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Microcirculation of the Newborn

Ian M.R. Wright and Rebecca M. Dyson

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Abstract

Appropriate regulation of microvascular blood flow in the neonate is crucial for cardiorespiratory stability and survival in the period immediately following birth. Inappropriate microvascular dilatation in the first few days of extrauterine life is associated with poor outcomes in preterm neonates. Male very preterm neonates (≤ 28 weeks completed gestation) have significantly higher flows than females of the same gestational age. This is of clinical importance as preterm males are twice as likely to die as females. Very little is known about the mechanisms underlying microvascular tone regulation in the perinatal period. Previous studies suggest a role for the gasotransmitters nitric oxide and carbon monoxide; however, differences in levels of these molecules do not account for all the variation observed, suggesting another player. In this chapter, the role of the third gasotransmitter—hydrogen sulphide—as a potential mediator of microvascular (dys)function in the preterm is explored.

Keywords: microcirculation, preterm, neonate, gasotransmitters, vasodilatation

1. Introduction

The newborn period represents a time of high mortality and morbidity. In the developed and developing world, perinatal mortality is the major contributor to infant, and thus child mortality [1], with the preterm infant at the greatest risk of poor outcomes. In Western countries, approximately 10% of infants are delivered prematurely (defined as less than 37 completed weeks of gestation) [2]. Both mortality and morbidity increase substantially with decreasing gestation at birth, as does the risk of long-term disability. Thus, the neonatal period, and prematurity in particular, represents a significant burden for the infant, the family and society. There is a marked sex difference in many of these outcomes, with males doing significantly worse, the causes of which are not fully elucidated. Studies of the microcirculation may be

important in understanding both the general and sex-specific risks as peripheral blood flow is subject to considerable changes during the first days of postnatal life, a period of marked circulatory vulnerability, especially in preterm infants.

2. Circulatory transition

There is an increasing body of recent work demonstrating the relationship between peripheral microvascular blood flow and measures of neonatal physiological and cardiorespiratory stability during the transitional period. These studies have shown that the evaluation of the peripheral microcirculation is useful for the assessment of cardiovascular changes within the initial extrauterine period [3, 4]. In the newborn, the skin represents a significant microvascular bed, both because of its size and functional perinatal changes. Measurement of cutaneous microvascular behaviour may be more useful at identifying individuals at risk of cardiovascular compromise than traditional blood pressure monitoring, as decreases in peripheral blood flow can be identified before blood pressure drops in states of compensatory peripheral vasoconstriction. Peripheral blood flow may increase early in vasodilatory shock, despite stable systemic blood pressure for some time after onset [5]. This suggests that peripheral microvascular tissue flow evaluation may be useful for early detection of cardiovascular compromise. In addition, the regulatory mechanisms of the peripheral microcirculation contribute to (and may reflect) the underlying neuroendocrine responses to cardiovascular compromise [6, 7]. Finally, the skin allows non-invasive microvascular measurement, a prerequisite for human newborn studies of the microcirculation.

In adults, blood enters the skin through small arterioles, penetrating the subcutaneous tissue toward the skin surface. One artery often branches into several precapillary arterioles (30–80 μm), which pass into the venous plexuses that are organized parallel to the skin surface. Each arteriole can divide into eight to ten capillary loops orientated perpendicular to the skin surface, with one to three loops perfusing each skin papilla [8]. In neonates, however, this is much less organized and the arteriolar-venular anastomoses, and the capillary loops are immature, with a less defined network. Capillary loops are not detectable (outside the nail beds, palms, or soles) until 2 weeks post-partum (and even then, are not distinguishable in all sites until 14 weeks postnatal age or more) [9]. Development and refinement of the vascular network continues until the formation of a more “adult-type” network at approximately 4 months postnatal age. Despite this structural immaturity, the cutaneous microvasculature of both term and preterm infants is capable of responding to external stimuli, such as occlusion or local heating, from shortly after birth [4, 10].

