Molecular, Genetic, and Pharmacological Modulation of the Ductus Arteriosus: K_{ATP} Channels as Novel Drug Targets

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Elaine L. Shelton and Jerod S. Denton

Abstract

Maintaining fetal patency and facilitating neonatal closure of the ductus arteriosus are complex processes that involve nuanced developmental programming and a precise balance of perinatal vasodilators and constrictors. Work over the past 40 years has identified “master regulators” of ductus tone, namely oxygen, prostaglandins, and nitric oxide signaling. Yet, prolonged neonatal patency of the ductus remains a significant clinical problem with limited therapeutic options. Understanding the molecular and genetic underpinnings of normal ductus function and PDA pathophysiology will facilitate the development of new therapeutic strategies to regulate ductus tone.

Keywords

Ductus arteriosus · PDA · K_{ATP} channel

E. L. Shelton (✉)

Department of Pediatrics, Monroe Carell Jr. Children’s Hospital at Vanderbilt and Vanderbilt University Medical Center, Nashville, TN, USA

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA
e-mail: Elaine.L.Shelton@vanderbilt.edu

J. S. Denton

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA
Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN, USA

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34.1 Introduction

The ductus arteriosus (DA) is an essential fetal vessel connecting the main pulmonary artery and aorta. In utero, high pulmonary vascular resistance coupled with relatively low systemic resistance promotes right-to-left blood flow through the DA, allowing blood oxygenated by the placenta to bypass the developing lungs [1, 2]. Soon after birth, the DA must close in order to facilitate proper perfusion of the newly inflated lungs, a process normally complete by 12–48 h in full-term neonates.

In some cases, the DA fails to close after birth, a condition termed patent ductus arteriosus (PDA). PDA is a common congenital heart defect, occurring in up to 80% of premature infants weighing <1000 g [3]. Several comorbidities including neuro-developmental impairment, intraventricular hemorrhage, pulmonary hemorrhage, respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis, and spontaneous intestinal perforation have been attributed to or associated with prolonged patency of the DA [4]. In contrast, keeping the DA open to preserve pulmonary or systemic circulation is essential in certain cardiovascular conditions where blood flow to the lungs or body is disrupted [5]. Unfortunately, there are surprisingly few therapies currently available to promote DA closure or maintain vessel patency. Pharmacology-based therapeutics are non-specific and relatively inefficient. While surgical ligation, catheter-based closure, and stent implantation are effective alternatives, these mechanical approaches come with their own risks and are sometimes limited by the anatomical size constraints of extremely premature newborns [6].

34.2 Molecular Regulation of the DA

The fetal DA has intrinsic tone and requires factors to maintain its patency in utero (summarized in Table 34.1) [2, 7, 8]. While nitric oxide (NO) and prostaglandin E₂ (PGE₂) are typically considered the primary mediators of DA dilation, other factors clearly play a role. Potassium (K⁺) channels are well-characterized

Table 34.1 Molecular regulators of DA tone

Promote DA patency	Promote DA constriction
Blood flow	Oxygen
Nitric oxide (cGMP/PKG)	Endothelin 1
Prostaglandin E ₂ (cAMP/PKA)	8-iso-PGF ₂ α
Adenosine (cAMP)	Reactive oxygen species (H ₂ O ₂)
Atrial natriuretic peptide (cGMP)	TRPM3
Voltage-gated K ⁺ channels: Kv1.2, Kv1.5, Kv2.1	Angiotensin II
Large-conductance voltage-dependent and calcium-activated (BK _{Ca}) channels	RhoA/B, Rock1/2
ATP-gated K ⁺ (K _{ATP}) channels	Bradykinin
	Acetylcholine, norepinephrine

DA dilators. Voltage-gated K^+ channels, large-conductance voltage-dependent and calcium-activated K^+ (BK_{Ca}) channels, and ATP-gated K^+ (K_{ATP}) channels are all specifically enriched in the DA compared to other vascular beds [9]. K_{ATP} channels are hetero-octameric complexes of pore-forming inward rectifier K^+ channel subunits (Kir6.1 or Kir6.2) and regulatory sulfonylurea receptor subunits (SUR1, SUR2a, or SUR2b) [10]. Different combinations of these subunits are expressed in a tissue-specific manner and exhibit different pharmacological properties, thereby making them attractive targets for DA-specific therapies. This notion is supported by animal studies demonstrating that activating or inhibiting K_{ATP} channels directly modulates DA tone [11, 12].

