New Mediators in the Biology of the Ductus Arteriosus: Lessons from the Chicken Embryo

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Abstract

The chicken embryo is an ideal model for the study of new hypotheses on the developmental biology of ductus arteriosus (DA). A unique characteristic of chicken DA is that it is the result of the fusion of two vessels with different embryological origins, morphologies, and functionalities. The pulmonary side (PulmDA) consists almost exclusively of neural crest-derived cells, shows the structure of a muscular artery, and responds to O₂ with contraction whereas the aortic part is of mesodermal origin, shows the morphology of an elastic artery and relaxes in response to O₂. In addition the two parts of the DA show marked differences in responsiveness to other contractile and relaxant agents.

In mammals, the most accepted model of O₂-induced DA constriction involves a rise in O₂ modulating the function of the mitochondrial electron transport chain (the sensor), leading to an increased production of H₂O₂ (the mediator) that causes the inhibition of K_V channels (the effector) with Rho kinase acting as another downstream effector of the O₂-sensing system in the DA. In the chicken embryo, we verified the very same pathway, proving a conserved mechanism for O₂ sensing/signaling in mammalian and nonmammalian DA. Moreover, we demonstrated a developmentally regulated response to O₂, which is restricted to the mature PulmDA and involves parallel maturation of the three components: sensor, mediator, and effectors. Besides O₂, we used the chicken embryo model to investigate the possible ductal effects of vasoactive mediators such as ceramide, H₂S, isoprostanes, or platelet-derived vasoactive mediators.

Keywords

Ductus arteriosus · Oxygen sensing · H₂S · Isoprostanes · Ceramide

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35.1 The Chicken Embryo as a Model for Ductus Arteriosus' Developmental Biology

Several mammalian models have helped to unravel the biology and pathobiology of the ductus arteriosus (DA) and to understand the factors responsible for its closure or patency after birth. However, these models are technically complex and experimental manipulations affect both the mother and the fetus [1, 2]. Therefore, there is a need for additional models, addressing these limitations. In the last years, the chicken embryo has emerged as a suitable model for the study of DA vascular biology [3–15].

A unique characteristic of chicken DA is that it is the result of the fusion of two vessels with different embryological origins, morphologies, and functionalities [1, 3, 6, 7]. The pulmonary side (PulmDA) consists almost exclusively of neural crest-derived cells, shows the structure of a muscular artery, and responds to O₂ with contraction whereas the aortic part (AoDA) is of mesodermal origin, shows the morphology of an elastic artery and relaxes in response to O₂ [1, 3, 6, 7]. The two parts of the DA show marked differences in responsiveness not only to O₂ but to other contractile and relaxant agents as well [4, 5]. These differences are summarized in Table 35.1. Another advantage of the chicken DA model is that we have an exhaustive developmental calendar. The developmental changes in PulmDA responsiveness to contractile and relaxant agents are summarized in Table 35.2.

Table 35.1 Effect of vasoactive mediators in the pulmonary (PulmDA) and the aortic (AoDA) side of chicken DA

Vasoactive agent	Nature or mechanism of action	Effect in PulmDA		Effect in AoDA	Ref.
Oxygen	Gaseous mediator	Contraction	≠	Relaxation	[3]
KCl	Depolarizing agent	Contraction	<	Contraction	[3]
PGE ₂	Prostaglandin	Contraction ^a	>	Contraction	[4]
Norepinephrine	Adrenoceptor agonist	Contraction	>	Contraction	[3]
U46619	TP receptor agonist	Contraction	>	Contraction	[3]
Isoproterenol	β-Adrenoceptor agonist	Relaxation	>	Relaxation	[4]
Forskolin	Adenylyl cyclase activator	Relaxation	>	Relaxation	[4]
Milrinone	Phosphodiesterase-3 inhibitor	Relaxation	>	Relaxation	[4]
Acetylcholine	Muscarinic agonist	Relaxation ^b	<	Relaxation	[5]
SNP	NO-donor	Relaxation	<	Relaxation	[5]
BAY 41-2272	sGC stimulator	Relaxation	<	Relaxation	[5]
CO ₂	Gaseous mediator	Relaxation	=	Relaxation	[8]
15-E ₂₁ -IsoP	Isoprostane	Contraction	>	Contraction	[14]
15-F ₂₁ -IsoP	Isoprostane	Contraction	>	Contraction	[14]
H ₂ O ₂	Reactive oxygen species	Contraction	≠	Relaxation	[6]
Hydroxyfasudil	Rho-kinase inhibitor	Relaxation	=	Relaxation	[6]
Y27632	Rho-kinase inhibitor	Relaxation	=	Relaxation	[6]
CO	Gaseous mediator	Relaxation	=	Relaxation	[13]
H ₂ S	Gaseous mediator	Relaxation	=	Relaxation	[13]
17α-estradiol	Estrogen hormone	Relaxation	=	Relaxation	[15]
17β-estradiol	Estrogen hormone	Relaxation	=	Relaxation	[15]
Ceramide	Membrane sphingolipid	Contraction	≠	Relaxation	[9]
Serotonin	Amine	Contraction	>	Contraction	[16]
Thrombin	Protease	Contraction	>	Contraction	[18]