2.1. Preterm infants

Circulatory transition is a period of marked circulatory vulnerability, especially in preterm infants. The preterm heart is structurally and functionally immature and is not capable of adapting to relatively small changes in preload and afterload in order to function effectively and deliver oxygen and nutrients to tissues. Animal studies in neonatal pigs have demon-

strated that the preterm heart requires significantly higher levels of preload than term hearts in order to function effectively [11, 12]. Thus, abnormal regulation of vascular resistance, with net effects on preload and/or afterload, may play a major role in the development of cardiovascular compromise in preterm infants [4, 13, 14].

The peripheral microcirculation undergoes rapid and considerable change during the initial extrauterine period [15, 16]. Studies examining microvascular behaviour in the first 3 days of life have reported that peripheral microvascular blood flow is significantly higher in very preterm infants (≤ 28 weeks gestational age [GA]) as compared to infants born moderately preterm (29–36 weeks GA) [4] or at full term [17]. This increased peripheral blood flow is associated with low blood pressure, physiological instability, and adverse outcome in the initial extrauterine period [4, 18]. Very preterm male infants, those at the greatest risk for poor short- and long-term outcomes [19], have greater baseline microvascular blood flow than female infants of the same gestational age at 24 h age [20], suggesting sex-specific differences in the neonatal ability to control vascular tone.

These studies demonstrate a strong relationship between microvascular flow and mean arterial pressure. Taken together, they suggest that a large proportion of the blood volume is being taken up by the capacitance vessels and the microcirculation in (male) preterm newborns, leading to “functional hypovolaemia” and decreased preload. This may explain why males are more at risk of complications in the first 24–48 h of life [19, 21].

3. Control of microvascular tone in the newborn

During rapid postnatal growth, autoregulatory mechanisms play a major role in maintaining adequate perfusion of developing tissues. Neural and myogenic control of skin blood flow must be rapidly established following birth to allow an effective thermoregulation in the newborn and to ensure that metabolic demands of tissues are met [22]. It is well established that the autonomic nervous system, particularly peripheral sympathetic nervous system activity, plays a central role in the regulation of vascular tone in the transitional circulation of term neonates [23]. The myogenic response contributes to autoregulation of tissue blood flow and is not dependent upon neural innervation or the endothelium in the adult vasculature. However, the myogenic response of juvenile arterioles isolated from Wistar rats is significantly reduced following endothelial removal, suggesting that in juvenile arterioles endothelium-derived factors normally augment myogenic activity over a wide range of pressures [24]. Thus, the endothelium may be more important in the regulation of vascular tone in the transitional circulation compared to the more mature individual. This is likely to be even more important in the preterm infant, where the balance of parasympathetic (vagal) tone to sympathetic drive is relatively increased compared to term infants [25].

Endothelial cells coordinate the release of vasoactive substances which act directly upon vascular smooth muscle cells (VSMCs) and thus elicit constriction or dilation of the blood vessel [26]. How various vascular mediators may interact in the initial extrauterine period is unknown at present, but elucidation of this interplay and the underlying mechanisms may

help to understand microvascular dysfunction and cardiorespiratory instability in the early neonatal period.

3.1. Vasoconstriction

Peripheral vascular resistance is known to correlate with the degree of sympathoadrenal activation at birth [27]. This is due to the fact that autonomic nervous system activity is an essential component of circulatory transition and adaptation to extrauterine life. The peripheral sympathetic nervous system plays a central role in regulation of vascular tone during this period largely via the action of norepinephrine, the main sympathetic neurotransmitter regulating the cardiovascular system in the neonate and eliciting vasoconstriction [28]. Its metabolite, normetanephrine, is excreted in the urine and has been used as a measure of total body sympathoadrenal activity [29] with levels shown to be higher in female than male newborns [23]. In that study, normetanephrine was inversely related to both baseline microvascular blood flow and physiological instability immediately following birth in preterm neonates.

