Pediatric RESEARCH

SPECIAL ARTICLE

Understanding the pathobiology in patent ductus arteriosus in prematurity—beyond prostaglandins and oxygen

Tim Hundscheid[1], Martijn van den Broek[2], Robin van der Lee[1] and Willem P. de Boode[1]

The ductus arteriosus (DA) is probably the most intriguing vessel in postnatal hemodynamic transition. DA patency in utero is an active state, in which prostaglandin E_2 (PGE_2) and nitric monoxide (NO), play an important role. Since the DA gets programmed for postnatal closure as gestation advances, in preterm infants the DA frequently remains patent (PDA). PGE_2 exposure programs functional postnatal closure by inducing gene expression of ion channels and phosphodiesterases and anatomical closure by inducing intimal thickening. Postnatally, oxygen inhibits potassium and activates calcium channels, which ultimately leads to a rise in intracellular calcium concentration consequently inducing phosphorylation of the myosin light chain and thereby vasoconstriction of the DA. Since ion channel expression is lower in preterm infants, oxygen induced functional vasoconstriction is attenuated in comparison with full term newborns. Furthermore, the preterm DA is more sensitive to both PGE_2 and NO compared to the term DA pushing the balance toward less constriction. In this review we explain the physiology of DA patency in utero and subsequent postnatal functional closure. We will focus on the pathobiology of PDA in preterm infants and the (un)intended effect of antenatal exposure to medication on both fetal and neonatal DA vascular tone.

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INTRODUCTION

Under physiological circumstances the ductus arteriosus (DA) remains patent in utero, mainly due to prostaglandins (PG) produced by the placenta and the DA itself.[1–3] The fetal DA diverts oxygenated blood from the pulmonary to the systemic circulation. After term birth the DA functionally closes within hours to days, secondary to a rise in oxygen tension (PaO_2) and a decline in PG concentration, both by cessation of placental production and an increase in PG catabolism in the lungs, as pulmonary bloodflow increases due to a decreased pulmonary vascular resistance after lung aeration.

In case of a duct dependent congenital heart disease clinicians attempt to (re)open the DA to improve or restore either pulmonary or systemic circulation. Most of these patients will receive PGE_1 as a bridge to therapeutic intervention.[4] On the contrary, clinicians might consider to actively close a patent DA (PDA), most often seen in preterm and very low birth weight (VLBW) infants.[5] In an attempt to prevent pulmonary hyperperfusion and systemic hypoperfusion secondary to high volume transductal left-to-right shunting. Drugs used for active closure inhibit PG synthesis by two different strategies (Fig. 1), namely cyclooxygenase (COX) inhibition with indomethacin (INDO) since 1976,[6,7] or ibuprofen (IBU) since 1995,[8] and peroxidase inhibition with paracetamol (PCM) since 2011.[9,10] A recent network meta-analysis revealed that PDA treatment with aforementioned drugs is effective in DA closure, but failed to show beneficial clinical effects in comparison to placebo or expectant management.[11] Furthermore, a substantial part of (extreme) preterm infants fails to close their DA, even after pharmacological treatment.[12,13]

As illustrated, all available pharmacological treatments aim to influence neonatal DA vascular tone by interfering in the PG pathway. However, one should note that PG is only one of the many mediators involved in the vascular tone of the DA. Postnatal DA vasoconstriction, initiating functional closure, is induced by a rise in intracellular $[Ca^{2+}]$. Consequently Ca^{2+} binds to Calmodulin and activates myosin light chain (MLC) kinase (MLCK), thereby inducing phosphorylation of the MLC and eventually constriction of the DA smooth muscle cell (DASMC). The mechanisms involved in the pathobiology of the DA lead to either a rise or decline in $[Ca^{2+}]$ and consequentially phosphorylation or dephosphorylation of the MLC (Fig. 2; Table 1).[14] By understanding the different mechanisms influencing these pathways, one can comprehend why preterm infants have a higher incidence of a PDA and why they might fail to respond to current treatment.

In this review we will first discuss the physiological in utero state and postnatal closure in term infants. The pathways that will be addressed are PG, O_2, nitric monoxide (NO), carbon monoxide (CO), hydrogen sulphide (H_2S), osmolality, glutamate, natriuretic peptide (NP), ion channels, and the MLCK or MLC phosphatase (MLCP) (Fig. 2). We will elaborate on the pathobiology in these pathways in preterm infants predisposing to postnatal PDA. Finally, we provide insight in candidate pathways for future research and possible novel therapeutic approaches. We will mainly focus on data available from animal research in rodents, sheep, and primates. It should be noted that there are conflicting results in different experimental models and avian models are not discussed in this review. Given the many interspecies differences, it is difficult to translate all findings in animal models directly to the newborn human in the neonatal intensive care unit.

[1]Radboud Institute for Health Sciences, Amalia Children's Hospital, Division of Neonatology, Department of Pediatrics, Radboud university medical center, Geert Grooteplein zuid 10, Postal code 804, 6525 GA Nijmegen, The Netherlands and [2]Medical Biology, Radboud university, Heyendaalseweg 135, 6526 AJ Nijmegen, The Netherlands
Correspondence: Tim Hundscheid (tim.hundscheid@radboudumc.nl)

