Cerebral vascular regulation and brain injury in preterm infants

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Brew N, Walker D, Wong FY. Cerebral vascular regulation and brain injury in preterm infants. Am J Physiol Regul Integr Comp Physiol 306: R773–R786, 2014. First published March 19, 2014; doi:10.1152/ajpregu.00487.2013.—Cerebrovascular lesions, mainly germinal matrix hemorrhage and ischemic injury to the periventricular white matter, are major causes of adverse neurodevelopmental outcome in preterm infants. Cerebrovascular lesions and neuromorbidity increase with decreasing gestational age, with the white matter predominantly affected. Developmental immaturity in the cerebral circulation, including ongoing angiogenesis and vasoregulatory immaturity, plays a major role in the severity and pattern of preterm brain injury. Prevention of this injury requires insight into pathogenesis. Cerebral blood flow (CBF) is low in the preterm white matter, which also has blunted vasoreactivity compared with other brain regions. Vasoreactivity in the preterm brain to cerebral perfusion pressure, oxygen, carbon dioxide, and neuronal metabolism is also immature. This could be related to immaturity of both the vasculature and vasoactive signaling. Other pathologies arising from preterm birth and the neonatal intensive care environment itself may contribute to impaired vasoreactivity and ineffective CBF regulation, resulting in the marked variations in cerebral hemodynamics reported both within and between infants depending on their clinical condition. Many gaps exist in our understanding of how neonatal treatment procedures and medications have an impact on cerebral hemodynamics and preterm brain injury. Future research directions for neuroprotective strategies include establishing bedside, real-time clinical reference values for cerebral hemodynamics and vasoregulatory capacity and to demonstrate that these thresholds improve long-term outcomes for the preterm infant. In addition, stimulation of vascular development and repair with growth factor and cell-based therapies also hold promise.

cerebral blood flow; cerebral vasoreactivity; infants; newborn; preterm brain injury

Understanding How Cerebral Blood Flow Is Regulated in the Preterm Brain

With many infants now born preterm at 24–32 wk of gestation (117), a full understanding of the physiological regulation of cerebral blood flow (CBF) in the immature brain is becoming increasingly important. Survival of infants born extremely preterm (<28 wk gestation) has increased sharply (50–70%) over the last two decades (38, 42), but this has been accompanied by higher rates of neurodevelopmental disability, which exceed 50% in most studies (42, 127, 218), and at least a quarter of survivors develop substantial neurological morbidity, such as cerebral palsy (170). Most follow-up studies of extremely and very preterm infants (<32 wk gestation) show sequelae, such as cognitive deficits, academic underachievement, and the need for increased remedial assistance during mid-childhood and adolescence (169). Extremely and very preterm birth contributes disproportionately to the medical care costs of prematurity, not only because of the intensive medical care required after birth, but also because survivors have disproportionately high rates of disabling conditions that generate high lifetime medical care costs (1, 167).

The pathological correlates of neurodevelopmental disability with preterm birth include various cerebral lesions, most notably periventricular leukomalacia (PVL) and germinal matrix-intraventricular hemorrhage (GM-IVH) (132, 223). Such lesions are present from early in the neonatal period, as shown by cranial ultrasound or magnetic resonance imaging (MRI), and they contribute significantly to neonatal mortality, including deaths due to the decision to withdraw or limit life support for poor neurological prognosis (40).

Although the etiology of cerebral lesions in the preterm infant is complex, it is generally accepted that hypoxia and poor cerebral perfusion lead to some regions of the preterm brain and, for reasons to be discussed, particularly white matter, falling into a condition best described as hypoxic-ischemia (94). Therefore, as neuromorbidity increases in severity with decreasing gestational age (170), it is not unreasonable to think that immaturity in the regulation of the cerebrovascular circulation may be a prime factor in determin-
Reviewing the severity of preterm brain injury. However, effective brain-oriented neonatal intensive care is still impeded by two major obstacles: 1) the lack of clinically relevant bedside monitoring of cerebral perfusion and oxygenation; and 2) a lack of knowledge about whether maintaining the cerebral hemodynamic parameters within a "normal" range would translate into improved outcomes for preterm infants. Current understanding of how the circulation of the immature brain is regulated is based in large part on data from experimental fetal animal models (13, 143) and studies of preterm infants, in which, necessarily, measurements are made indirectly and noninvasively. Although both approaches have provided important insights, gaps remain in our knowledge of the normal and abnormal regulation of CBF in the preterm brain.

The aim of this review is to integrate information on the regulation of cerebral hemodynamics and oxygenation in the preterm brain, and to discuss the relevance of such findings for the management of the preterm infant at risk of hypoxic-ischemic brain injury. This review will also discuss the challenges and directions for informed "brain-oriented" intensive care of preterm infants and possible new treatments to minimize cerebrovascular lesions.

Techniques for Noninvasive Measurement of CBF in the Human Brain

Clinically, investigation of CBF in infants is constrained by the necessity of using noninvasive techniques. Considerable insight into the cerebral circulation of preterm infants has been provided by application of the techniques summarized in Supplemental Table S1. Each of these techniques has its own merits and limitations regarding use in newborn infants.