Endothelin (ET)-1 is a primary vasoconstrictor in the pulmonary vasculature of the foetus and neonate. ET-1 is produced by endothelial cells in response to a number of stimuli, including shear stress, hypoxia and ischemia [30]. ET-1 binds to one of the three receptors: ET_A, ET_{B1} or ET_{B2} receptors, located in the underlying VSMCs. Studies in neonatal pigs suggest that all three ET receptor subtypes are highly expressed following birth, and play distinct roles in the newborn: in line with previous studies, ET_A and ET_{B2} receptors appear to be responsible for the vasoconstrictive action of ET-1, whereas binding of ET-1 to ET_{B1} receptors elicits vasodilatation. Importantly, these receptors follow a distinct expression profile: the pro-constrictive ET_A and ET_{B2} receptors are more abundant in proximal vessels [31], for example, in the pulmonary arteries and veins, respectively [32]; whereas ET_{B1} receptors are more abundant in the distal vasculature, with relatively low expression in arteries [31, 32]. Evidence from newborn piglets suggests that receptor-affinity for ET-1 is higher in veins than arteries, suggesting that in the immediate postnatal period at least, the majority of the ET-1 vasoconstrictive effect is mediated through ET_{B2} receptors [32].

ET-1 can also elicit vasodilatation through endothelium-derived nitric oxide (NO) and prostacyclin, by binding to endothelial ET_{B1} receptors. Endothelial ET_B receptors are expressed more highly in the distal than the proximal vessels [31, 33]. In the pulmonary vasculature, ET_A-mediated contraction decreases and ET_{B1}-mediated NO-dependent relaxation increases with advancing postnatal age [34], suggesting a switch from a constricted to dilated state, which follows the known decrease in pulmonary vascular resistance following birth, allowing for blood oxygenation [35]. In line with this, plasma ET-1 levels peak immediately after birth and then gradually decrease, with concentrations in healthy term newborns more than threefold greater than at 5 or 30 days postnatal age [36–38]. In a recent study of preterm newborns [23], a significant increase of ET-1 after birth was observed in very preterm neonates, but not in more mature neonates. Interestingly, umbilical arterial ET-1 was significantly higher in female preterm neonates, which is of interest given the well characterised sexual dimorphism in the development of respiratory distress [39]. Plasma ET-1, however, did not correlate

with peripheral microvascular blood flow or illness severity, suggesting that while ET-1 may play a significant role in the regulation of pulmonary vascular tone, it does not appear to exert a dominant effect over systemic or peripheral microvascular tone [23].

Isoprostanes (prostaglandin-like bioactive molecules generated by free radicals and reactive oxygen species) are also vasoconstrictive in a number of vascular beds in the foetus and neonate. Under normal conditions in the adult, isoprostanes are present at nanomolar concentrations. In the newborn, however, levels are significantly higher due to the oxidative stress of the adaptation to a rapid increase in blood oxygen tension that occurs during the transition from fetal to neonatal life. The isoprostanes elicit vasoconstriction through a number of pathways, including activation of prostanoid and thromboxane receptors, and tyrosine kinase and Rho kinase pathways [40, 41]. As preterm infants are more susceptible to oxidative stress, they have greater isoprostane concentrations, with an inverse correlation between isoprostane levels and gestational age being observed [42]. At term, umbilical cord arterial isoprostane concentrations are higher in male neonates compared to females of the same age [43], which is consistent with the known vulnerability of males to oxidative stress compared to females [44]. Whether this relationship exists at earlier gestational ages is unclear, however, it seems unlikely that this is a major contributor to peripheral vascular tone regulation in the preterm neonate, as it is well documented that these neonates have a loss of peripheral vascular tone (vasodilatation) [20]. Isoprostanes, along with prostaglandins, play a major role in the closure of the ductus arteriosus after birth, suggesting these molecules may play a more significant role in central, rather than microvascular, vasoactive effects during circulatory transition [41].

Overall, the balance of vasoconstrictors to vasodilators in the immediate postnatal period appears to be relatively increased in females and more mature infants and is associated more with the control of central than peripheral vascular tone.

3.2. Vasodilatation

Whilst the above suggests that microvascular dysregulation in the preterm newborn may be associated with impaired vasoconstriction, there is also a potential role for abnormal peripheral vasodilatation leading to the development of cardiovascular compromise and poor outcome [45]. Changes in enzyme expression, receptor density, ion channel activity and intracellular signalling pathways are all likely responsible for the changes in vascular tone regulation observed during circulatory transition. Studies have shown, for example, that the mechanisms mediating endothelium-dependent vasodilatation in the femoral artery of piglets undergo a maturational change during the first weeks of life [46].