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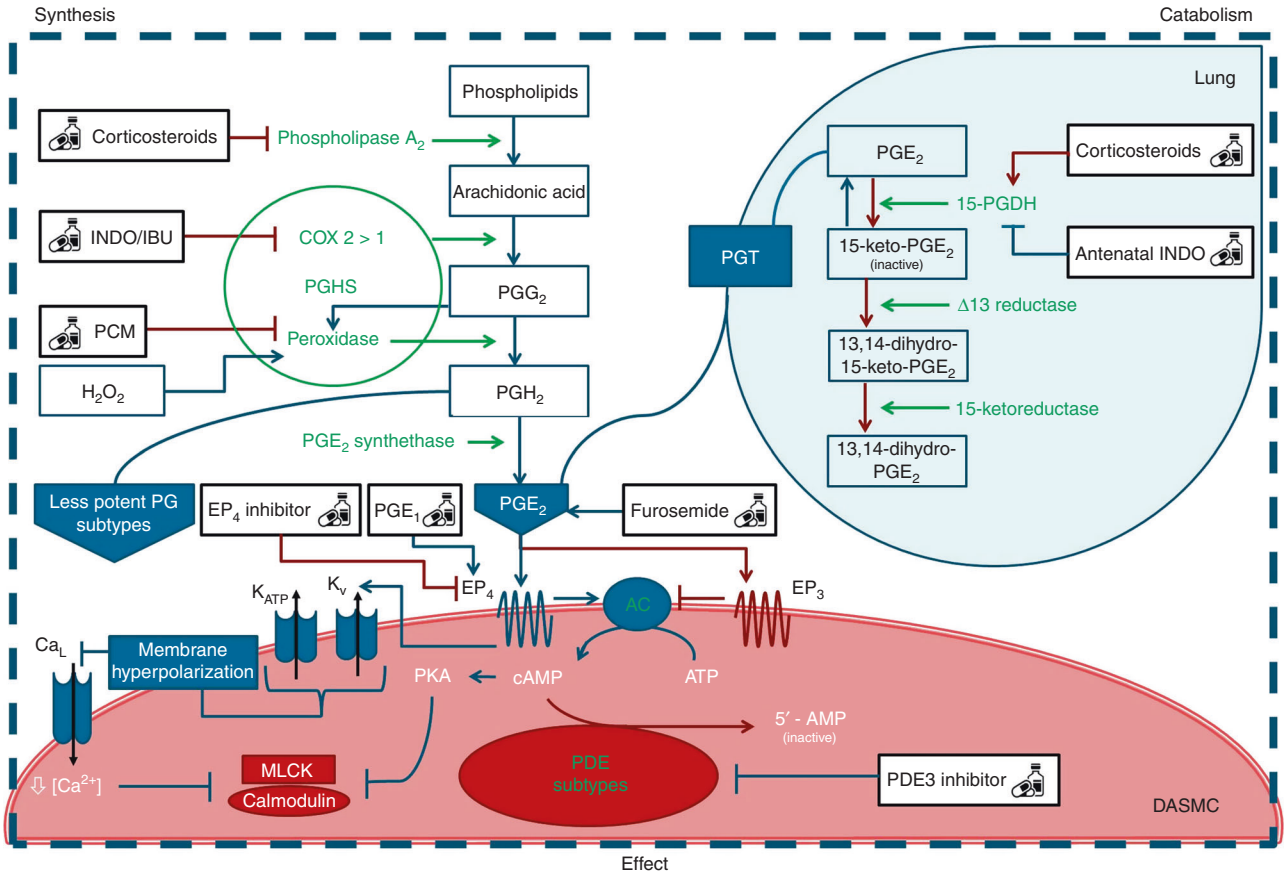


Fig. 1 Prostaglandin pathway with synthesis, catabolism and effect related to DA vascular tone. Blue indicates vasodilatory pathways, red indicates vasoconstrictive pathways, enzymes are depicted in green and drugs/pharmaceuticals are represented by boxes with the medication symbol. Black arrow headed lines indicate the normal direction of ions across the membrane. Arrow headed lines indicate stimulation and bar-headed lines indicate inhibition, the net effect of these actions on the vascular tone are indicated by the color of the lines *15-PGDH*, 15-hydroxy prostaglandin dehydrogenase; *AC*, adenylyl cyclase; *AMP*, Adenosine monophosphate; *PCM*, N-acetyl-para-aminophenol/acetaminophen; *ATP*, adenosine triphosphate; *BK*, bradykinin; BK_2, BK-2 receptor; *CaL*, L-type voltage-gated calcium channel; *cAMP*, cyclic adenosine monophosphate; *COX*, cyclooxygenase; *DASMC*, ductus arteriosus smooth muscle cell; EP_3, prostaglandin E2 receptor 3; EP_4, prostaglandin E2 receptor 4; *IBU*, ibuprofen; *INDO*, indomethacin; K_{ATP}, ATP sensitive potassium channel; K_V, voltage-gated potassium channel; *PDE*, phosphodiesterase; *PGHS*, prostaglandin H synthase; *PGT*, prostaglandin transporter; *PKA*, cAMP-dependent protein kinase

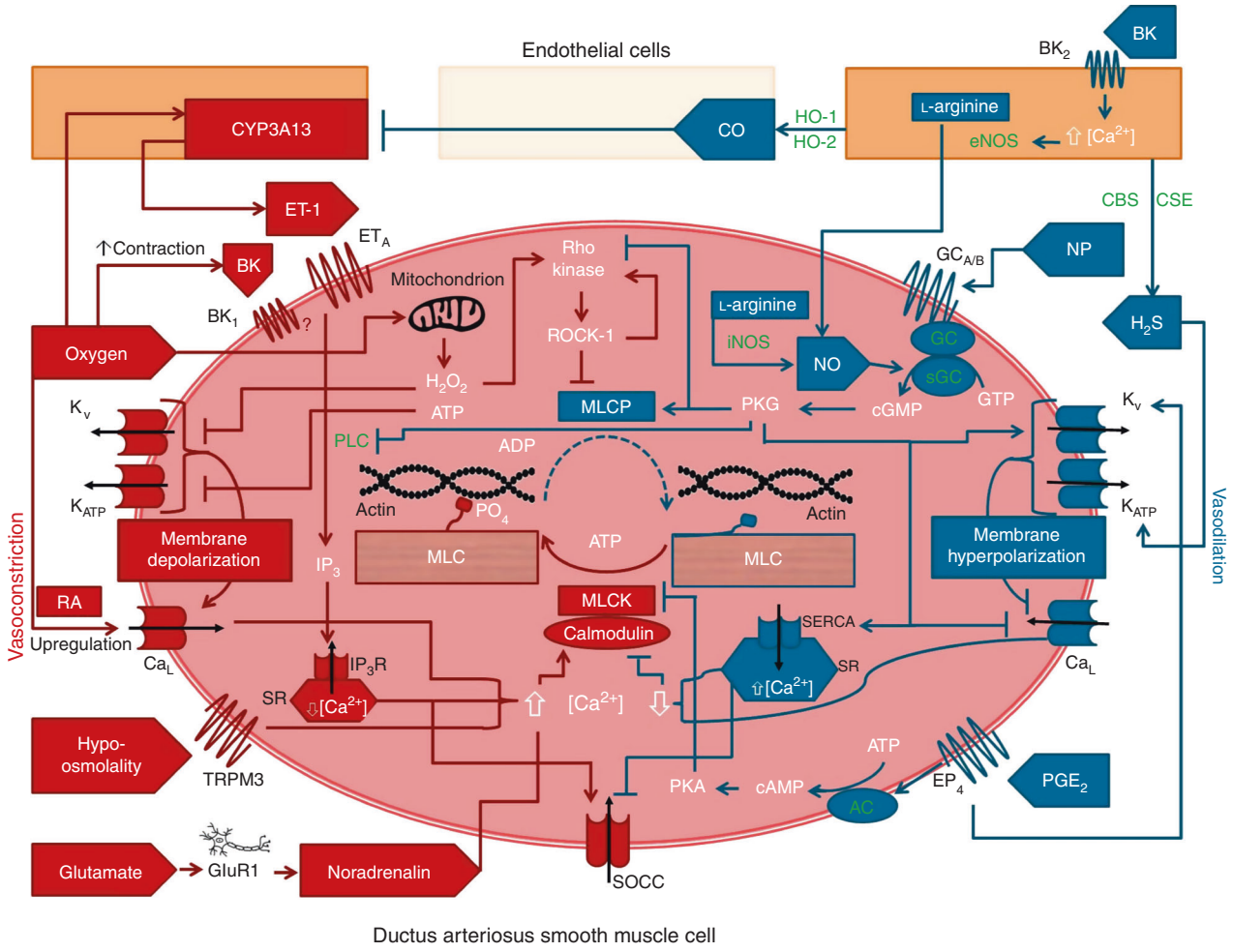
PATENCY OF THE DA IN THE FETUS

PG play a major role in fetal DA patency. PG are synthesized from phospholipids (Fig. 1) and PGE_2 is the most potent subtype.[15–17] PGE_2, produced in the placenta and fetal DA,[18] activates the PGE_2 receptor 4 (EP_4), which induces several effects.[2,19–22] It increases the K^+ current (outward) by voltage-gated potassium channel (K_v channel) activation, thereby inducing membrane hyperpolarization which inhibits Ca^{2+} influx via the voltage-gated L-type calcium channel (Ca_L channel) and decreases intracellular [Ca^{2+}].[21] PGE_2 also induces the formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) by activating adenylyl cyclase.[2,19,20,23] cAMP activates cAMP-dependent protein kinase (PKA), which inhibits MLCK.[22] As inhibition of MLCK avoids phosphorylation of the MLC, vasoconstriction is inhibited, hence vasodilation is maintained.

