Ontogeny of chicken ductus arteriosus response to oxygen and vasoconstrictors

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The ductus arteriosus (DA) is a vessel that connects the pulmonary artery to the aorta and provides, during the fetal life, a pulmonary-to-systemic diversion that shunts more than half of the right ventricle output away from the nonventilated lungs into the systemic circulation (43, 47). The main factors maintaining patency of the DA in utero are low O2 tension, high levels of circulating PGE2, and locally produced PGE2 and PGI2 (24, 43). Failure of DA closure after birth is a common complication of premature delivery that is presenting challenges in terms of diagnosis, assessment, and treatment options (43).

Although the isolated DA is sensitive to a wide range of contractile agonists, the major factor actively stimulating contraction at birth is increasing O2 tension, which has a profound effect on the DA, both directly and by modulating its response to vasodilators and vasoconstrictors (43). To constric properly after birth, the DA prepares itself for this specific task from a quite early onset during development (3). This preparation is reflected by changes in responsiveness with advancing gestational age. These have been extensively characterized in numerous mammalian species including human, lamb, mouse, rat, guinea pig, dog, and rabbit (43). However, we are not aware of any reports dealing with the maturational changes in responsiveness to O2 and other vasoactive agents of a nonmammalian DA.

The chicken (Gallus gallus) embryo represents an excellent model for investigating developmental physiology of the cardiovascular system. Chicken embryos have a mammalian-like circulation, with an extraembryonic circuit involved in the gas exchange (the chorioallantois), analogous to the placenta, and they maintain bilaterally developed DA (3). During the past few years, our group has analyzed developmental changes in reactivity of chicken embryo systemic and pulmonary arteries (26, 38, 51, 52). In the present study, we hypothesized that the responsiveness of the chicken embryo DA to O2 and other vasoconstrictors is developmentally regulated. Therefore, our goal was to characterize the contractile properties of chicken embryo DA and to analyze how they are influenced by in ovo development and by transition to ex ovo life.

METHODS

Incubation of Chicken Embryos and Vessel Isolation

Experiments were performed in accordance with Dutch law for animal experimentation. Fertilized eggs of White Leghorn chickens were incubated at 37.8°C, 45% humidity, and rotated once per hour (Incubator model 25HS; Masalles Comercial). Embryos incubated for 15, 19, and 21 days of the 21-day incubation period were studied. The 19-day embryos were defined as noninternally-pipped embryos, as verified by candling, while the 21-day embryos were defined as externally-pipped when the beak of the embryo was observed in an opening of the eggshell. The embryos were taken out, immediately

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R485
killed by decapitation, placed on the dorsal side on a petri dish coated with silicon and a midline laparotomy and sternotomy were performed. With the aid of a dissecting microscope, the right DA was carefully dissected free from surrounding connective tissue and severed distal to the takeoff of the right pulmonary artery and proximal to the insertion into the aorta (see Fig. 1). The in situ length (in mm) of right DA was 3.48 (SD 0.27), 4.71 (SD 0.40), and 5.58 (SD 0.40) at embryonic days 15, 19, and 21, respectively, whereas the maximal vascular segment length allowed in the myograph was 2 mm. Therefore, a similar amount of ductal tissue was severed from the pulmonary and aortic ends of the vessel to obtain vascular rings with a length of ~2 mm. The right DA was selected because its shorter length allowed a higher proportion of the vessel to be represented in the myograph. In another set of experiments, and to analyze putative functional differences along the DA, rings obtained from the pulmonary and aortic side of the same right DA were compared (see Fig. 7). The boundary between pulmonary and aortic segments was determined based on the marked differences of diameter observed, along the vessel in the 19- and 21-day embryos (see Fig. 1). In some experiments, rings of the pre- and postductal pulmonary arteries were also isolated.

Recording of Arterial Reactivity

Two stainless steel wires (diameter 40 μm) were inserted into the lumen of the endothelium-intact vessels, which were mounted as 1.5- to 2-mm ring segments between an isometric force transducer and a displacement device in a myograph (model 610M; Myo Technology, Aarhus, Denmark). The myograph organ bath (5 ml vol) was filled with Krebs-Ringer bicarbonate (KRB) buffer maintained at 39°C and continuously aerated with one of the following gas mixtures: 95% N₂-5% CO₂ (P₀₂ = 2.6–3.3 kPa), 5% O₂-90%N₂-5% CO₂ (P₀₂ = 6.8–7.2 kPa), 21% O₂-74% N₂-5% CO₂ (P₀₂ = 16–20 kPa), or 95% O₂-5% CO₂ (P₀₂ = 72–76 kPa). The final pH was 7.38–7.42 and P₀₂ was 4.6–5.6 kPa in all solutions. The P₀₂ of the KRB buffer was measured with a blood gas analyzer (model 510 radiometer; ABL, Copenhagen, Denmark) and, in some experiments, was recorded continuously with an oxygen electrode (OXELP; World Precision Instruments, Berlin, Germany). Each DA ring was stretched to its individual optimal lumen diameter (i.e., the diameter at which it developed the strongest contractile response to 62.5 mM KCl) using a diameter-tension protocol as previously described (26, 52). During the first phase of stabilization and determination of optimal diameter, the DA rings were maintained in KRB buffer aerated with 0% O₂. Afterward, and depending on the specific protocol, one of the above-described gas mixtures was used.