While nitric oxide (NO) and prostaglandin E_2 (PGE_2) are typically considered the primary mediators of DA dilation, other factors clearly play a role. Potassium (K^+) channels are well-characterized DA dilators. Voltage-gated K^+ channels, large-conductance voltage-dependent and calcium-activated K^+ (BK_{Ca}) channels, and ATP-gated K^+ (K_{ATP}) channels are all specifically enriched in the DA compared to other vascular beds [9]. K_{ATP} channels are hetero-octameric complexes of pore-forming inward rectifier K^+ channel subunits (Kir6.1 or Kir6.2) and regulatory sulfonylurea receptor subunits (SUR1, SUR2a, or SUR2b) [10]. Different combinations of these subunits are expressed in a tissue specific manner and exhibit different pharmacological properties, thereby making them attractive targets for DA-specific therapies. This notion is supported by animal studies demonstrating that activating or inhibiting K_{ATP} channels directly modulates DA tone [11, 12].

At birth, the DA constricts in response to a sharp increase in oxygen (O_2) tension coupled with a loss of vasodilators. Several mechanisms have been proposed to explain the DA's unique ability to sense and respond to O_2 . These include cytochrome P450-mediated induction of endothelin 1 and production of "constrictor" isoprostanes (8-iso- $PGF2\alpha$), and mitochondrial-mediated reactive oxygen species inhibition of Kv1.5 and Kv2.1 [8]. O_2 can also inhibit K_{ATP} channels resulting in membrane depolarization, activation of voltage-dependent calcium (Ca^{2+}) channels, and increased intracellular Ca^{2+} accumulation [12]. Other factors known to regulate DA contraction are listed in Table 34.1 [2, 6–8]. While it is clear that multiple mechanisms are involved, all pathways eventually converge on Ca^{2+} -mediated phosphorylation of myosin light chain, leading to actin/myosin interaction and ultimately DA smooth muscle cell contraction [2, 8].

34.3 Genetic Regulation of the DA

While occurring most often in the setting of prematurity, twin studies gave the first indication that PDA has a genetic component [6]. Several mouse models of PDA have been created and are summarized in Table 34.2 [6–8]. Not surprisingly, many of these models result from disruption of genes involved in smooth muscle function or prostaglandin signaling.

Syndromes featuring PDA (Table 34.2) can also be informative regarding genetic regulation of DA development and function [6, 13]. For instance, clinically

Table 34.2 Genetic regulators of DA tone

Mouse models of PDA	Human syndromes	Non-syndromic SNPs
<i>Smooth muscle related:</i>	Char (TFAP2B)	<i>Increased risk of PDA:</i>
Tfap2b ^{-/-}	Mowat-Wilson (SMADIP1)	TFAP2B (rs987237)
Myocd ^{-/-}	Loeys-Dietz (TGFBRI/2)	TRAF1 (rs1056567)
Myh11 ^{-/-}	Noonan (PTPN11)	AGTR1 (rs5186)
<i>Prostaglandin pathway:</i>	Holt-Oram (TBX5)	<i>Decreased risk of PDA:</i>
Ptgs2 ^{-/-}	Rubinstein-Taybi (CREBP)	PTGIS (rs493694,
Ptgs1/Ptgs2 double ^{-/-}	DiGeorge (TBX1)	rs693649)
EP4 ^{-/-}	Periventricular heterotopia	ESR1 (rs2234693)
Sloco2a1 ^{-/-}	(FLNA)	IFN- γ (rs2430561)
Hpdg ^{-/-}	Cantu (ABCC9, KCNJ8)	
<i>Others:</i>		
Notch2 ^{-/-}		
Notch2/Notch3 double ^{-/-}		
Jag1 ^{-/-}		
Brg1 ^{-/-}		
Bmp9 ^{-/-} with loss of		
Bmp10		
Nfe2 ^{-/-}		
Itga2b ^{-/-}		

significant PDA occurs in 50% of patients with Cantu syndrome, a condition caused by gain-of-function mutations in K_{ATP} channel genes, *KCNJ8* and *ABCC9*, which encode the vascular-specific K_{ATP} channel subtype Kir6.1/SUR2B. In these cases, PDA is resistant to indomethacin therapy and often requires surgical ligation to achieve closure [14].

While only 10% of PDA cases are associated with chromosomal abnormalities [7], identifying single-nucleotide polymorphisms (SNPs) associated with non-syndromic PDA may be more informative regarding the more common sporadic cases of PDA (Table 34.2). Variants in *TFAP2 β* , TRAF1 (TNF receptor-associated factor 1), and *AGTR1* (Angiotensin II type 1 receptor) were associated with an increased risk of PDA while SNPs in *PTGIS* (prostaglandin I2 synthase), *ESR1* (estrogen receptor- α), and *IFN- γ* (interferon- γ) were found to be protective [6]. Unfortunately, replicating many of these findings in other cohorts has proved difficult to this point [15].