Noteworthy, animal models have shown that both EP_4- and COX-knockout is associated with PDA.[20,24–26] Furthermore, these animals fail to respond to postnatal therapy with INDO or IBU.[20,24,25] This is probably the result of concurrent alteration in other vasodilatory or vasoconstrictive pathways in the absence of PGE_2 mediated vasodilation.[27] Additionally, since EP_4 signaling induces anatomical remodeling of the DA, EP_4 knockout may further attenuate DA closure.[20,26]

NO is formed in the DA wall by the endothelial nitric oxide synthase (eNOS) isoform (Fig. 3).[28,29] After endothelial production NO diffuses into the adjacent DASMC.[29] There it binds with soluble guanylyl cyclase, leading to the production of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate.[14] cGMP activates cGMP-dependent protein kinase (PKG) which eventually induces vasodilation.[30] PKG decreases intracellular [Ca^{2+}] by inhibiting Ca^{2+} influx due to inhibition of Ca_L channels and stimulation of uptake of Ca^{2+} in the sarcoplasmic reticulum (SR). The uptake of Ca^{2+} in the SR is the result of inhibition of Ca^{2+} efflux through the inositol triphosphate (IP_3) receptor (IP_3R) and stimulation of Ca^{2+} influx via sarco/endoplasmic reticulum calcium ATPase (SERCA). This subsequently leads to inhibition of the store-operated calcium channels (SOCC), which also prevents a rise in intracellular [Ca^{2+}].[31,32] Furthermore, PKG stimulates MLCP direct and indirect by inhibiting the inhibitory effect of Rho kinase, which will be discussed in the section about postnatal DA constriction (Fig. 2; Fig. 3).[30]

Apart from endothelial production, NO can be formed in the DASMC itself by inducible NOS (iNOS) and neuronal NOS (nNOS), although they are only found in minimal amounts in the DA. Despite their low mRNA expression, these isoforms might contribute to DA relaxation since bradykinin (BK) induced relaxation was suppressed after non-selective NOS inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) in wildtype mice, but was preserved in untreated eNOS knockout mice.[33] It was found in



Ductus arteriosus smooth muscle cell

Fig. 2 Overview of pathways involved in regulation of DA vascular tone (based on Zhao et al.[14] and Hung et al.[148]). Blue indicates vasodilatory pathways, red indicates vasoconstrictive pathways and enzymes are depicted in green. Black arrow headed lines indicate the normal direction of ions across the membrane. Arrow headed lines indicate stimulation and bar-headed lines indicate inhibition, the net effect of these actions on the vascular tone are indicated by the color of the lines. *AC*, adenylyl cyclase; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *BK*, bradykinin; *BK_1*, BK-1 receptor; *BK_2*, BK-2 receptor; *Ca_L*, L-type voltage-gated calcium channel; *cAMP*, cyclic adenosine monophosphate; *CBS*, cystathionine β-synthase; *CSE*, cystathionine γ-lyase; *cGMP*, cyclic guanosine monophosphate; *CYP3A13*, Cytochrome P_{450} 3A13; *eNOS*, endothelial nitric oxide synthase; *EP_4*, prostaglandin E_2 receptor 4, *ET-I*, endothelin-I; *ET_A*, endothelin type A receptor; *GC*, guanylyl cyclase; *$GC_{A/B}$*, guanyl cyclase A/B receptor; *GluR1*, glutamate inotropic receptor subunit 1; *GTP*, guanosine triphosphate; *HO-1*, heme oxygenase 1; *HO-2*, heme oxygenase 2; *iNOS*, inducible nitric oxide synthase; *IP_3*, inositol triphosphate; *IP_3R*, IP_3 receptor; *K_{ATP}*, ATP sensitive potassium channel; *K_v*, voltage-gated potassium channel; *MLC*, myosin light chain, *MLCK*, MLC kinase; *MLCP*, MLC phosphatase; *PGE_2*, prostaglandin E_2; *NO*, nitric oxide; *NP*, natriuretic peptide; *PKA*, cAMP-dependent protein kinase, *PKG*, cGMP-dependent protein kinase; *PLC*, phospholipase c; *RA*, Retinoic acid; *ROCK*-1, Rho-associated coiled-coil forming protein kinase 1; *sGC*, soluble GC; *SERCA*, sarco/endoplasmic reticulum calcium-ATPase; *SOCC*, store-operated calcium channel; *SR*, sarcoplasmic reticulum; *TRPM3*, transient receptor potential melastatin 3

a lamb model that inhibition of nNOS and eNOS was fifty times more potent in DA constriction than inhibition of iNOS.[29] In utero, at low PaO_2 and low BK concentration, vasodilation occurs via BK-2 receptor activation.[34,35]

Heme oxygenase-1 (HO-1) and 2 (HO-2), enzymes responsible for the synthesis of CO, have been identified in the DA.[36] CO, produced by the DASMC, dilates the lamb DA via inhibition of O_2-sensing cytochrome P_{450} (CYP) 3A13 and thus interrupting endothelin-1 (ET-1) signaling (Fig. 2).[37] HO-1/HO-2 inhibition contracted mice DA to a similar degree as INDO.[33] However, deletion of HO-2 is not followed by an upregulation of the NO pathway, as is seen after COX deletion.[28,33] Contrarily, in the lamb DA HO-1/HO-2 inhibition showed no effect on the resting tone.[36] Of interest, when PGE_2, NO and CO were suppressed in a mouse model another endothelium derived hyperpolarizing factor became evident after stimulation with BK,[33] which was later identified as H_2S.[38]

In mice DA two H_2S forming enzymes, namely cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), were identified.[38] H_2S-induced DA vasodilation occurs at high [H_2S] by opening of ATP sensitive potassium channels (K_{ATP} channel).[39] Inhibition of CBS or CSE attenuated the vasodilation. In avian DA no vasodilatory effects of H_2S were found.[40]

Transient receptor potential melastatin 3 (TRPM3), which is more expressed in the DASMC than in the aorta, acts as a calcium channel which increases intracellular [Ca^{2+}] independently of Ca_L channels.[41] Progesterone, a natural TRPM3 inhibitor in human, might prevent DA closure in utero.[42]