Contractile Responses

Concentration-response curves to KCl (4.75–125 mM), norepinephrine (NE; 10 nM-0.1 mM), phenylephrine (Phe; 10 nM-0.3 mM), the thromboxane A₂ mimetic U-46619 (10 nM-10 μM), and endothe-
lin (ET)-1 (0.01 nM-0.1 μM) were constructed by increasing the organ chamber concentration of the drug, by cumulative increments after a steady-state response had been reached with each increment. When two or more agonists were studied in the same arterial preparation, the vessels were repeatedly washed and allowed to equilibrate for at least 30 min. If the tone did not recover to resting level, the vessels were discarded for further experiments. Sympathetic neuroeffector mechanisms were studied by using electrical field stimulation (0.25–16 Hz, 2 ms, 85 mA) via two platinum electrodes that were placed in the axial direction of the vessel. Constant-current pulses were delivered by a stimulator (Technical Services, Universiteit Maastricht, The Netherlands).

To determine the role of K+ channels in DA contraction, we performed concentration-response curves to 4-aminopyridine (4-AP; 1 mM-10 mM), glibenclamide (1 μM-10 μM), and tetraethylammonium (TEA; 1 mM-10 mM), preferential inhibitors of voltage-gated (Kv), ATP-sensitive (KATP), and Ca2+-activated (KCa) K+ channels, respectively. To analyze the putative contractile effects of cyclooxygenase (COX) inhibition, DA rings were incubated for 30 min in the presence of the nonselective COX inhibitor indomethacin (10 μM), the COX-1 inhibitor valeryl salicylate (0.5 mM), or the COX-2 inhibitor nimesulide (0.1 μM).

*Response of chicken DA to O2.* In a first set of experiments, DA rings were incubated for (at least) 30 min with 95% N2-5% CO2. Then the gas mixture was switched to 21% O2-74% N2-5% CO2. This gas was maintained for 10 min (a stable response to O2 was observed after 2–3 min, see RESULTS). In a second set of experiments, and to analyze the involvement of the nitric oxide (NO)/cGMP pathway and of ET-1 against smooth muscle

**Drugs and Solutions**

KRB contained (in mmol/l): 118.5 NaCl, 4.75 KCl, 1.2 MgSO4-7H2O, 1.2 KH2PO4, 25.0 NaHCO3, 2.5 CaCl2, 5.5 glucose. Solutions containing different concentrations of K+ were prepared by replacing part of the NaCl by an equimolar amount of KCl. Arterenol bitartrate (NE), Phe, indomethacin, 4-AP, glibenclamide, TEA, and l-NAME were obtained from Sigma (St. Louis, MO); ET-1, valeryl salicylate, nimesulide, and PD-142,893 were from Alexis Biochemicals (Lausen, Switzerland); U-46619 was from Cayman Chemical (Ann Arbor, MI), and ODQ was from Tocris (Ballwin, MO). All drugs were dissolved initially in distilled deionized water (except indomethacin, valeryl salicylate, and nimesulide in ethanol and U-46619, glibenclamide, and ODQ in DMSO) to prepare adequate stock solutions, and further dilutions were made in KRB.

**RESULTS**

**Chicken DA Anatomy and Histology**

On its emergence from the right ventricle, the main pulmonary trunk is directly divided into right and left pulmonary arteries that pass caudally and bifurcate into the DA and the postductal pulmonary arteries (Fig. 1, A–C). The postductal pulmonary artery segments arise in an angle of 90° and enter immediately the lungs, whereas the two DA appear as the natural continuation of the preductal pulmonary arteries (Fig. 1, A–C). The right DA runs caudally adhered to descending aorta. As both DA pass caudally, they become progressively broader. Differences in diameter between proximal and distal segments of DA become more prominent with advancing incubation age (Fig. 1, A–C). Finally, both DA join the dorsal aorta with an acute angle of insertion.