^aIn the presence of TP receptor blockade, PGE₂ evokes relaxation

^bConcentrations >3 μM of acetylcholine evoke contraction

Table 35.2 Developmental changes in the response of chicken DA (pulmonary side) to vasoactive mediators

Vasoactive agent	Nature/mechanism of action	15-day DA		19-day DA		21-day DA	Ref.
Oxygen	Gaseous mediator	No response	≠	Contraction	<	Contraction	[3]
KCl	Depolarizing agent	Contraction	<	Contraction	<	Contraction	[3]
4-AP	K _v -channel blocker	Contraction	<	Contraction	<	Contraction	[3]
Norepinephrine	Adrenoceptor agonist	No response	≠	Contraction	<	Contraction	[3]
Phenylephrine	α-Adrenoceptor agonist	No response	≠	Contraction	<	Contraction	[3]
U46619	TP receptor agonist	Contraction	<	Contraction	=	Contraction	[3]
Endothelin-1	Polypeptide	Contraction	<	Contraction	>	Contraction	[3]
ACh (≥3 μM)	Muscarinic agonist	Contraction	<	Contraction	>	Contraction	[10]
PGE ₂	Prostaglandin	Relaxation	≠	Contraction	≠	No response	[4]
PGE ₂ (+TP-blockade)	Prostaglandin	Relaxation	>	Relaxation	>	Relaxation	[4]
Acetylcholine (<3 μM)	Muscarinic agonist	Relaxation	>	Relaxation	>	Relaxation	[5]
SNP	NO-donor	Relaxation	=	Relaxation	>	Relaxation	[5]
Isoproterenol	β-Adrenoceptor agonist	Relaxation	=	Relaxation	>	Relaxation	[4]
BAY 41-2272	sGC stimulator	Relaxation	=	Relaxation	=	Relaxation	[5]
8-Br cGMP	cGMP analog	Relaxation	=	Relaxation	=	Relaxation	[5]
Forskolin	Adenylyl cyclase activator	Relaxation	=	Relaxation	=	Relaxation	[4]
Milrinone	Phosphodiesterase-3 inhibitor	Relaxation	<	Relaxation	=	Relaxation	[4]
H ₂ O ₂	ROS	No response	≠	Contraction			[6]
BAY K8644	Calcium-channel activator	No response	≠	Contraction			[6]
Nifedipine	Calcium-channel blocker	Relaxation	<	Relaxation			[6]
Hydroxyfasudil	Rho-kinase inhibitor	Relaxation	<	Relaxation			[6]
Y27632	Rho-kinase inhibitor	Relaxation	<	Relaxation			[6]
H ₂ S	Gaseous mediator	Relaxation	=	Relaxation			[13]
CO	Gaseous mediator	Relaxation	=	Relaxation			[13]
15-E ₂ -IsoP	Isoprostane	Contraction	<	Contraction			[14]
15-F ₂ -IsoP	Isoprostane	Contraction	<	Contraction			[14]

35.2 Oxygen Sensing and Signaling in the Chicken DA

In mammals, the most accepted model of O₂-induced DA constriction involves a rise in O₂ modulating the function of the mitochondrial electron transport chain (the sensor), leading to an increased production of H₂O₂ (the mediator) that causes the inhibition of K_v channels (the effector) with Rho kinase acting as another downstream effector of the O₂-sensing system in the DA. In the chicken embryo the very same pathway has been verified, proving a conserved mechanism for O₂ sensing/signaling in mammalian and nonmammalian DA [1, 6, 7]. Moreover, we demonstrated a developmentally regulated response to O₂, which is restricted to the mature PulmDA and involves parallel maturation of the three components: sensor, mediator, and effectors [6].