Regulation of Cerebral Hemodynamics

Cerebral vascular development in the preterm brain. The developmental immaturity of the cerebral vasculature has long been thought to determine the regional vulnerability of the preterm brain to hypoxia-ischemia (94). Accordingly, cerebral vascularization and vasoregulation play a part in determining the differing patterns of cerebral injury that occur in preterm infants born between 24 and 36 wk of gestational age.

Anatomical Development. Development of the cerebral vasculature is closely associated with structural development of the brain and involves a tightly regulated process of vasculogenesis and angiogenesis. In the human, arterial development is completed earlier in the brain stem and cerebellum (between 24 and 36 wk of gestation), and then in the basal ganglia and diencephalon by 28 wk of gestation, and lastly in the cortex and germinal matrix. Initially, a single vascular plexus covers the cerebral surface. Thinner penetrating vessels descend from the cerebral surface, and by 24 wk of gestation, long penetrating vessels extend from the pial surface down into the periventricular regions, forming arterial end zones. Numerous growth factors are implicated in the vascular development in the brain. Among these, vascular endothelial growth factor (VEGF) is a key regulator of both vasculogenesis and angiogenesis, and expression of this hypoxia-responsive gene is critical for endothelial proliferation and migration, and in determining solute permeability in these nascent vascular conduits (55). For example, in mouse embryos, formation of pial collaterals, which provide an alternative route around a vascular obstruction and determine severity of ischemic conditions, requires VEGF stimulation of liver kinase 1-Notch signaling during a narrow developmental window (113). The formation of collateral arteries early in brain development is a determinant of their presence in the adult brain, such that disturbance of collaterogenesis results in a life-long deficiency (113), and potentially increased risk of stroke and cerebral ischemia in adult life.

Angiogenesis and Neurogenesis. Angiogenesis and neurogenesis utilize similar molecular cues to guide migration and development of endothelial cells, neurons and axons (102). For example, the temporal and spatial expression of VEGF in the developing brain is closely correlated with vascularization and endothelial cell growth. Increased VEGF expression in many cell types (e.g., neuroblasts, neuroepithelial cells, radial glia, astrocytes, pericytes, and endothelial cells) coincides with increased neuronal metabolism, which stimulates angiogenesis (12, 174, 205). Disruption of neuronal activity by chronic excessive sensory stimulation and chemically induced seizures decreases angiogenesis in neonatal but not adult mice (213). Endothelial cells provide a microenvironment supporting the maintenance and proper differentiation of neural progenitor cells (194), as shown by in vitro co-culture systems. For example, when neural stem cells are co-cultured with endothelial cells, they develop greater self-renewal activity followed by extensive neurogenesis (179).

Germinal Matrix. Angiogenesis is especially active in the germinal matrix in the preterm brain. The germinal matrix is located in the thalamostriate groove and contains a richly vascularized collection of neural progenitor cells. Endothelial cell proliferation, VEGF and angiopoietin-2 expression are substantially increased in the preterm human germinal matrix relative to the white matter and cortex (16). This enhanced angiogenesis is associated with less astrocyte end-feet and pericyte capillary coverage in the germinal matrix vasculature than in the cortical gray or white matter in human fetuses and preterm infants (25, 47). Consequently this is thought to result in a more fragile vasculature with increased propensity for mechanical rupture and bleeding. Indeed, GM-IVH is relatively common in preterm infants, and predisposes them to long-term morbidities, including hydrocephalus, cerebral palsy, and impaired neurodevelopment (45).

Cerebral blood flow in the preterm brain. Low global CBF. A dramatic change in CBF occurs at birth. In the newborn lamb CBF decreases to approximately one-third (relative to the term fetus) in the first 24 h after birth (164) and arterial oxygen content increases by more than 50% (61). In the preterm human infant, low CBF (Supplemental Table S2) and high cerebral oxygen extraction (COE) immediately after birth have been reported in several studies (64, 69, 153). Using near-infrared spectroscopy (NIRS) in extremely preterm infants, CBF values between 5 and 17 ml·100g⁻¹·min⁻¹ were measured on the first day of life (121, 133, 219), comparable to (or even less than) the minimal CBF estimated as necessary to maintain neuronal viability and metabolism in the adult brain (15 ml·100g⁻¹·min⁻¹) (17). Postnatally, CBF then rises over the first few days of life (121), typically to a
Cerebral Vasoreactivity in the Preterm Brain

Cerebral vascular resistance is fundamentally regulated by the muscularis layer of the arterioles which, during brain development, is initially confined to the pial vessels and superficial penetrators, before descending into the cerebral parenchyma with further maturation (130). Consequently, cerebral vasoreactivity occurs predominantly in the superficial and peripheral regions of the preterm brain (Fig. 1), remote from the distal vascular beds, with the deeper vessels remaining relatively passive (44). This spatial restriction of vasoreactivity of the immature cerebral circulation is likely to contribute to the pattern of injury—most often, of the white matter—observed in the very preterm brain.