Double staining for elastin and smooth muscle α-actin demonstrated the presence of abrupt changes in vessel wall structure along the DA. As shown in Fig. 1, D and G, in the aortic side of the DA, elastic fibers fill the vascular wall arranged in concentric lamellae with α-actin-positive smooth muscle cells embedded between the lamellae. From this elastic segment, and in aortopulmonary direction, a progressive reduction of medial thickness and elastin staining was observed (Fig. 1D). The narrow central segment of the DA displayed the structure of a muscular artery with a dense α-actin-positive media subjacent to the endothelium and few layers of elastic fibers around the muscular layer (Fig. 1F). Finally, dense elastic fibers can again be observed in the vicinity of the pulmonary artery (Fig. 1E). These fibers completely fill the media but do not assemble into the neatly organized lamellar structures observed in the aortic side.

**Contractile Responses in Isolated DA Rings**

Isolated DA obtained from 15-, 19- and 21-day chicken embryos responded to depolarizing high-K+ solution with a tonic contraction and the amplitude of this response significantly increased with age (Fig. 2A). The diameter at which a maximal response to 62.5 mM KCl was obtained did not significantly change between day 15 (761 μm, SD 76, n = 48) and day 19 (774 μm, SD 94, n = 81, P = 0.42 vs. 15-day), but decreased at day 21 (549 μm, SD 192, n = 36, P < 0.001 vs. 15- and 19-day).

To identify the K+ channel types involved in the control of DA tone, we tested the effects of glibenclamide, TEA, and 4-AP, which preferentially block KATP, KCa, and Kv channels, respectively.
respectively. Neither glibenclamide nor TEA had any effect on basal tone at any age (not shown). However, the Kv channel inhibitor 4-AP caused a dose-dependent increase in tension, which increased significantly with age (Fig. 2B).

In another set of experiments, concentration-response curves to the polypeptide ET-1 (Fig. 2D) and the thromboxane A2 receptor (TP) agonist U-46619 (Fig. 2C) were performed. Both agonists contracted the 15-day DA and the responses were higher in older animals. Weak but significant contractile responses to ET-1 were observed at concentrations above 0.03 nM in 19-day and 21-day DA, whereas concentrations ≥3 nM induced a clear contractile response in the three age groups. The maximal response to ET-1 could not be reached at concentrations up to 0.1 μM at any age. The responses induced by U-46619 were markedly increased in the 19-day embryos [maximum contraction \(E_{\text{max}}\) = 0.81 N/m, SD 0.18, n = 6] and the 21-day embryos \(E_{\text{max}}\) = 0.86 N/m, SD 0.15, n = 5) compared with those obtained in the 15-day DA (\(E_{\text{max}}\) = 0.33 N/m, SD 0.10, n = 8, \(P < 0.001\) vs. 19- and 21-day), although no significant changes in pD2 values (15-day: 6.91, SD 0.21; 19-day: 7.08, SD 0.25; 21-day: 7.17, SD 0.22) were observed.

Maturational responses to adrenoceptor activation. In contrast to KCl, 4-AP, ET-1, and U-46619, the nonselective adrenergic receptor agonist NE (Fig. 3A) and the \(\alpha_1\)-adrenergic receptor agonist Phe (Fig. 3B) failed to contract the 15-day DA. NE caused a concentration-dependent contraction of the 19-day and 21-day DA, reaching a higher maximal response in the older animal. Thus, the \(E_{\text{max}}\) induced by NE averaged 0.55 N/m (SD 0.31) at day 19 (n = 18) and 0.94 N/m (SD 0.15) at day 21 (n = 8, \(P = 0.0029\)), but pD2 values were not significantly different at both ages (19-day: 6.22, SD 0.52; 21-day: 6.21, SD 0.18). The \(E_{\text{max}}\) induced by Phe averaged 0.78 N/m (SD 0.18) at day 19 (n = 6) and 1.17 N/m (SD 0.46) at day 21 (n = 8, \(P = 0.08\)) and mean pD2 were 5.56 (SD 0.16) at days 19 and 21, respectively. In contrast to the marked contractile responses to NE in the 19-day DA, we found no responses to NE in either pre- or postductal pulmonary arteries at this age (Fig. 3C).