Additionally, there are considerable variations in the contribution of vasoactive substances to endothelium-dependent relaxations in different tissues—it appears that nitric oxide (NO) is the principal vasodilator in conduit arteries, whereas other mediators, such as endothelial-derived hyperpolarising factors (EDHF), make a significant contribution at the level of the resistance arteries [47, 48]. Despite this clear role for other mediators, NO is the most extensively studied vasodilator in the preterm newborn [49–53], partly due to its therapeutic use for the treatment of persistent pulmonary hypertension of the newborn.

Vasodilatation of cerebral arterioles in the newborn pig is largely mediated by endothelial prostanoids, with endothelial NO assuming a progressively greater role in vascular tone regulation during subsequent postnatal maturation. Underlying this difference is a twofold to threefold increase in both the expression and activity of endothelial nitric oxide synthase (eNOS) between birth and 3–4 months postnatal age in cerebral microvessels [54–57]. In the cerebral arteries of newborn sheep, NO plays a major role in endothelium-dependent dilatation during the first week of life [58, 59]. Interestingly, it has also been shown that in newborn lambs, the contribution of NO to peripheral vascular tone is relatively low compared to its contribution in the cerebral circulation [60]. This suggests a role for the involvement of other factors regulating systemic vasodilatation, either independently or in concert with NO, during circulatory transition; and that considerable interspecies and regional differences in vasodilator mechanisms exist. Human studies of the perinatal cerebral microvasculature rely on non-invasive measures such as near infrared spectroscopy or minimally invasive techniques such as xenon cerebral blood flow assessment. Neither has been assessed in this age group in relation to the mediators and their interactions described earlier.

3.2.1. Gasotransmitters

As the gasotransmitters, NO, carbon monoxide (CO) and hydrogen sulphide (H₂S) appear to be crucial to the dilatory component in the newborn circulation these will each be dealt with in the following sections.

3.2.1.1. Nitric oxide

NO is known to play a central role in maintaining vascular homeostasis in the transitional circulation [61]. Hypoxic events occurring at birth are known to upregulate eNOS expression leading to increased NO production, eliciting vasodilatation in the systemic microvasculature of the newborn [62, 63]. It has been hypothesised that the overproduction of NO in the perinatal period may lead to poor control of blood flow throughout the peripheral microcirculation of preterm infants, increasing their risk of circulatory compromise [64]. Blood pressure in the preterm neonate is inversely correlated to cGMP levels [61]. A role may exist for NO, its substrates or second messengers as potential regulators of peripheral blood flow in the preterm infant [61, 65].

Our group, however, has previously shown that changes in human skin microvascular blood flow in the first 3 days of life are not associated with changes in systemic NO production [45]. Additionally, it has been hypothesised that the rate of NO production by eNOS in the endothelium of peripheral microvessels is lower than would be required to activate the downstream sGC pathway in VSMCs responsible for the excessive vasodilatation seen in premature neonates [66, 67]. This has led to the speculation that other mechanisms may be involved in both the production of NO in the microvasculature and its vasoactive effects on VSMCs during the transition from fetal to neonatal circulatory systems, with NO contributing primarily to the maintenance of baseline tone throughout this period, rather than the pathophysiological variations that are seen in microvascular tone.

3.2.1.2. Carbon monoxide

Carbon monoxide (CO) is produced endogenously in endothelial cells and VSMCs as a result of catabolism of heme by the enzyme heme oxygenase (HO) [68]. Two functional HO isoforms have been identified in humans and other species—HO-1, the inducible isoform which is upregulated by various stress stimuli (oxidative stress, cytokines, endotoxin and hypoxia) [69] and plays an important role in cellular defence, and HO-2, the constitutive isoform. Vasodilation by CO is thought to be through similar pathways to NO, with CO inducing VSMC relaxation by the activation of cGMP-dependent pathways [70]; however, evidence of cGMP-independent pathways also exists—with several papers now suggesting that CO-induced vasodilatation is also mediated by large conductance calcium-activated potassium channel (BKCa) activation leading to VSMC hyperpolarisation [71, 72].