It is worth noting that regardless of the stimulus, the cerebral vascular response is dependent on resting tone and diameter, so that vessels already dilated by hypercarbia or hypoxia have limited capacity for further vasodilation in response to additional stimulation, such as systemic hypotension, a relatively common occurrence in preterm infants. Although there are numerous physiological and pathologic stimuli capable of eliciting cerebrovascular responses, the most important ones are the following.

Pressure autoregulation. Cerebral autoregulation refers to the maintenance of a relatively constant CBF over a broad range of perfusion pressures (104), the so-called “autoregulatory plateau”. The autoregulatory plateau in the adult human operates over mean blood pressures ranging from ~60 to 150 mmHg, and it takes 10–90 s for CBF to stabilize after a change of systemic blood pressure (27). Autoregulation is not an all-or-none phenomenon, as the plateau usually has a small positive slope. In preterm infants, the plateau range and the position of lower and upper limits have been suggested to be much narrower and lower, but importantly, this varies significantly between and within individuals for reasons that are not yet fully understood (182, 221).

MECHANISMS OF PRESSURE AUTOREGULATION. Several hypotheses have been proposed for the mechanisms that underlie autoregulation. One is that autoregulation is the direct result of a myogenic reflex (Fig. 1A), acting via a calcium-dependent pathway in smooth muscle in response to changes of transmural pressure, (and presumably wall tension), caused by changes in perfusion pressure (192). With decreased transmural pressure, endothelial-derived nitric oxide, carbon monoxide, and calcium-activated potassium channels induce vasodilation (89). Shear-generated endothelial nitric oxide synthase is likely to be the dominant source of nitric oxide to mediate autoregulatory responses (19); for example, rat studies demonstrate that while inhibition of neuronal nitric oxide synthase impairs autoregulation, cerebral vasodilatation during reduced arterial pressure still occurs and vessel wall nitric oxide rises to near-normal levels. The vasoconstrictor effects of nitric oxide synthase inhibition are significantly less in cerebral vessels from young (4–5 wk old) compared with adult rats, indicating that the contribution of endothelial nitric oxide in pressure autoregulation increases with age (115) and, therefore, may be less important in the preterm brain where other mechanisms may dominate. Apart from the pressure-sensitive myogenic mechanism, pressure- and flow-induced vasoreactivity have been proposed to function together in maintaining effective autoregulation. Increased constriction to flow in isolated hu-
man internal carotid cerebral arteries is mediated by 20-hydroxyeicosatetraenoic acid (20-HETE, an arachadonic acid metabolite) acting via thromboxane A2/prostaglandin H2 receptors (200). Notably, many conflicting observations of flow-induced responses in cerebral vessels have been reported, varying from constriction to dilatation to biphasic responses; these differences in observed responses to flow may be dependent upon species, vessel type, and methods used (99), all of which needs to be resolved.

Another proposed mechanism underlying cerebral autoregulation is the metabolic hypothesis, which is that a local reduction in blood flow results in the release of vasoactive factors from neurons or astrocytes, which then elicit dilatation of the adjacent cerebral vessels. The release of adenosine, a known cerebral vasodilator, is increased with reductions in blood pressure and is considered a prime mediator of metabolically regulated autoregulation (216). Other studies suggest that cerebral vasodilatation, following reduced arterial blood pressure, is dependent on reduced oxygen tension within the tissues, which then either activates a local reflex (210), or causes the secondary release of vasodilator metabolites, such as adenosine (100) or nitric oxide (168).

Finally, perivascular nerves may also contribute to the modulation of cerebral autoregulation. Extraparenchymal and intraparenchymal blood vessels are richly innervated by autonomic nerves (111) and have been shown to modify autoregulation. For example, the lower limit of CBF autoregulation is shifted toward higher blood pressure in parasympathetically denervated rats (128), and a similar shift is observed after stimulation of the cerebral sympathetic system (142). In newborn lambs, sympathetic denervation by cervical ganglionectomy results in greater CBF increases associated with blood pressure surges (111). Importantly though, cerebral autoregulation is still functional after cervical ganglionectomy (111, 216).

Fig. 1. A: Major factors contributing to cerebral blood flow (CBF) regulation in newborn infants. Cerebral perfusion pressure, hypercarbia, hypoxia, regional neuronal activation, and immature vascular anatomy (left side of dotted line) are major determinants of CBF in newborns. The vascular tone of cerebral resistance arterioles is modulated by numerous factors, primarily transmural pressure, perivascular pH, neuronal metabolism, as well as numerous vasodilatory molecules, such as potassium ($K^+$), hydrogen ions ($H^+$), carbon monoxide (CO), nitric oxide (NO), prostaglandins, prostanoids, and adenosine. Intracranial pressure and cerebral venous pressure (right side of dotted line) play a more significant role in CBF regulation in the adult brain. B: Neurovascular unit in cerebral resistance arteriole, illustrating relationship between arteriole, interneuron, astrocyte, and neuron-secreted factors on smooth muscle cells, which regulate vascular diameter. A: CO, carbon monoxide; CSF, cerebrospinal fluid; WM, white matter. B: SMC, smooth muscle cell; EC, endothelial cell; Ach, acetylcholine; PGs, prostaglandins; EETs, epoxyeicosatrienoic acids; VIP, vasoactive intestinal polypeptide; CGRP, calcitonin gene related peptide; NA, noradrenaline; DA, dopamine; NPY, neuropeptide Y; EDHF, endothelium derived hyperpolarizing factor; ET, endothelin; GABA, γ-amino butyric acid; ROS, reactive oxygen species.
It has been proposed that under normotensive conditions, myogenic control of cerebral vasomotor tone is the dominant mechanism determining the reactivity of cerebral autoregulation, with neurogenic and metabolic mechanisms having minimal influence. However, when systemic blood pressure is abnormally increased, sympathetic activity is increased commensurately to attenuate the increase in CBF (28, 30).

