of COX-2 not only in brain neuron including hippocampus, cerebral cortex, amygdalae and hypothalamus, but also in the cerebral endothelium. Injection of NS-398, a COX-2 inhibitor, can inhibit vascular contraction of the MCA from the first hour in LPS-treated rats, suggesting vasoconstrictors derived from COX-2 are involved in early endotoxin-induced cerebral vasoconstriction. As these changes occur within one hour of LPS injection, they have the potential to explain the cerebral vasoconstriction that is illustrated in Figure 1.

4. **Proinflammatory cytokines.** Inhibition of endothelium-dependent relaxation following treatment with proinflammatory cytokines has been shown in peripheral arteries. Proinflammatory cytokines such as TNF-α was found to be increased in septic neonates. It was also found in post-mortem brain tissue from patients suffering from bacterial meningitis. Moreover, TNF-α has been found to reduce cerebral blood volume by 15-30%, and causes cerebral vasoconstriction via an endothelin- and TNF-α-type-2 receptor dependent pathway.

**Cerebral Vasodilatation**

1. **iNOS.** iNOS expression increases after endotoxin exposure in the MCA in rats around 5 hours post-LPS, and may be responsible for subsequent reduction in vascular resistance and systemic hypotension. However, in our own observations, CVR increased but hypotension did not develop. Therefore, iNOS induction was not a mitigating factor in the early vasoconstriction responses.

2. **COX-2 derived vasodilators.** LPS not only induces COX-2 expression causing vasodilatation in systemic circulation, but also increases COX-2 expression in brain tissue. LPS application induces PGE₂-dependent dilatation of cerebral arterioles, with the maximum dilatation occurring at 4 hours. It is possible that LPS induces cyclooxygenase which in turn may also produce reactive oxygen species which are vasodilators in the brain.

**Anaesthetic Agents**

These should be taken into account when comparing experimental results between anaesthetised and conscious animals. For example, halothane produces a dose-dependent vasodilatation of intraparenchymal cerebral microvessels, which is similar to those of the potent vasodilator sodium nitroprusside, an endothelium dependent NO donor.

**Clinical Implications**

Our experimental data demonstrating that a reduced CBF and increased cerebral vasoconstriction occur in normotensive lambs post-LPS administration, suggest that impaired cerebral perfusion is possible despite normal blood pressure during the early stages of endotoxaemia in the newborn. Thus, disturbances in cerebral perfusion should be considered in managing the septic newborn, even when the blood pressure remains in the normal range.

Potential treatments including anti-LPS antibody, NOS inhibitors, endothelin-1 (ET-1) antagonists, and proinflammatory cytokine blockers are still under experimental study, but they are thought to be an important potential additions to future haemodynamic therapy in
septic shock.

In conclusion, endotoxin-induced cerebral circulatory changes in the newborn may be altered by a series of factors following proinflammatory cytokines mediation. By disrupting the balance of vasoconstrictor and vasodilator factors, cerebral vasoconstriction and flow impairment become the net results in the early stage of neonatal endotoxaemia.

References

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