In summary, vasodilation is induced by dephosphorylation of MLC by MLCP. In utero MLCP is enhanced due to the inhibitory effect of PKG on the Rho-kinase pathway (Fig. 2). PKG also activates K_V channels, leading to hyperpolarization and thereby inhibiting Ca^{2+} influx via Ca_L channels. Another effect of PKG is stimulation of the SERCA, which leads to uptake of Ca^{2+} in the SR

Table 1. Ion channels as common pathway and myosin light chain kinase of phosphatase as the final common pathway in the regulation of DA vascular tone, mediators and effect

	Vasoconstriction	Vasodilation
Common pathway 1	Inhibition of K_v and K_{ATP} channels	Stimulation of K_v and K_{ATP} channels
Mediators	H_2O_2, ATP	PKG, PGE_2 via EP_4, H_2S
Common pathway 2	Activation of Ca_L channels	Inhibition of Ca_L channels
Mediators	Membrane depolarization by inhibition of K_v and K_{ATP} channels, Ca_L channels upregulated by retinoic acid	Membrane hyperpolarization by stimulation of K_v and K_{ATP} channels, PKG
Common pathway 3	Increased [Ca^{2+}]	Decreased [Ca^{2+}]
Mediators	Activation of Ca_L channels, activation of $TRPM_3$ by hypo-osmolality, glutamate induced noradrenalin production, ET-1 induced IP_3R and SOCC stimulation	Inhibition of Ca_L channels, stimulation of SERCA and inhibition IP_3R and SOCC
Final common pathway 1	MLCP inhibition	MLCP activation
Mediators	ROCK-1, formed after H_2O_2 induced Rho-kinase activation	PKG, both directly, and indirectly by inhibiting the inhibitory effect of ROCK-1 via Rho-kinase inhibition
Final common pathway 2	MLCK activation	MLCK inhibition
Mediators	Ca^{2+}/Calmodulin complex formed with increased [Ca^{2+}]	PKA and decreased [Ca^{2+}]

ATP adenosine triphosphate, *Ca_Lchannel* L-type voltage-gated calcium channel, *EP_4* PGE_2 receptor 4, *ET-1* endothelin-1, *PGE_2* prostaglandin E_2,*IP_3R* Inositol triphosphate receptor, *K_{ATP}* ATP sensitive potassium channel, *K_v* voltage-gated potassium channel, *MLCK* myosin light chain kinase, *MLCP* myosin light chain phosphatase, *PKA* cAMP-dependent protein kinase, *PKG* cGMP-dependent protein kinase, *ROCK*-1 Rho-associated coiled-coil forming protein kinase 1, *SERCA* sarco/endoplasmic reticulum calcium-ATPase, *SOCC* store-operated calcium channel, *TRPM3* transient receptor potential melastatin 3

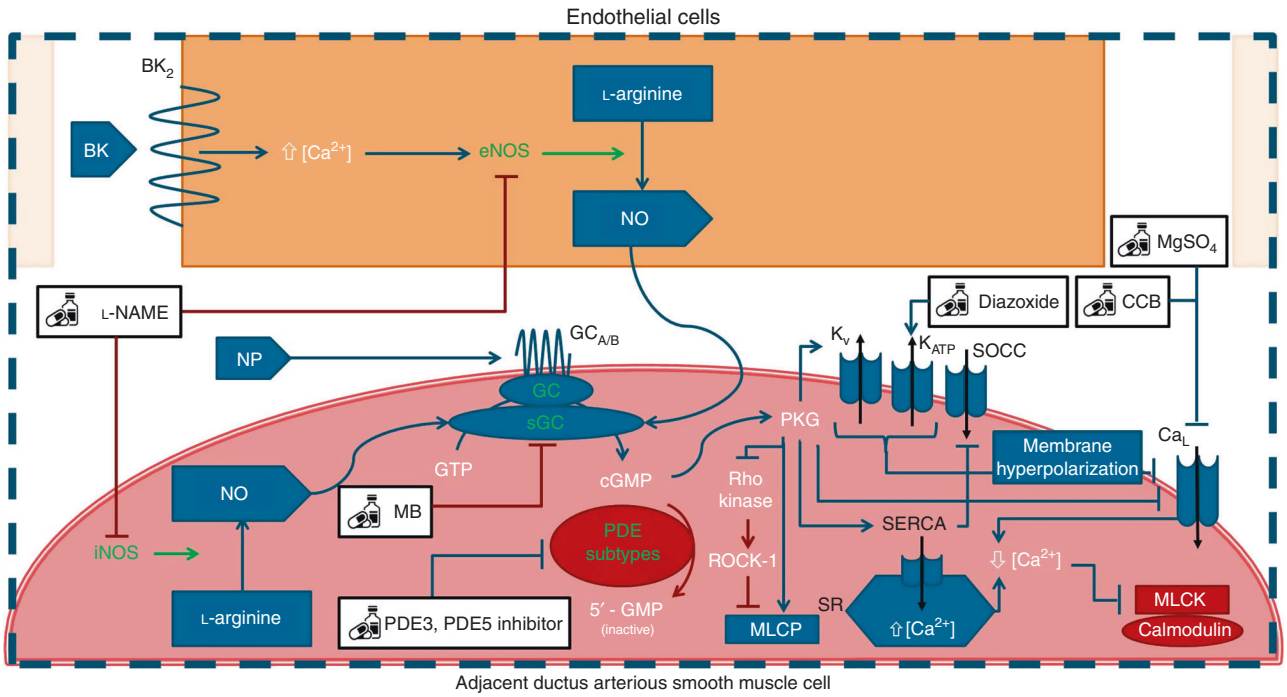


Fig. 3 NO pathway with mechanisms of intervention. Blue indicates vasodilatory pathways, red indicates vasoconstrictive pathways, enzymes are depicted in green and drugs/pharmaceuticals are represented by boxes with the medication symbol. Black arrow headed lines indicate the normal direction of ions across the membrane. Arrow headed lines indicate stimulation and bar-headed lines indicate inhibition, the net effect of these actions on the vascular tone are indicated by the color of the lines. *BK*, bradykinin; *BK_2*, BK-2 receptor; *Ca_L*, L-type voltage-gated calcium channel; *CCB*, calcium channel blocker; *cGMP*, cyclic guanosine monophosphate; *eNOS*, endothelial nitric oxide synthase; *GC*, guanylyl cyclase; *$GC_{A/B}$*, guanyl cyclase A/B receptor; *GTP*, guanosine triphosphate; *iNOS*, inducible nitric oxide synthase; *K_{ATP}*, ATP sensitive potassium channel; *K_v*, voltage-gated potassium channel; *L-NAME*, NG-nitro-L-arginine methyl ester; *MB*, methylene blue; *MLCK*, myosin light chain kinase; *MLCP*, myosin light chain phosphatase; *NO*, nitric oxide; *NP*, natriuretic peptide; *PKG*, cGMP-dependent protein kinase; *ROCK*-1, Rho-associated coiled-coil forming protein kinase 1; *sGC*, soluble GC; *SERCA*, sarco/endoplasmic reticulum calcium-ATPase; *SOCC*, store-operated calcium channel; *SR*, sarcoplasmic reticulum