HO-1 plays an important role as an antioxidant, anti-inflammatory and anti-apoptotic mediator in prenatal and postnatal development and in the transitional circulation of neonates [69, 71]. HO-2 expression in the cerebral vasculature is developmentally regulated, with significant increases in expression observed with advancing gestational age [73, 74]. HO-2 is highly expressed in cerebral blood vessels in the newborn piglet and in these studies CO was shown to be a potent vasodilator in this microcirculatory bed [71].

CO is thought to play a role in maintaining vascular homeostasis in the fetal circulation, and endogenously produced CO is known to play a role in maintaining the patency of the ductus arteriosus (DA) in utero [75]. We have previously shown that CO is relatively increased in males and younger infants, that is, those who exhibit increased vasodilatation and adverse clinical outcomes [45]. However, the CO findings only explain a proportion of the difference present in early vasodilatation events ($r=0.495$ at 24 h postnatal age), with differences in NO levels apparently occurring beyond the crucial early period, suggesting at least one other vasodilator is involved.

3.2.1.3. Hydrogen sulphide

In adults, interest is increasing in the role of a third gasotransmitter, H_2S , as a vascular mediator important in regulation of microvascular tone. H_2S is produced endogenously in the vasculature in amounts capable of causing vasodilatation and thus may play a role in the regulation of vessel dilatation and control of blood pressure [76–78]. The majority of endogenous H_2S synthesis occurs by two pyridoxal-5'-phosphate (PLP)-dependent enzymes in the transsulphuration pathway: cystathionine- β -synthase (CBS) and cystathionine- γ -lyase (CSE). The substrates for these enzymes include the amino acids cysteine, homocysteine and cystathionine [79]. A third non-PLP-dependent enzyme 3-mercaptopyruvate sulphurtransferase (MST) also contributes to H_2S production by the metabolism of mercaptopyruvate.

In contrast to NO and CO, H_2S is proposed to exert the majority of its vasodilatory effects through the activation and opening of transmembrane ATP-sensitive potassium channels (K_{ATP}) in VSMCs [80]. In VSMCs, opening of K_{ATP} channels leads to hyperpolarisation of the cell membrane, inactivating voltage-dependent calcium channels and resulting in VSMC relaxation [81]. Thus, H_2S has been implicated as a crucial physiological mediator of vascular

tone that represents an alternative pathway of vascular tone control to NO and CO. Like all the gasotransmitters, H₂S is lipophilic, ensuring rapid diffusion of the gas throughout the endothelium and VSMCs, despite its intrinsic reactivity with a diverse range of substrates [82, 83]. This diffuse production across the vessel wall and its mechanism of action qualify H₂S as an EDHF [84].

The H₂S producing enzyme CBS is expressed in multiple tissues during development. In the adult, many of these tissues, including the heart and lungs, do not express CBS [85]. In comparison, many studies have suggested that CSE is not expressed during early mammalian development—in human liver, CSE activity is not detectable in fetal liver or in premature or full-term neonatal liver samples and is not detected until several days postnatal age, with levels comparable to that in the adult reached by 3 months postnatal age [86, 87]. Recent studies, however, suggest that this developmental expression pattern may not be consistent across different tissues and vascular networks. Baragatti et al. [82] have recently demonstrated gene expression of CSE, CBS and MST in the ductus arteriosus in fetal mice (MST expression was very low compared with CSE and CBS). The expression of CSE and CBS was localised to the ductus with a specific distribution: CSE staining was more intense in the endothelial layer (45% higher than in the smooth muscle layer). Conversely, CBS staining was 15% higher in the smooth muscle layer compared to the endothelium. This supports an endothelial, CSE-derived source of H₂S in the mammalian foetus. Leffler et al. [88] showed that H₂S may also be important in the transitional cerebral circulation of newborn piglets with endogenous H₂S produced by CSE, but not CBS, in concentrations capable of eliciting pial arteriolar dilatation.