35.3 New Mediators in the Biology of the DA

35.3.1 Role of Ceramide in DA Oxygen Sensing and Signaling

Ceramide is synthesized from membrane sphingomyelin by sphingomyelinases (SMases) which are activated by multiple membrane receptors and non-receptor stimuli. Using the chicken model, we observed that inhibition of the ceramide-generating enzyme neutral SMase reduced the hypoxic vasoconstriction in the pulmonary artery (PA) and the normoxic contraction of the DA [9]. Moreover, ceramide content and H_2O_2 production were increased by hypoxia in PAs and by normoxia in the DA. Accordingly, ceramide mimicked the contractile responses of hypoxia in PAs and those of normoxia in the DA. In addition, ceramide inhibited K_v currents in PA and DA smooth muscle cells. Finally, the role of nSMase in acute oxygen sensing was confirmed in human PA and DA [12]. However, the mechanisms matching the putative O_2 sensor(s) (i.e., mitochondria and/or NADPH oxidase) and ceramide remain unknown.

35.3.2 Vasoactivity of H_2S in the Chicken DA

Oxygen is not the only gaseous mediator with a relevant role in DA reactivity. CO_2 and non-respiratory gases, such as NO and CO, are also ductal vasoactive mediators [1, 16]. In the last years H_2S has been identified as an endogenous gaseous transmitter, playing multiple physiological roles in animal systems [13]. Furthermore, H_2S has been proposed as a vascular O_2 sensor. We have investigated the vasoactivity of H_2S and CO in the chicken DA. We observed that either H_2S or CO induces relaxation of chicken DA. However, this relaxation is not vessel-specific since PulmDA, AoDA, and chicken embryo pulmonary and femoral arteries were similarly relaxed by H_2S and CO [13]. Moreover, the presence of precursors/inhibitors of H_2S and CO synthesis did not significantly affect the response of the chicken DA to normoxia/hypoxia and did not affect endothelium-dependent or -independent relaxation [13]. Therefore, our results indicate that [the gasotransmitters](#) H_2S and CO are vasoactive in the chicken DA but they do not suggest an important role for endogenous H_2S or CO in the control of chicken ductal reactivity.

35.3.3 Response of Chicken DA to Isoprostanes

Isoprostanes (IsoPs) are a unique series of prostaglandin-like compounds formed *in vivo* from the free radical-catalyzed peroxidation of arachidonic acid independent of the cyclooxygenase enzymes [17]. Initially, IsoPs were recognized as valuable markers of oxidative stress and numerous pathological conditions have been shown to be associated with increases in urinary, plasma, and tissue levels of IsoPs. Later it became apparent that IsoPs exhibit significant bioactivity and could contribute to the functional consequences of oxidative stress and participate in oxygen signaling, particularly during the transition to extra-uterine life [17]. Surprisingly,

until our study, the possible vascular effects of IsoPs in the DA had not been investigated. We observed that 15-E₂₁-IsoP evoked a potent and efficacious, TP receptor-mediated, contraction in the chicken DA [18]. On the other hand, 15-F₂₁-IsoP is probably acting as a partial agonist at TP receptors. The contractile response of the PulmDA to IsoPs increased with development and both 15-E₂₁-IsoP and 15-F₂₁-IsoP were particularly efficacious in contracting the PulmDA in comparison with the AoDA and the femoral or the pulmonary arteries [14]. Altogether, our data suggest that IsoPs, acting through TP receptors, may play a role in the control of chicken DA tone and could participate in its closure.

35.3.4 Role of Platelet-Derived Vasoactive Mediators in DA Biology

Several cohort studies have shown an association between low platelet counts in the first day(s) of life and patent DA (PDA) in preterm infants [19]. Activated platelets release different substances with vasoactive properties, like serotonin, thromboxane A₂ (TXA₂), platelet activating factor, or thrombin. The two former agonists are vasoactive in the chicken DA [10, 16]. In addition, we investigated the possible vasoactive effects of thrombin in the DA. Cellular effects of thrombin are mediated by protease-activated receptors (PARs), members of the G-protein-coupled receptors that carry their own ligand, which remains cryptic until unmasked by proteolytic cleavage. PAR-1 and PAR-2 have been shown to be involved in regulating vascular tone. Our preliminary results indicate that thrombin, acting through PAR-1, is a selective vasoconstrictor in the DA of the chicken, providing the first functional evidence for a role of PARs in DA biology [18]. Currently, we are analyzing the expression of PARs in the DA of several species (human, lamb, rat, mouse, and chicken).

35.4 Future Direction and Clinical Implications

The hope for future targeted therapies and for the prevention of patent DA (PDA) requires knowledge of the fundamental mechanisms controlling its development and pathogenesis [20]. In addition, new drugs or even those that are commonly used in the neonatal intensive care unit may have unexpected effects on the ability of the DA to spontaneously close, resulting in increased risks for PDA [20]. The chicken DA is a feasible model to test the possible ductal effects of drugs or therapeutic strategies to which the infants are exposed in utero or after birth.

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