34.4 Pharmacological Regulation of the DA

Only three drugs are currently available to treat PDA (indomethacin, ibuprofen, and acetaminophen). All are non-selective cyclooxygenase inhibitors that suppress prostaglandin signaling. Alternately, infusion of prostaglandin E₁ (PGE₁) is the only pharmacologic option used to maintain DA patency in cases of ductus-dependent congenital heart defects. None of these therapies specifically target the DA, leading to off-target effects on other vascular beds. Furthermore, indomethacin and ibuprofen have been associated with spontaneous intestinal perforation and necrotizing

Table 34.3   Pharmacological regulators of DA tone

Maternal drugs	Neonatal drugs
<i>Increased risk of PDA:</i> Tocolytics (indomethacin, nifedipine, magnesium sulfate) ACE inhibitors Antihistamines Anticonvulsants (valproic acid, phenytoin) Amphetamines Alcohol	<i>Dilates DA:</i> PGE ₁ Gentamicin PDE inhibitors (milrinone, amrinone, sildenafil) Cimetidine Heparin Diazoxide
<i>Cause fetal DA constriction:</i> NSAIDs SSRIs antidepressants (sertraline, fluoxetine) Polyphenols Betamethasone	<i>Constricts DA:</i> NSAIDs Caffeine K _{ATP} inhibitors (glibenclamide)

enterocolitis while PGE₁ has been associated with apnea, fever, and other physiologic disturbances [16, 17].

The fetal DA can also be affected by drugs administered to pregnant women (summarized in Table 34.3). Use of tocolytics, ACE inhibitors, antihistamines, anti-convulsants, amphetamines, and alcohol during pregnancy have all been associated with PDA [18]. In contrast, maternal use of NSAID pain-relievers and antidepressants can cause fetal DA constriction [18, 19].

Furthermore, drugs commonly administered to neonates often have vasoactive properties and may inadvertently affect postnatal DA closure [18]. Several drugs have been shown to specifically dilate the DA or cause resistance to indomethacin therapy (Table 34.3). Of note, DA reopening has been reported after exposure to diazoxide, a K_{ATP} channel activator used to treat neonatal hyperinsulinism [20].

34.5   Clinical Implications and Future Directions

PDA remains a significant problem that is inefficiently managed with currently available therapies. Therefore, a greater emphasis must be placed on identifying other factors that can be targeted to regulate DA tone.

The identification of drug targets outside of the prostaglandin pathway is informed by the molecular findings, mouse models of PDA, and human genetic studies mentioned above. While these provide several pathways that could be targeted therapeutically, one with a high likelihood of success is K_{ATP} channels. The vascular form of K_{ATP} channels (i.e., Kir6.1/SUR2B) is enriched in smooth muscle cells of the DA [9, 21] making it a promising target for developing DA-preferring dilators and constrictors. Advances in molecular target-based high-throughput screening of large compound libraries have enabled the ability to discover small molecules that target specific K_{ATP} channel subtypes [22]. We recently performed a thallium flux-based high-throughput screen of compounds for modulators of Kir6.1/SUR2 and identified several novel-scaffold inhibitors of this channel (Denton,

Fig. 34.1 Term-gestation mouse DAs were treated with increasing concentrations of glibenclamide, VU063, or VU278. Changes in lumen diameter were measured and plotted as a percent change from the initial lumen diameter reading under baseline (bl) conditions. Glibenclamide and VU279 caused DA constriction while VU063 had no effect

unpublished data). The most potent inhibitor, termed VU278, exhibited preferential activity toward the vascular form of K_{ATP} channels (Kir6.1/SUR2) over the pancreatic beta/neuronal cell form (i.e. Kir6.2/SUR1). Using isolated vessel pressure myography assays, we evaluated the specificity and vasoactive potential of these drugs (Fig. 34.1). Fetal mouse DAs were exposed to increasing concentrations of glibenclamide (non-specific sulfonylurea receptor inhibitor), VU063 (pancreatic/neuronal Kir6.2/SUR1 activator) [22], or VU278 (vascular Kir6.1/SUR2 inhibitor). As expected, glibenclamide constricted the DA in a dose-dependent manner. Importantly, VU063 had no effect on the DA while VU278 induced constriction.

In conclusion, an emerging body of genetic, physiological, and pharmacological evidence paints a bright future for the treatment of PDA. Developing a comprehensive understanding of the molecular mechanisms and signaling pathways that regulate DA patency, coupled with focused efforts to develop specific pharmacological modulators of these new targets, is creating unprecedented opportunities for improving PDA outcomes. Among several potential drug targets emerging from these studies, vascular-specific Kir6.1/SUR2 channels hold significant promise due to their enriched expression in the DA and unique pharmacological properties. Ongoing efforts by our laboratories will explore the therapeutic potential of Kir6.1/SUR2B channels in regulating DA patency.

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