Until recently, very little was known about the role of H₂S in the transitional circulation of the neonate. The above studies demonstrated at least some activity of both CBS and CSE in the transitional circulation of the preterm newborn [89]. CBS expression, and to a lesser extent CSE, is crucial for the survival of newborn animals: CBS-deficient mice (*Cbs*^{-/-} knockout) display endothelial dysfunction, severe growth retardation and profound lethality at weaning age, as well as the features of homocysteinemia seen in human CBS-deficient patients [90, 91]. CSE may be important in protecting against oxidative stress in the preterm newborn; *Cth*^{-/-} mice display greater sensitivity to oxidative stress, despite normal serum biochemistry (for example, levels of bilirubin and glutathione) and the absence of histological abnormalities [90, 92].

Studies have now shown evidence of a role for H₂S in physiological microvascular tone regulation during circulatory transition in human infants, with higher production potentially associated with dysregulation in the human preterm male neonate [93, 94]. In preterm neonates (29–36 weeks completed gestation), total body turnover of H₂S (measured as urinary thiosulphate) positively correlates with peripheral microvascular blood flow and negatively correlates with blood pressure, supporting both a role for H₂S in microvascular tone regulation [93]. This increased total body turnover of H₂S was shown to be related independently to all the major risk factors for poor outcome: gestational age, postnatal age and male sex. In these studies, thiosulphate differences were not present in the first few hours immediately after birth, which suggests that very preterm neonates are not born with inherently higher levels of H₂S production, but that this increases significantly following birth [93]. Potential triggers for this could include oxidative stress [95] or inflammation [96], both of which are increased in preterm

neonates [97]. The positive relationship of H₂S turnover with microvascular blood flow, and the inverse relationship with blood pressure in more mature neonates, suggests a physiological role of H₂S in this age group, perhaps as a counter to the increased vasoconstrictors (see above) [98], or as a reflection of an organ specific vascular dilatation, such as in the pulmonary circulation [99, 100].

The contribution of H₂S to vasodilatation via different pathways to those of NO and CO, or interactions with these mediators, may represent a crucial role for this gasotransmitter in regulation, or dysregulation of microvascular tone in the neonatal microvasculature.

4. Interactions of the gasotransmitters in the neonate

It is becoming increasingly evident that the complexity of hemodynamic microvascular control is not through the activity of single factors working in isolation, but by the interaction of all these elements [101, 102]. This includes interactions between the three gasotransmitters in the neonatal microcirculation (**Figure 1**).

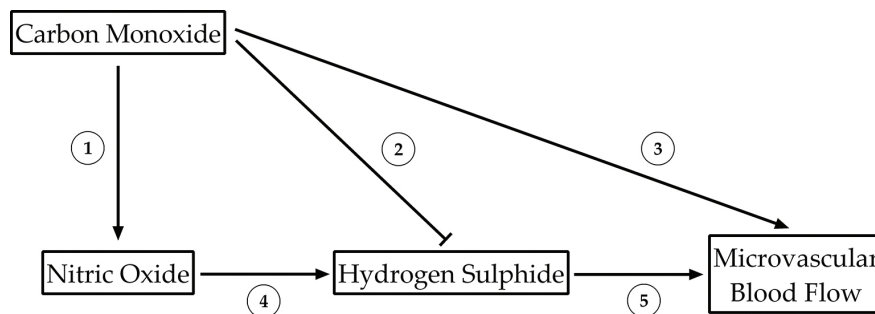


Figure 1. Structural equation modelling of gasotransmitter and microvascular flow interactions in the preterm newborns (males and females combined). NO promotes H₂S production (Interaction 4; overall $p = 0.002$; males $p = 0.06$, females $p < 0.0001$), whilst CO inhibits H₂S only in female infants (Interaction 2; overall $p = 0.18$, males $p = 0.84$, females $p < 0.0001$). The net result is a mild increase in the effect of all vasodilators acting on the microvasculature in males (Interaction 5, $p = 0.006$) compared to the effect of H₂S in isolation (model not shown). In females the model predicted a lower contribution of H₂S on microvascular blood flow when CO and NO were included in the model compared to its effect in isolation. The model predicted covariance in the levels of NO and CO (Interaction 1) but CO had no direct effect on microvascular blood flow (Interaction 3). Inclusion of this pathway, however, improved goodness of fit, most markedly in females (with CO effect $\chi^2 = 0.03$, without $\chi^2 = 0.29$). The inclusion of a direct effect of CO on flow additionally increased the predicted blood flow effect of H₂S in the overall model in both sexes, suggesting a synergistic or permissive action between these molecules. Adapted from Dyson et al. [94].