**Pressure Autoregulation in the Preterm Brain.** Coincident changes in both CBF and arterial blood pressure in preterm infants, which appear to indicate little or no autoregulatory capacity (cerebral pressure-passivity), were initially reported more than 25 years ago; on the basis of which it was proposed that autoregulation was either impaired by preterm birth or was not yet fully developed in the immature brain (112). In either case, it might follow that the immature brain of the preterm infant is particularly susceptible during episodes of hypotension, with this perhaps leading to ischemia, as well as to hemorrhage during periods when blood pressure is either high or fluctuating, all of which are common events in the preterm infant undergoing intensive care.

Studies in fetal lambs showed that autoregulation is present at 0.9 but not at 0.6 of gestation (74); these fetal ages in sheep correspond with brain maturation approximately equivalent to term and 26–28 wk gestation in humans, respectively (162). Despite extensive investigation, it is still not clear when cerebral autoregulation really does develop in the human infant, or what the upper and lower limits of the autoregulatory range actually are. Some studies using NIRS, or electroencephalography detection of reduced cerebral activity (indicative of cerebral hypoperfusion), have found the lower blood pressure limit of the autoregulatory plateau to be between ~23 and 30 mmHg in very preterm infants (129, 203, 212). It is now apparent that the threshold and range of the autoregulatory plateau are quite variable both between and within infants, and that the general clinical condition of the infant, in addition to blood pressure and brain injury, are contributory factors (220, 221). A pressure-passive blood flow relationship is more likely to be present during episodes of hypotension than hypertension, suggesting that baseline MAP may be close to the lower threshold of the autoregulation plateau in very preterm infants (60). Furthermore, when hypotensive preterm infants are treated with inotropic agents, both CBF and MAP increase together (82, 129, 148, 176), indicating that pressure-passivity of the cerebral circulation persists over a range of arterial pressures in these infants.

Other studies suggest that MAP alone is a poor indicator of CBF and autoregulatory capacity in the early postnatal period. While Soul et al. (187) demonstrated an association between systemic hypotension and cerebral pressure-passivity in preterm infants, in fact, the ability to predict the cerebral autoregulatory characteristics from blood pressure values was poor (182). This could be due to variation in the position of the autoregulatory plateau and its upper and lower thresholds within and between infants, and according to changes in their clinical condition (221). Not surprisingly, cross-sectional studies are particularly poor at identifying the characteristics of autoregulation in the human preterm cerebral circulation.

When and how autoregulatory thresholds change with gestational or postnatal age in the preterm infant is not clear. In one study of preterm infants (<1,500 g birth weight), cerebral pressure-passivity (i.e., absence of autoregulation) was identified consistently at different times over the first five postnatal days (182). Cerebral pressure-passivity was also more prevalent in younger preterm infants of lower gestational age (182). Differences in autoregulation related to gestational age were also reported in a study comparing hypotensive term and preterm infants treated with low-dose dopamine. Autoregulation was present in hypotensive term infants who received dopamine therapy because CBF velocity did not increase as MAP increased, whereas in hypotensive preterm infants, CBF velocity increased together with the rise in MAP, indicating the absence of autoregulation (176).

There is clear evidence from fetal sheep of regional variations in autoregulation in the developing brain (189). For example, the brain stem has a lower autoregulatory threshold than the cerebrum, and as a result, a higher tolerance of hypotension. In contrast, CBF in white matter in these fetal sheep is particularly vulnerable to hypotension because autoregulatory adjustment of CBF is weak or nonexistent (189). These studies are consistent with imaging studies in hypotensive preterm infants that show perfusion of periventricular white matter may decrease when systemic blood pressure falls below 29 mmHg (22).

**Carbon dioxide vasoreactivity.** **Mechanisms of Carbon-dioxide Vasoreactivity.** Decreased CBF induced by hyperventilation and hypocapnia was first described 60 years ago in healthy adults (93). The effect of arterial carbon dioxide tension (PaCO₂) on vascular smooth muscle is due to the changes in perivascular pH that occur after CO₂ crosses the blood-brain barrier (Fig. 1A) and equilibrates with intraparenchymal bicarbonate (2). The pH-mediated changes in contractility of the small cerebral vessels result from activation of the electrogenic sodium pump, and ATP-sensitive, voltage-gated potassium channels leading to hyperpolarization of smooth muscle cells (155), inhibition of voltage-dependent calcium channels (2), and the activation and release of numerous secondary vasoactive substances, such as prostanoids, adenosine, and nitric oxide (105, 119, 152, 215). The larger upstream arteries and arterioles seem to respond to nitric oxide release, whereas both adenosine receptor activation and nitric oxide release mediate pial arteriolar responses to increases in CO₂ levels (152). Sympathetic activity may also influence the reactivity of CBF to PaCO₂ (31). Nevertheless, with chronic changes in PaCO₂, CBF returns to baseline over a period of hours as the pH in cerebrospinal fluid, and hence, extracellular fluid is normalized as a result of the action of carbonic anhydrase, and in accordance with the Henderson-Hasselbalch equation (26, 103).