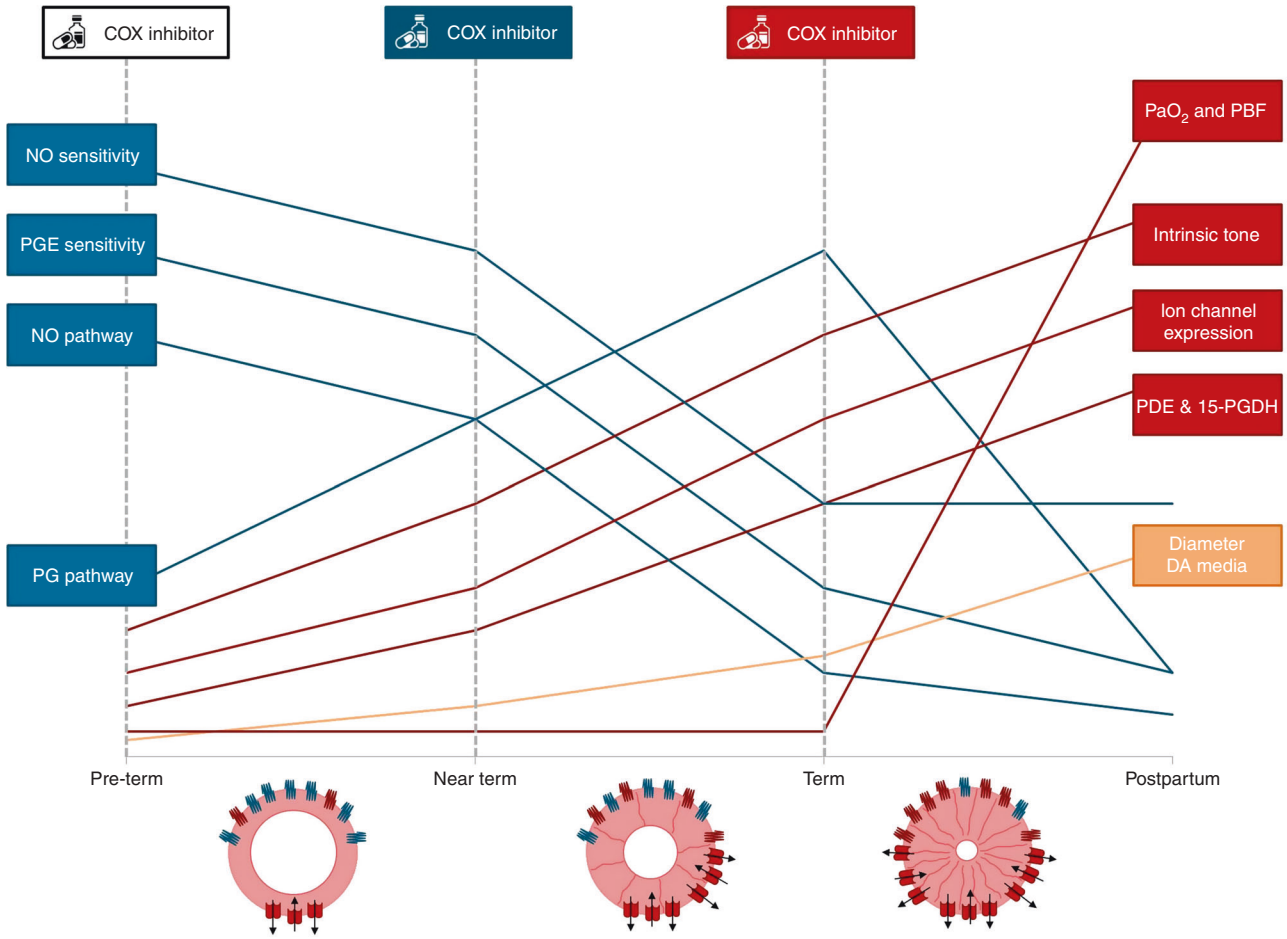


Fig. 4 Schematic drawing of the main changes in pathways involved in DA patency (blue diagrams), functional DA closure (red diagrams), and anatomical closure (orange diagrams) and the effect of antenatal COX inhibition at different moments during gestation on postnatal DA closure or patency. Under the *x*-axis are schematic drawings of the DA at different moments with available receptors (blue vasodilatory and red vasoconstrictive), vasa vasorum (pink lines in DA media) and ion channel expression. *15-PGDH*, 15-hydroxy prostaglandin dehydrogenase; *COX*, cyclooxygenase; *NO*, nitric oxide; *PaO_2*, oxygen tension; *PBF*, pulmonary bloodflow; *PDE*, phosphodiesterase; *PG*, prostaglandin

and inhibition of Ca^{2+} influx through SOCC, further lowering the intracellular [Ca^{2+}]. PKA, activated by the PG pathway, inhibits MLCK, thereby preventing phosphorylation, and thus leading to vasodilation (Figs. 1, 2; Table 1).

The effect of antenatal exposure to various maternal medications on the fetal DA

Exposure to pharmacological COX inhibition during pregnancy is associated with both DA closure in utero and postnatal PDA, depending on the timing of administration during gestation (Fig. 4).[24,27,43] In mice, early COX inhibition (day 11–14, term = 19 days) did not interfere with DA patency in utero, nor DA closure after birth.[43] However, near-term exposure (from day 15 to 18–19) made the DA unresponsive to acute COX inhibition postnatally and was associated with PDA. Acute treatment with COX inhibition at day 18–19 in mice resulted in DA constriction in utero.[27] In sheep, after fetal exposure to INDO the expression of eNOS and muscle media cell death increased, which made the DA unresponsive to postnatal vasoconstrictive treatment.[44] In fetal mice, inhibition of the PG pathway by INDO, promoted the vasodilatory effect of NO on the DA.[45] It appears that NO and PGE_2 are coupled for reciprocal compensation at least during fetal life, meaning that in the absence of PGE_2 the NO pathway will be upregulated and vice versa.[28] Furthermore, antenatal COX inhibition decreases PGE_2 catabolism by 15-hydroxy prostaglandin dehydrogenase (15-PGDH), predisposing the DA to stay patent (Fig. 1).[46] On the other hand, antenatal steroids attenuate the sensitivity of the DA to PGE_2 and increase PGE_2 catabolism by 15-PGDH.[47,48]

In a recent systematic review 116 cases of fetal DA closure or constriction were described, that occurred at a mean gestational age of 32.2 ± 3.8 weeks, of whom 58 (50%) had received non-steroidal anti-inflammatory drugs.[49] Since postnatal treatment with high dose PCM appears as effective as IBU or INDO in DA closure, the safety of maternal PCM use was recently questioned.[50–52] However, PCM is widely used during pregnancies (37–53%) and fetal closure seems to be extremely rare.[52,53]