Knecht et al. [103] found in neonatal pig pial arterioles that CO responses are biphasic: dilatation occurred in response to an acute elevation, such as that produced by inducible HO activity following birth [104], while prolonged or sustained CO exposure caused constriction via NOS inhibition. They speculated that such interaction between CO/HO and NO/NOS could form a negative feedback system for the regulation of cerebrovascular tone. Importantly, the balance of NO and CO systems is different in neonatal and adult cerebrovascular circulations [103]. The role of NO in cerebrovascular control is less in newborn piglets compared to juvenile

pigs, consistent with findings in human newborns [45, 55, 57, 61]. In contrast, the pial arteriolar response to CO is greater in newborn piglets compared to older pigs or adult rats. Additionally, they showed that whilst CO-induced dilation in older pigs and rats occurred independently of co-factors, NO and prostacyclin were required for CO-mediated vasodilatation to occur in the newborn [71].

In the mature circulation endogenous production of H₂S appears to be enhanced by NO. H₂S is also known to promote endothelial cell NO release, leading to a threefold increase in vasorelaxation [105, 106]. In adult rats with pulmonary hypertension, the administration of L-arginine (the pre-cursor for NO) increases CSE expression in pulmonary artery VSMCs and also plasma H₂S concentrations [107]. It is unclear whether L-arginine itself or its metabolites induce this upregulation but evidence that NO upregulates CSE expression and H₂S synthesis in VSMCs exists [105]. H₂S can also induce an upregulation of HMOX1 – the gene coding for HO-1. By upregulating HO-1 expression, H₂S can thus increase CO production eliciting further vasodilatation. Conversely, administration of an inhibitor of CSE leads to a decrease in CO synthesis [108, 109].

In preterm newborns, a significant positive relationship exists between NO and H₂S [94]. Previous studies have reported that NO inhibits H₂S production via CBS [110, 111] but induces CSE H₂S production [105]. This may suggest that in the human preterm newborn, CSE expression is significantly modulated by NO. Evidence from animal models of prematurity suggest that increases in H₂S associated with microvascular dysregulation are driven by CSE-dependent mechanisms: in preterm animals, H₂S increases during fetal-to-neonatal transition, with significantly higher levels of H₂S produced by tissues collected at 24h postnatal age compared to fetal tissues. Additionally, CSE contribution to total H₂S increases postnatally. H₂S produced by the vasculature (both total H₂S and CSE-derived H₂S) correlates with microvascular blood flow at 24h postnatal age [112]. This suggests that CSE-dependent mechanisms drive the observed increase of H₂S production, and potentially the increased microvascular blood flow and decreased blood pressure, over the first 3 days of life in preterm human neonates [93]. In modelling known interactions of the gasotransmitters in human preterm neonates, a lower contribution of H₂S to microvascular tone regulation in females was predicted when the other gasotransmitters were added into the model [94]. This suggests that the effects of either NO or CO, singly or in combination, negate the effect of H₂S in these female infants to such a degree that there is no net effect on vascular tone. This may be primarily due to CO, which is inversely correlated with H₂S and may reflect an inhibitory action of CO on H₂S [94], in line with published reports [113–115]. Thus in the preterm neonate, because of these dimorphic responses, comparable levels of CO could be associated with microvascular dysregulation and cardiovascular compromise in males but help protect against inappropriate vasodilation in females [45].