**Carbon-dioxide Vasoreactivity in the Preterm Brain.** While vasoreactivity to PaCO₂ has been shown to be present in the neonatal cerebral circulation, the underlying mechanisms differ somewhat from that in the adult brain in which the role of nitric oxide appears to predominate (Fig. 1A). In newborn piglets and the extremely preterm human brain, nitric oxide has a minor role in CO₂ vasodilatation (140, 190), whereas endothelium-derived prostaglandins, including PGE₂, PGF₁α, and PGF₂α seem to mediate most of the CO₂ vasoreactivity (75, 141, 145). In fetal sheep, CO₂-induced vasodilatation is a robust and nitric oxide-independent response and is present from at least 0.65 gestation (~26–28 wk human) to term (71). In healthy term human infants, CBF changes by ~25% per kPa of PaCO₂ (159), which is similar to the value of 30% per kPa of PaCO₂.
Presumably, this robust vasodilatory response in the preterm brain stem perfusion exceeds cerebellar perfusion, which, in only produces a global increase in CBF in fetal sheep, but despite the response to mediators of hypoxic dilatation, such as relatively underdeveloped state of the cerebral vasculature, arterial oxygen content. This is likely due to the emerging and vasodilatation is sufficient to fully compensate for decreased in humans) (143), although it is not clear whether the hypoxic vasodilation remains unclear, although CBF-PaCO2 reactivity does have an important role in breathing control. Systemic hypocapnia tends to increase CBF and “wash out” CO2 from brain tissue, thereby affecting tissue pH and the central chemoreceptor stimulus which, in turn, determines the overall ventilatory response to CO2 (3). Reduced CBF-PaCO2 reactivity typically results in an enhanced ventilatory response, as the central chemoreceptors sense a relatively lower tissue pH and provide a greater drive to ventilation. However, when CBF is markedly reduced (say, by 50%), CBF-PaCO2 reactivity is nearly abolished, and the ventilatory response to CO2 is markedly blunted, possibly as a consequence of hypoxic depression of the respiratory neurons (3). Whether these mechanisms apply to preterm infants who require mechanical ventilation, and in whom cerebral hypoxia and hypoperfusion are often present, remains unclear and is an important area of research.

Oxygen vasoreactivity. MECHANISMS OF OXYGEN-VASOREACTIVITY. During hypoxia, the normal cerebrovascular response is vasoconstriction, which increases CBF and serves to maintain oxygen delivery (Fig. 1A). CBF can increase as much as 2-3-fold in response to hypoxia. With hypoxia, there is local, rapid release of metabolic factors favoring vasodilation, such as perivascular hydrogen ions, potassium ions, adenosine, carbon monoxide, and prostanoids (e.g., prostacyclin, PGII), along with decreased release of vasoconstricting factors, such as calcium ions (88, 206). Oxygen vasoreactivity depends, in part, on an intact endothelium and nitric oxide production. During development, the endothelial (143) and neuronal (140) contribution of nitric oxide to hypoxic vasodilation is relatively weak, but this increases throughout postnatal life to become more prominent in the adult (19, 43, 76), and is probably due to the increased synthesis of endothelial nitric oxide (143) and maturation of nitric oxide responsiveness (9).

OXYGEN-VASOREACTIVITY IN THE PRETERM BRAIN. The ability to increase cerebral perfusion in response to acute hypoxia appears early in fetal development and has been demonstrated at less than 0.7 gestation in fetal sheep (~28–30 wk gestation in humans) (143), although it is not clear whether the hypoxic vasodilatation is sufficient to fully compensate for decreased arterial oxygen content. This is likely due to the emerging and relatively underdeveloped state of the cerebral vasculature, despite the response to mediators of hypoxic dilatation, such as adenosine, already being present (143). Global hypoxia not only produces a global increase in CBF in fetal sheep, but results in a redistribution of blood flow in the brain, so that brain stem perfusion exceeds cerebellar perfusion, which, in turn, is greater than blood flow to the cerebrum (32, 144). Presumably, this robust vasodilatory response in the preterm brain stem makes it more resistant to hypoxic injury than other brain regions (198).