The role of prostaglandin E_2 in programming the fetal DA for postnatal closure

Although PGE_2 is the most potent DA vasodilator in fetal life, there is growing evidence that supports a dual role for PGE_2 in late gestation in preparing the DA for both functional and anatomical closure.[27,43,54–56] PGE_2 is involved in the expression of contraction-related genes.[27,43,55] PGE_2 deficient mice showed lower mRNA expression of the Ca_L (α1C and β2 subunits) and potassium channels (K_V 1.5 and Kir6.1 subunit), which leads to decreased O_2-induced DA contractility.[27,43] This will be discussed in the next paragraph. The decreased contractile response to O_2 might

explain why inhibition of the NO pathway, although upregulated in the absence of PGE_2, did not prevent delayed DA closure after antenatal COX inhibition.[27] With increasing gestational age, the lamb DA shows upregulation of several phosphodiesterase (PDE) subtypes that degrade cAMP to the biologically inactive 5′-adenosine monophosphate (Fig. 4).[43,54,57,58] This implies a limited intracellular accumulation of cAMP and therefore an attenuation of the vasodilator effects of PGE_2 when the fetus matures. Furthermore, PGE_2-mediated upregulation of PDE subtypes was found in term rat, lamb and neonatal human DA treated with PGE_2.[43,54,58]

POSTNATAL FUNCTIONAL DA CLOSURE IN TERM INFANTS

As stated above, several adaptations during late gestation contribute to an increase in intrinsic tone and rapid decline in cAMP in DASMC after birth, which in synergy with O_2-induced ion channel activation, initiates DA contraction (Fig. 4). PGE_2 concentration drops postnatally, due to absence of placental production and increased PGE_2 catabolism in the lungs.[3,59] The rate of breakdown of PG in the lung is controlled and limited by the PG transporter (PGT) that controls PGE_2 influx specifically into type II alveolar epithelial cells (Fig. 1).[60,61] This is supported by the fact that PGT knockout mice fail to close their DA.[61] Postnatal COX inhibition in these mice closed their DA,[61] further supporting the upregulation of other vasodilatory pathways in the absence of PGE_2. After PGE_2 influx, 15-PGDH reversibly metabolizes PGE_2 to the biologically inactive 15-keto-PGE_2. 15-PGDH activity increases with gestational age in rats (Fig. 4), while in lambs the 15-PGDH activity decreases as gestation advances.[59,62] 15-keto-PGE_2 is irreversibly metabolized by Δ13-reductase, which concentration remained constant in rats but increased in lambs during fetal maturation.[59,62] Apart from the PGE_2 catabolism, the lungs also play a role in postnatal BK production.[34,35] Although BK induces vasodilation in utero as described earlier, at higher concentrations it induces vasoconstriction via the BK-1 receptor.[34,35]

After birth, a change in EP receptor expression is seen in several animal models. The postnatal expression of EP_4 decreases[2,63] and the increase in PaO_2 reduces DA sensitivity to PGE_2 in the rabbit.[64,65] Activation of PGE_2 receptor 3 (EP_3), although inhibiting cAMP production by adenylyl cyclase after stimulation by PGE_2 (Fig. 1), dilated the DA both in vitro and in vivo in sheep.[2] However, in term rabbits high EP_3 levels were found minutes after birth which induced vasoconstriction.[63] In pigs, fetuses expressed PGE_2 receptor 2 (EP_2), EP_3, and EP_4.[66] Neonatal pigs expressed EP_2 only, at the same level as fetuses. EP_2 has similar effects as EP_4 leading to an overall decrease in PGE_2 receptor expression.[66]

During fetal-to-neonatal transition a major change occurs in the exposure to O_2, from hypoxemia (40 mmHg) to normoxemia (>80 mmHg). This postnatal increase in PaO_2 plays a pivotal role in DA closure (Fig. 4). Both K_V and K_{ATP} channels appear to function as O_2-sensors, since they are inhibited under normoxemic conditions.[67–70] Inhibition of these channels results in membrane depolarization, which opens Ca_L channels. The resulting increase in intracellular [Ca^{2+}] initiates vasoconstriction (Fig. 2).[71]

The mitochondrion plays a role in O_2-induced contraction by increasing H_2O_2 and ATP production (Fig. 2).[69,72] Mitochondrial ATP production through oxidative phosphorylation increases with rising PaO_2 levels and inhibits K_{ATP} channels in the rabbit.[69] Mitochondrial production of H_2O_2 increases with maturation,[73] which inhibits K_V channel activity.[21,72,74–77] Furthermore, H_2O_2 increases the expression of Ras homolog gene family member B (RhoB) relatively specific in the DA, and both expression and activation of Rho-associated coiled-coil forming protein kinase 1 (ROCK-1).[73] Since ROCK-1 inhibits MLCP and thereby dephosphorylation of the MLC, induced vasoconstriction is sustained. RhoB gene expression in the DA increased in rats with advanced gestation,[78] while Rho-kinase inhibitors led to vasodilation in rabbits.[79] O_2-induced contraction decreased when either complex I or complex III of the mitochondrial electron transfer chain was inhibited.[72,75] Of interest, grossly the same O_2-sensors and effectors play a role in vasoconstriction in the pulmonary vasculature, however these are stimulated by hypoxemia instead of normoxemia/hyperoxemia.[80,81] Isoprostanes (i.e., 8-iso-PGE_2) are produced by free radical peroxidation of arachidonic acid. In term mice exposure to isoprostanes induced DA constriction via the Thromboxane A_2 receptor (TP).[82]

Retinoic acid, a metabolite of vitamin A, also has a role in O_2-induced DA constriction, and might be an O_2-sensing factor.[55,83] Maternal administration of vitamin A upregulated the expression of Ca_L channels in rat,[55] thereby increasing the effect of O_2-induced inhibition of K_V channels.[83] However, in a double-blind, placebo-controlled trial in 40 preterm human infants postnatal vitamin A treatment had no effect on DA closure.[84]

Several studies showed that CYP and ET-1 play a role in DA closure,[85–91] although one study in human did not find an effect.[76] O_2 stimulates the release and synthesis of ET-1, via CYP3A13, acting as vasoconstrictor via the endothelin type A receptor (ET_A). Stimulation of ET_A induces IP_3 production by phospholipase c, which leads to an increase in intracellular [Ca^{2+}] by activation of the IP_3R (Fig. 2).[92] Depletion of Ca^{2+} in the SR activates SOCC, which further increases Ca^{2+} influx.[32,79] Both non-selective endothelin receptor antagonists in rats, and ET_A antagonists in lambs have been found to inhibit spontaneous DA constriction.[85,93] In another study, no effect of ET_A on postnatal DA constriction was found both in vivo and in vitro in sheep.[94]

Newborns show a transient decline in their serum osmolality after birth, which recovers to "adult" levels over the next few days.[95] A potential physiological role of this temporary decrease in osmolality in facilitating DA closure was recently postulated.[41] In rats, this decrease in serum osmolality, leads to increased activation of TRPM3, and thereby increased [Ca^{2+}]. On the contrary, the DA vasodilates in hyperosmolar states in a TRPM3 independent way.