4.1. Proposed mechanism of gasotransmitter-regulated microvascular vasodilatation in the newborn

We propose a model of gasotransmitter-dependent vasodilation in the preterm newborn, including a role for oxidative stress in driving dysregulation. It is now evident that all three

gasotransmitters may protect against oxidative damage, but may also be cytotoxic via their inhibition of cellular respiration (through inhibition of mitochondrial respiratory chain cytochrome-C-oxidase) and through their marked pro-inflammatory effects [116]. As in the studies performed by Knecht et al., an acute increase in CO, driven in part by the HO-1 response to birth and exposure to high-concentration oxygen during resuscitation [117], drives CO-mediated vasodilatation in the male preterm newborn. Due to lowered antioxidant defences, HO-1 upregulation persists, and the prolonged CO production inhibits NO [103]. This would account for the transient nature of both the CO rise and increased microvascular blood flow observed in the preterm newborn around 24 h postnatal age, which has been shown to not be associated with NO [45]. Additionally, the transsulphuration pathway is upregulated by oxidative stress to produce the antioxidant glutathione. H₂S production is increased by this response, driving aberrant vasodilatation and contributing to hypotension and cardiovascular compromise in the preterm newborn.

We propose varying roles for the three gasotransmitters, allowing for regional and temporal control of blood flow:

- Taking into account gasotransmitter level time course and actions, we propose that NO during circulatory transition is responsible for the maintenance of basal vascular tone—as it is in adults [118, 119]. Levels are relatively stable in the first few days of life, with increases occurring outside the crucial early period of the first 24–48 h following birth [45]. NO in the transitional neonate is not, however, correlated with aberrant peripheral microvascular flow.
- CO has been shown to be a crucial regulator of vascular tone in the cerebral circulation during circulatory transition [71, 120]. It may also explain a proportion of the variance observed in early peripheral vasodilator events [45, 94], both directly and via the sexually dimorphic effects on other vasodilator pathways. The cerebral circulation effects of a CO increase, such as that observed by Stark et al [45], could contribute to the greater incidence of cerebral injury in preterm males.
- Based on our more recent studies, including metabolite measurement, production enzyme assays and interaction modelling, we suggest that H₂S is a key player in the systemic microvasculature in the sick or preterm newborn. Further we propose that H₂S is produced by the peripheral vasculature, particularly via endothelial CSE, and that this closely regulates microvascular tone during this critical period [112].

5. Summary

The microcirculation is structurally and functionally different in the neonate. The ability of the newborn to appropriately distribute blood flow to key vascular beds and to maintain adequate cardiac output is strongly linked to their survival. As cardiac function in the neonate is strongly influenced by both preload and afterload, sex differences in peripheral vascular function in preterm neonates may lead to differences in cardiac function, contributing to physiological instability and thus poor outcomes. In support of this, sexual dimorphism in the functional

integrity of the microvasculature, including appropriate control of vasodilatation, is observed in very preterm neonates and is linked to outcome. Gestational age, postnatal age and sex all exert significant effects on peripheral microvascular function, dysregulation of which is associated with clinical illness severity in the neonate; however, the mechanisms by which these factors exert their effects are largely unknown. The gasotransmitters NO, CO and H₂S represent novel mediators which may play a significant role in the regulation of microvascular tone in the newborn: both through their individual roles but also through their potential interactions. The studies outlined above suggest that the gasotransmitters and their interactions could influence the adverse cardiovascular outcomes seen in this population.

6. Conclusions

Cardiovascular compromise is associated with poor outcome in the preterm neonate, with gestational age and male sex as independent risk factors for the development of hypotension and cardiovascular compromise, amongst several other morbidities. A growing body of work has highlighted the importance of the microvasculature in the development of cardiovascular compromise in the preterm newborn, and thus the importance of understanding the contribution of inappropriate peripheral vasodilatation to hypotension, cardiovascular compromise and poor neonatal outcomes.

Potential therapies aimed at either preventing or ameliorating cardiovascular compromise in the preterm neonate must consider the role of microvascular dysfunction, driven by an imbalance in vascular tone mediators, including the gasotransmitters, NO, CO and H₂S. Understanding the aetiology of cardiovascular compromise is crucial for the development of better strategies for monitoring, prevention and treatment of these at risk infants.

Author details

Ian M.R. Wright* and Rebecca M. Dyson

*Address all correspondence to: iwright@uow.edu.au

University of Wollongong and Illawarra Health & Medical Research Institute, Wollongong, NSW, Australia

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