Conversely, hyperoxia decreases CBF, and this response to high levels of oxygen has been reported in fetal, neonatal, and adult animals and in human subjects (32, 84, 92, 214). In preterm infants, hyperoxia decreases CBF by 15–30% per kPa of PaO2 (106, 161), even exceeding the increase in arterial oxygen content, whereas in the mature adult brain, the CBF reduction to hyperoxia is proportional to the increased oxygen content in the blood (180). Importantly, decreased CBF induced by hyperoxia persists in preterm infants beyond the time that blood oxygenation was raised; e.g., CBF was lower in very preterm infants 2 h after brief exposure to oxygen in the delivery room, compared with a similar group of infants randomized to receive room air (114). As with CBF-PaCO2 reactivity, CBF-PaO2 reactivity also shows regional differences in the immature brain, with white matter being the least vasoreactive in response to changes in oxygen availability (32, 188).

Cerebral oxygen vasoreactivity differs from CO2 vasoreactivity in that, unlike the response to CO2, cerebral oxygen vasoreactivity occurs outside the physiologic range of PaO2 usually experienced by preterm infants. In addition, unlike chronic hypercapnea, during which CBF returns to baseline after several hours, hypoxic vasodilation persists during sustained hypoxia (143). These observations provide grounds for insisting that hypoxia, as well as hyperoxia, should be avoided, if possible, in preterm infants.

Cerebral blood flow-metabolism coupling. CBF has been shown to vary in relation to the cerebral metabolic rate of both glucose and oxygen (35), and tight coupling of cerebral function, metabolism, and blood flow is well established and has been demonstrated in numerous physiological, biochemical, and clinical studies (27, 79). Neurons and astrocytes are present in close physical proximity and are functionally coupled to the smooth muscle cells and endothelial cells of cerebral arterioles. The interactions of these cells have led to the concept that they constitute a functional unit termed the “neurovascular unit” (78). The hyperemic response to neuronal activation occurs within seconds and is highly localized (33, 107).

MECHANISMS OF CEREBRAL BLOOD FLOW-METABOLISM COUPLING. CBF-metabolism coupling (Fig. 1, A and B) is mediated by vasoactive factors that are released locally under the influence of neural activity, and include hydrogen ions, potassium ions, nitric oxide, oxygen, adenosine, calcium ions and arachadonic acid metabolites of the cyclooxygenase (COX) and cytochrome P-450 (CYP) pathways (78). Neuronal metabolic activity leads to local changes in the concentration of these vasoactive factors and, therefore, can lead to local changes in extracellular fluid osmolarity and, hence, cerebral vasodilatation, resulting in increased CBF. For example, nitric oxide, a potent vasodilator, is released in the brain as a consequence of increased synaptic activity (59), and is likely to play an important role in linking neural activity and CBF. COX-2 is present in glutamatergic neurons and COX-2-derived prostanoids participate in the increase of CBF induced by somatosensory activation (79). The importance of perivascular astrocytes, which produce CYP metabolites such as epoxyeicosatrienoic acids, as me-
diators of the rapid neurovascular response, has been discussed at length elsewhere (98, 225).

CEREBRAL BLOOD FLOW-METABOLISM COUPLING IN THE PRETERM BRAIN: CBF-metabolism coupling is probably developed in the preterm infants by 32 wk of gestation, if not earlier (67). Excessive neuronal activity during seizures has been associated with increased CBF and CBF velocity in term newborn and preterm infants, respectively (23, 150). Changes in the regional distribution of CBF during infancy and childhood correlate with changes in the local pattern of glucose utilization in the brain (35), supporting the concept of CBF-metabolism coupling in the developing brain.

However, hemodynamic responses to neuronal activation appear to be incompletely developed in the neonatal and infant brain, compared with the adult brain. For example, in extremely preterm infants there is no correlation between CBF and spontaneous changes in the cerebral metabolic rate of oxygen (CMRO\textsubscript{2}) during the first 48 h after birth; instead, changes in COE rather than CBF meet changes in oxygen requirement arising from variations of CMRO\textsubscript{2} (95, 219). Furthermore, brain activation studies in neonates and young infants report increases in cerebral deoxyhemoglobin, as measured by functional NIRS (120), and decreases in the blood oxygen level-dependent (BOLD) signal as detected by functional MRI (50), whereas in older infants and adults, the opposite changes in NIRS and BOLD signals occur (50, 73). Another study comparing the BOLD signal response in preterm infants, term infants, and healthy adults identified decreasing response time and increasing signal amplitude with increasing postnatal age (8). These findings suggest that in young infants the increase in cerebral oxygen consumption may be relatively greater than the corresponding increase in CBF during functional activation.

Among the mechanisms that determine CBF-metabolism coupling, changes in astrocyte-dependent processes during development may explain these age-related differences in CBF and brain activity. Animal studies have found marked increases in astrocyte number, size, and their connectivity with each other and blood vessels with increased brain development (72, 91). Additionally, the capacity of the local arterioles to increase local CBF through the neurovascular coupling cascade is likely to increase with age, in accordance with histological studies showing enhanced structural development of the arterioles and capillary beds, which continues until close to term age in the human fetal cortex (87, 101, 134). Moreover, cerebral vessel density and volume have been shown to approximately double in the cortex when newborn and adult primates are compared, with the bulk of this increase occurring at the capillary level (166). It is probable that this increase in vascularity translates into faster and increased local CBF responses when cerebral metabolic activity is increased.