It was shown in rats that the amino acid glutamate promotes DA contraction.[96] The mechanism of action is by postsynaptic noradrenalin production via glutamate inotropic receptor subunit 1 stimulation (Fig. 2). This suggests that adequate nutritional intake of amino acids also influences DA closure, at least in rats since human studies are currently lacking. In mice, glutamate induces cortical vasodilation in the brain, via increased NO production after stimulation of the GluN1-NMDA receptors.[97] In the DA glutamate-induced vasodilation is not to be expected, since the GluN1-NMDA receptor was not expressed in rat DA.[96]

Apart from the earlier discussed K_V and K_{ATP} channels other potassium channels might play a role in regulating the DA vascular tone. The large-conductance voltage-dependent and calcium-activated potassium channel (BK_{Ca} channel) is expressed in the rat DA,[98] although its role is not yet clarified.[75,98] Even though BK_{Ca} channels respond to O_2, it is thought to be unlikely that they are involved in O_2-induced DA constriction, since activation of these channels would lead to a decrease of intracellular [Ca^{2+}] and thus vasodilation.[75,98–100] Apart from the Ca_L channel, there might be a role for the T-type voltage-dependent Ca^{2+} channel, not only in O_2-induced constriction,[69,101] but also in anatomical closure of the DA.[101]

In summary, after birth the drop in PGE_2 impairs DA vasodilation, whereas a rise in PaO_2 leads to vasoconstriction in a temporal sequence.[73] It starts after an increase in intracellular [Ca^{2+}] via activated Ca_L channels, secondary to inhibition of K_V and K_{ATP} induced membrane depolarization.[67,76,102] This rise leads to Ca^{2+}/Calmodulin-dependent MLCK activation, which phosphorylates MLC.[103] Calmodulin1 expression is upregulated in the rat DA after birth.[78] As MLCK depends on the influx of extracellular [Ca^{2+}], ET-1 synthesis is increased to release intracellular Ca^{2+}

from the SR and subsequently SOCC.[32,79,85,87] Finally, the Rho-kinase pathway inhibits MLCP, thereby inducing Ca^{2+}-sensitization by reducing the requirement for Ca^{2+} influx, thus maintaining vasoconstriction.[73]

PATENT DA IN THE PRETERM INFANT

The preterm DA shows a higher sensitivity to PGE_2 than near-term DA (Fig. 4).[15,104–106] This is supported by the fact that PGE_2 concentrations in blood did not differ between human infants with or without a PDA.[107,108] PGE_2 catabolism is reduced due to decreased 15-PGDH activity in early gestation.[59] In a sheep and baboon model the increased sensitivity to PGE_2 in preterm compared to term infants was attributed to increased PGE_2 binding to EP_2, EP_3, and EP_4 with equivalent mRNA and protein expression.[109] Furthermore, the increased production of cAMP induces a more vasodilatory effect,[109] probably due to reduced breakdown by PDE3 which is less expressed in early gestation.[43,54,57,58] In preterm mice 8-iso-PGE_2 exposure caused vasodilation, which could be reversed with EP_4 inhibition.[82] Gene expression revealed low TP gene expression until near term and high EP_4 expression throughout gestation. Postnatal O_2 exposure-induced formation of isoprostanes might promote vasodilation in preterm infants via EP_4 activation, instead of vasoconstriction via TP activation.

The immature DA seemed six times more sensitive to dilation by NO than the mature DA in lambs (Fig. 4).[29] Under hyperoxemic (neonatal) conditions, L-NAME increased the vascular tone more in preterm than in term DA.[1] However, the contraction produced by inhibiting NO production with L-NAME, was not enhanced in the immature DA under hypoxemic (fetal) conditions.[29] This suggests that postnatal increase in PaO_2 might enhance NO production, which due to an increased sensitivity for NO in combination with decreased expression of ion channels in the preterm DA, might eventually induce vasodilation.

In rats the largest decrease in DA caliber was seen after L-NAME at day 19 (term = 30).[110] After INDO treatment the largest decrease was seen on day 21. This suggests that NO and PGE_2 are not only coupled for reciprocal compensation, but that NO predominates during early gestation and PGE_2 during advanced gestation (Fig. 4). A PDE3 inhibitor increases both cGMP and cAMP accumulation. Since cGMP is derived from the, in preterm more prominent, NO pathway one can explain that in rats, a PDE3 inhibitor dilated the preterm DA, that was preconstricted with INDO, more intense than the term DA.[111]

Conflicting results were found from gene expression studies, showing an increased expression of iNOS with advanced gestation in lambs.[57] In fetal baboons, immature gestation had no effect on both iNOS and eNOS mRNA expression.[112] However, the eNOS expression also increases in human fetuses with advancing gestation,[113] or after fetal inhibition of PGE_2 in mice.[45] eNOS is not only expressed by luminal but also vasa vasorum endothelial cells that are required for sufficient oxygenation of the DA muscle media.[29] The NO and PGE_2 coupling for reciprocal compensation might explain why a substantial part of preterm infants fail to close their DA after INDO, IBU, or PCM.[114] In non-Caucasian preterm infants, a better response towards INDO is seen.[115–117] This could be related to a decreased gene expression of SLCO2A1 (i.e., PGT) and eNOS,.[113] This better response indicates that non-Caucasian infants are at increased risk of PDA by extensive PGE_2 concentrations, which are attenuated after INDO.

In preterm baboons, combination of INDO with a non-selective NOS inhibitor had a greater vasoconstrictive effect than INDO alone.[118] This has also been observed in preterm and term rats.[119,120] A phase I and II trial in human preterm infants using INDO in combination with non-selective NOS inhibition showed increased DA constriction but had to be stopped early due to side effects, especially hypertension.[121]

Although H_2S induces vasodilation in utero, vasoconstriction occurs at lower [H_2S] in which K_{ATP} channels are not likely to be involved.[39] Due to this biphasic response, it was hypothesized that postnatal H_2S oxidation might play a role in DA closure, either by decreasing the [H_2S] or by the formation of a putative oxidation product that plays this role.[38]

A small cohort of human preterm infants with a PDA ($n = 15$) showed a significant faster increase in serum osmolality after initial hypo-osmolality compared to preterm infants without a PDA ($n = 12$).[41] A recent prospective study in 799 preterm infants only found a 5 mOsm/L higher osmolality in the hemodynamic significant PDA group compared to the non-hemodynamic significant PDA group.[122] In human PDA a lower protein expression of TRPM3 was found, compared to constricted DA.[123] Since studies in TRPM3 knockout mice did not report the occurrence of PDA, this pathway seems of minor importance than the PG, NO and O_2-induced pathways.[124,125]

NP increases intracellular cGMP via guanyl cyclase-A and -B receptors,[126] inducing vasodilation (Figs. 2, 3).[127] In PDA, the volume of left-to-right shunting is associated with NP level.[128] Higher baseline levels before INDO treatment predict a poor response.[114,128] The NP mediated increase in cGMP actually neutralizes INDO mediated reduction in cAMP, shifting the balance to vasodilation. These findings suggest that NP is not only a marker of cardiac failure, but could also play a role in DA tone and therapeutic failure.