Preterm Brain Injury and Cerebral Hemodynamics

Impact of cerebral hemodynamics on germinal matrix-intraventricular hemorrhage. In the third trimester, the dense, fragile vasculature of the germinal matrix lies within an arterial end zone. As a result, the germinal matrix is liable to be exposed to ischemic conditions resulting from periods of hypoperfusion, and to rupture and hemorrhage due to fluctuations in perfusion pressure, both of which are not unusual in the preterm infant. Protection against such events is provided by prenatal glucocorticoid administration, which decreases the incidence of GM-IVH in preterm infants (48) by suppressing VEGF and angiogenesis and possibly by stabilization of the existing germinal matrix vasculature (204).

In the mature brain, baseline CBF exceeds the threshold for ischemic injury by fivefold (154). However, as discussed above, global and regional CBF are significantly lower in the preterm brain, especially in the white matter. During the first 12–24 h of life, preterm infants are at significant risk of even lower cardiac output, lower systemic blood flow, and CBF (77, 97, 121), resulting in acute brain injury, including GM-IVH. The occurrence of GM-IVH is related to the nadir and duration of decreased CBF (52, 77, 97, 122) and may also occur as a “reperfusion” injury because it is strongly associated with abrupt increases in CBF (125) and fluctuating CBF (96, 135, 151, 201), presumably because there is also a loss of autoregulatory capacity as discussed above. Several studies have reported the association between impairment of pressure autoregulation in preterm infants and adverse clinical indices, including GM-IVH (125, 135, 158, 201, 220). There is also evidence that impaired autoregulation actually precedes, and may, therefore, contribute to the occurrence of the hemorrhage (4, 221). Blood pressure fluctuations are more likely to exceed the autoregulatory capacity in clinically unwell and unstable extremely preterm infants in whom the autoregulatory plateau has become very narrow or even pressure-passive. Indeed, there is increased evidence of brain injury in this group compared with well, physiologically stable infants at the same age (221).

Both high and low blood CO\textsubscript{2} levels have been implicated in causing GM-IVH (53). Hypercapnic hyperemia may trigger severe GM-IVH through vasodilation and engorgement of microvasculature (85). In addition, hypercapnea may limit the normal cerebrovasodilatory response to stimuli, such as hypotension and hypoxemia, predisposing brain tissue to hypoxic-ischemic insult. Doppler studies show that permissive hypercapnea [applied to reduce lung injury (124)] is associated with impaired cerebral pressure autoregulation (86).

Impact of cerebral hemodynamics on periventricular leukomalacia. Conditions leading to cerebral ischemia are also associated with periventricular leukomalacia (PVL). Severe hypotension is strongly associated with white matter injury and neurodevelopmental disability (39, 54, 62), with low CBF and impaired autoregulation likely to be contributing factors. Hyperventilation and severe hypocapnia are also strongly associated with PVL and cerebral palsy (63, 68, 177), and again, cerebral hypoperfusion will likely be present in these circumstances. While a small increase in intracranial volume in the preterm infant may be accommodated by the open fontanelles and sutures without changing intracranial or cerebral perfusion pressure, hydrocephalus arising as a result of GM-IVH does reduce CBF (183) and more severely so in the white matter (41). In such cases, white matter injury arises from a combination of an acute increase in parenchymal pressure, ischemic damage, inflammation, and free radical-mediated injury (34). Compared with other brain regions, immature white matter has the least vasoreactivity to changes in pressure (22, 66, 189), and infants with evidence of impaired pressure autoregulation have a higher risk for PVL (201). Interestingly, antenatal magnesium sulfate, a tocolytic and vasodilator, is associated
with increased CBF-velocity in very preterm infants from day 1 to day 5 of life (81), which may underlie its neuroprotective role in reducing hypoxic-ischemic brain damage in preterm infants.

Studies using the preterm lamb have improved our understanding of the role of inflammation and medications on cerebral oxygen metabolism and PVL. Intrauterine inflammation increases cerebral oxygen consumption (7) and decreases PaO2 with a compensatory increase in fetal CBF (171). In fetal lambs exposed to hypoxic or hypotensive stress, greater CBF fluctuations occur, and white matter injury is worse when there is superimposed intrauterine inflammation (171). The increased cerebral metabolic load and hemodynamic fluctuations imposed by intrauterine inflammation may help explain the clinical observation that the risk of white matter injury is increased when there is inflammation and/or infection present, even without a clear role for cytokines (49). On the other hand, antenatal steroids decreases the risk of GM-IVH, their administration to pregnant ewes decreases fetal CBF without increasing COE, which may be detrimental to brain oxygenation in conjunction with other hypoxic stressors (118).

**Future directions. PROANGIOGENIC THERAPY FOR PRETERM BRAIN INJURY.** Research for adult stroke therapies has shown that enhancement of angiogenesis may improve blood flow and support the regeneration and survival of cells after ischemic brain injury. In this regard, proangiogenic therapies may provide therapeutic opportunity also in preterm PVL, which occurs in a brain region with relatively poor vascularization. Prominent among the angiogenic factors investigated are VEGF and basic fibroblast growth factor (bFGF/FGF2). VEGF significantly augments neurogenesis and angiogenesis and reduces lesion volumes after traumatic brain injury in mice (195). Intracerebroventricular VEGF administration in a rat model of adult stroke reduces infarct size, improves neurological performance, enhances neuron survival, and stimulates angiogenesis (187). VEGF also reduces neurodegeneration and increases total vessel volume in the peri-infarct region in a neonatal rat model of stroke (46). However, VEGF overexpression in mice may worsen cerebral hemodynamics after stroke, as it induces angiogenesis with the “hemodynamic steal” phenomena, resulting in reduced blood flow in ischemic areas (208).

FGF2 is a biologically active polypeptide with mitogenic, angiogenic, and neurotrophic properties. Experiments in the chick indicate that FGF signaling is important for the initiation of angioblast specification (163). Increased expression of both FGF2 mRNA and protein occurs in the ischemic cortex of rats, corresponding with robust angiogenesis (110). FGF2 protects against hypoxic-ischemic insults in vitro and in vivo and enhances recovery of rat behavior following traumatic brain injury (211). FGF2 also mediates the positive effects of exercise on the brain, as exercise-induced FGF2 expression promotes functional recovery through enhanced angiogenesis in a mouse model of hypoxic-ischemic brain injury (175).

Cell-based therapies also constitute an attractive approach to enhance angiogenesis and neurogenesis after cerebral ischemia. Most notably, endothelial progenitor cells (EPCs) are able to mediate the repair of vascular injury after focal cerebral ischemia. EPCs are mobilized from bone marrow in response to acute hypoxia and are released into the circulation, where they act to maintain vascular integrity and promote tissue neovascularization. Systemic administration of umbilical cord blood-derived EPCs in an adult mouse model of stroke is neuroprotective, with reduced infarct volume and increased focal blood flow 48 h after ischemia (137). Coadministration of EPCs and smooth muscle progenitor cells to adult mice after stroke enhances angiogenesis and vascular remodeling, enabling the maintenance of neurogenesis and improved neuroblast survival (131). Interestingly, in preterm infants, hyperoxia impairs EPC signaling and proliferation (58). In neonatal mice, hyperoxia decreases vessel density and decreases the number of EPCs in the blood and bone marrow (14), suggesting that overoxygenation in preterm infants could impair the physiological actions of EPCs, whether released endogenously from bone marrow or following exogenous administration.

**Perspectives and Significance**

Improved knowledge of cerebral hemodynamics and metabolism during development and blood flow responses to physiological and external stimuli have aided understanding of the pathogenesis of preterm brain injury. Notwithstanding the ongoing debate around the “normal” or recommended ranges for systemic PaO2, PaCO2, and arterial oxygen saturation in preterm infants (124, 185) (Supplemental Table S3), the knowledge gained so far has been translated into the clinical recommendation that these parameters should be maintained steady to stabilize CBF and avoid hypocapnia- or hyperoxia-induced cerebral hyperperfusion (108). Application of a minimal infant-handling regime, to reduce stress and discomfort and minimize fluctuations in blood pressure, is also recommended to reduce potential fluctuations in CBF (109). In the context of preterm infants having low CBF and high baseline COE, treatment of anemia with blood transfusion reduces COE (209), improves cerebral oxygenation (172), and protects against GM/IVH and PVL (20). However, the effect of some common NICU practices, such as the prone body position for ventilated infants, on cerebral oxygen delivery remain unknown, albeit prone positioning compromises cerebral perfusion and oxygenation in older infants (139, 222).

Lastly, there is still relatively little understanding of the impact of neuronal activation, seizures, or drug-induced central nervous system depression on preterm cerebral hemodynamics and metabolism. In particular, it is still not known whether abnormal CBF metabolism coupling in the immature brain contributes to preterm neuropathology. Neuronal activation or depression is particularly important as many medications used in neonatal intensive care (e.g., caffeine, dopamine, morphine, midazolam, and various anticonvulsants) may alter brain activity. The effects of these medications on the coupling between CBF and metabolism remains to be explored in the preterm brain. Among the available devices to monitor cerebral perfusion, NIRS-based cerebral oximetry has extended its applicability to different research and clinical settings due to its noninvasiveness, instrument portability, and ease of use (147). However, a major limitation of most NIRS monitors is the lack of detail in the signal output from which the underlying change in cerebral oxygenation is derived. For example, reduced cerebral oxygenation may be due to reduced CBF, increased COE, increased oxygen demand, or a combination of these factors, but it is often difficult to differentiate between these possible mechanisms based on conventional NIRS measurements. Therefore,
findings in clinical studies regarding the relationships between NIRS measurements and clinical variables or outcomes are limited to being descriptive or associative, rather than explanatory or causative. This lack of mechanistic insight also makes the rational development of new treatments more difficult. A continuous, real-time, outside index of cerebral autoregulatory capacity can be derived from NIRS measurements, which may help in identification of autoregulatory impairment and pathophysiology, allowing for improved and timely management (24, 60). Future prospective research demonstrating that use of these tools in guiding treatment, and evidence that maintaining predefined and targeted cerebral hemodynamic measurements improves clinically relevant outcomes for preterm infants is clearly important.

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