Animal studies showed that the preterm DA contracts less intense upon higher PaO_2 than the near-term DA.[15,21,72,102] The attenuated O_2-induced contraction in the preterm DA is due to a reduced PGE_2 induced gene expression of ion channels.[27,43,55,68,72,102] This is supported by the observation that K_V channel inhibition does not induce depolarization in the preterm rabbit DA.[21,102] In a baboon model, three calcium- and potassium channel genes were similarly affected in prematurity.[112]

In summary, during gestation and after birth changes occur in both the concentration of various mediators and the sensitivity of the DA to these mediators (Fig. 4). Preterm infants fail to program their DA for postnatal constriction by reduced PGE_2 expression. Apart from this functional immaturity of the DA, one should note that the DA anatomy itself is also immature (i.e., a smaller diameter of the DA media with absence of vasa vasorum), which prevents sufficient muscle media hypoxia in the DA and thereby postponing anatomical closure (Fig. 4), which is beyond the scope of this review.[129]

THE EFFECT OF ANTE- OR POSTNATAL EXPOSURE TO VARIOUS MEDICATIONS ON THE DA IN THE (PRE)TERM INFANT

Mg^{2+}, a natural calcium channel blocker, administered antenatally as $MgSO_4$, results in a dose-dependent delayed DA closure and a higher incidence of PDA.[130–134] This effect seems to be short, since the responsiveness to postnatal INDO prophylaxis is reduced while the symptomatic treatment in a later phase is not influenced.[132] The only report on antenatal use of a calcium channel blocker, nifedipine, did not find a higher prevalence of PDA.[135] Antenatal steroids, increase 15-PGDH activity, thereby promoting PGE_2 breakdown (Fig. 1).[106] Diazoxide, a K_{ATP} channel activator used for example in hyperinsulinemic hypoglycemia, induced DA opening in rats.[136] A Japanese questionnaire-based cohort study analyzed the effect of diazoxide and showed 8% reopening of the DA in 25 VLBW infants, compared to 1.9% in 53 non-VLBW infants treated with diazoxide for hyperinsulinemic hypoglycemia.[137] Moreover, it was found in rabbits that sulfonylurea, a K_{ATP} channel inhibitor, induced DA constriction.[69] Furosemide increases renal PGE_2 production and has been shown to dilate the DA in rats.[138] A recent analysis of 4055 VLBW infants that were treated with furosemide revealed no association with PDA requiring treatment.[139]

FUTURE PERSPECTIVES

Research

Several, mainly mammalian animal models have been used to gain more insight in the pathobiology of a PDA in prematurity. Each model has its own strengths and limitations. For example, the avian model is very suitable to assess O_2-induced vasoconstriction, whereas the absence of vasa vasorum in the DA of rats mimics the preterm human. Recently, it was suggested to expand animal studies to lungfish, in which DA constriction is O_2-sensitive and reversible, for further exploration of these pathobiological processes.[140] As already stated in the introduction one should be aware of interspecies differences. In an attempt to translate these findings from animal studies to human, many researchers also tested their hypotheses on human DA tissue. Further research is needed in an attempt to better understand PDA in prematurity and explore innovative therapeutic interventions for PDA in preterm infants.

Novel treatment strategies

Current pharmacological treatment options for PDA, namely PCM, IBU, and INDO, all intervene in the PG pathway. Since treatment failure of these drugs is common,[13] PCM was combined with IBU in two pilot studies that suggested a higher DA closure rate in the combined treatment group.[141,142] Antenatal exposure to corticosteroids also plays a role in an attempt to reduce PDA in preterm infants by promoting DA constriction via PGE_2 breakdown,[59,106] decreasing PGE_2 sensibility of the DA,[47,48] and possibly enhancing ion channel expression.[112] A single study in mice with a microsomal PGE synthase type 1 inhibitor showed dual effects on DA tone, ranging from vasoconstriction to vasodilation.[143] However, since there was no reciprocal increase in the NO pathway, further research regarding this enzyme might be worthwhile. Recently it was found that neonatal EP_4 inhibition with a EP_4 antagonist contracted the DA, with fewer adverse effects due to the selective expression of EP_4 in the rat DA.[144]

All these therapeutic options intervene in the PG pathway but the pathobiology is far more complex. Our review shows other potential pathways of interest that could be used for active DA closure. Influencing the NO pathway by NOS inhibition seems to be promising.[118–120] Although the only available clinical study which appeared to be effective was stopped early due to side effects.[121] Especially in patients with high NP levels, intervening in the cGMP-pathway appears to be more effective from a pathobiological point of view. Enhancing ion channel expression might be a novel therapeutic approach since in vivo gene transfer of K_V channels partially restored the O_2-induced vasoconstriction in the preterm rabbit DA.[102] Retinoic acid enhances the expression of Ca_L channels and thereby O_2-sensing in animal models,[55,83] but showed no effect in a small human trial.[84] Prevention of hypoxemia with surfactant and caffeine will enhance O_2-induced vasoconstriction.[145] Glutamate supplementation might support DA closure.[96] Considering the induced cerebral vasodilation there is not enough data about the safety of higher dosage[97,146–148] Trying to prevent hyperosmolality might also support DA closure and is safe, but unfortunately seems to be a pathway of minor importance.[41,122] As illustrated in this review, one should also be aware of unintended adverse effects of medications given for other purposes, like antenatal $MgSO_4$ and postnatal diazoxide. The silver bullet in PDA treatment must be an agent that works selectively on the DA, since unintended effects on other vasculature might lead to unacceptable side effects.

CONCLUSION

The DA is a highly intriguing vessel in postnatal hemodynamic transition. Since DA patency is essential for fetal homeostasis, this is enhanced by several pathways to overcome its high intrinsic tone and prevent DA closure in utero. As gestation progresses the DA gets programmed for postnatal closure, both functional and anatomical. After preterm birth the DA, not yet programmed to close, might additionally be influenced by several factors. These factors include antenatal medication, the medication given to the newborn, the maturity and the postnatal condition of the newborn. The combination of these factors probably explains the high incidence of PDA in prematurity. DA vascular tone regulation is a complex process. One can understand that simply intervening in only one pathway as we do now with PCM, IBU, and INDO, is ineffective in preventing a prolonged exposure to a PDA in a substantial part of preterm infants.

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AUTHOR CONTRIBUTIONS

T.H. initiated the idea to critically review the literature regarding the pathobiology in DA patency and designed the data search with MvdB. T.H. and M.v.d.B. interpretated the data. T.H. and M.v.d.B. drafted the first concept of the review. W.d.B. and R.v.d.L. critically revised the article. All authors have read and approved the final version of the review.

ADDITIONAL INFORMATION

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