Oxygen-induced contraction and modulation of responses to vasoconstrictors. Switching the O2 mixture from 0 to 21% produced a progressive increase of the O2 concentration in the organ chamber that reached a steady state after ~3 min (Fig. 5A). This switch in O2 concentration resulted in a contractile response in the DA from 19- and 21-day embryos, but not in

Figure 2. Concentration-dependent contractile effects of KCl (A), 4-aminopyridine (4-AP; B), U-46619 (C), and endothelin-1 (ET-1; D) in 15-, 19- and 21-day DA rings aerated with 5% O2-90% N2-5% CO2. Each point represents the mean and SD of the number of embryos. *P < 0.05 for difference from 19-day embryos, #P < 0.05 for difference from 21-day embryos.
those from 15-days (Fig. 5A). Oxygen-induced contractions were tonic, reversible, and reproducible. In the presence of NE (1 μM) the change from 0 to 21% O2 caused a further tonic constriction, which was significantly higher in the 21-day than in the 19-day DA (Fig. 5, B and D). In 62.5 mM KCl-contracted DA the contractile response to O2 was markedly reduced compared with that obtained in NE-stimulated DA (Fig. 5, C and D).

The NO synthase inhibitor l-NAME (0.1 mM) and the soluble guanylate cyclase inhibitor ODQ (10 μM) induced a slight contraction of the 19-day DA (l-NAME: 0.04 N/m, SD 0.05, n = 8; ODQ: 0.07 N/m, SD 0.05, n = 8). Either the presence of l-NAME or ODQ produced a marked increase in O2-evoked constriction (Fig. 5E). l-NAME did not produce any significant contractile effect and did not modify the (absent) response to O2 of the 15-day DA (n = 4, not shown). Incubation with the peptidic ETA and ETB-receptor antagonist PD-142,893 (1 μM) did not significantly modify the basal wall tension of the 19-day DA. The presence of PD-142,893 produced a significant reduction (26.7%, SD 21.4, n = 12) of O2-evoked constriction (Fig. 5E). When the 19-day DA was incubated in the presence of both L-NAME and PD-142,893, no significant changes were observed in O2-induced constriction (Fig. 5E).

In another set of experiments, the contractions induced by NE or KCl were tested under different O2 concentrations (0, 5, and 95%) in 15- and 19-day embryos (Fig. 6). KCl-induced responses were not affected by changing the O2 tension at any age, whereas, in 19-day embryos, the maximal response to NE was significantly increased under 95% O2 (E_{max} = 0.80 N/m, SD 0.35, n = 10) compared with 5% O2 (E_{max} = 0.46 N/m, SD 0.29, n = 18, P = 0.01) but not compared with 0% O2 (E_{max} = 0.60 N/m, SD 0.30, n = 9, P = 0.2) (Fig. 6).

**DISCUSSION**

In air-breathing vertebrates varying from lungfish to mammals, the DA derives from the sixth pharyngeal arch artery and, in preparation for its specific task, undergoes a morphologic and functional differentiation program, starting early in development (3, 43). For the first time, developmental changes in DA responsiveness to O2 and other vasoconstrictors have been characterized in a nonmammalian species. Our results can be summarized as follows. First, on embryonic day 15, the chicken DA did not respond to O2, NE, or Phe but demonstrated depolarization-, thromboxane A2-, and ET-1-induced constriction that increased with age. Second, responsiveness to O2 and catecholamines were present in the 19- and 21-day

![Fig. 3. Maturation in the contractile effects of adrenoceptors stimulation. Concentration-dependent curves for norepinephrine (NE; A) and phenylephrine (Phe; B) in 15-, 19-, and 21-day DA rings aerated with 5% O2-90% N2-5% CO2. C: lack of responsiveness to NE in 19-day pre- and postductal pulmonary arteries compared with those obtained in DA from the same embryos. D: 19-day DA and femoral artery segments stained with glyoxylic acid. Bar = 100 μM. Each point represents the mean and SD of the number of embryos. *P < 0.05 for difference from 19-day embryos, #P < 0.05 for difference from 21-day embryos.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00863.2006)
embryo DA with some further developmental differences, particularly in the response to NE. Oxygen not only increased tension of 19- and 21-day chicken DA but also potentiated NE-induced contraction. Third, the response to O2 was augmented by NO synthase and soluble guanylate cyclase inhibition and was reduced by the presence of an ETA and ETB-receptor antagonist. Fourth, oxygen-induced contraction was located in the pulmonary side of the DA, whereas the aortic side demonstrated O2-evoked relaxation and a weaker NE-induced contraction. Finally, neither transmural stimulation of nerves nor COX inhibition consistently constricted the chicken DA, suggesting lack of involvement of neural vasoconstriction and locally formed dilatory PGs on chicken DA tone control, respectively.

Changes in Chicken DA Responsiveness with Advancing Gestational Age

We observed that the responses of chicken DA to O2 and to receptor-independent (i.e., high-K+ solution) and -dependent (i.e., NE, Phe, U-46619, and ET-1) vasoconstrictors increased with gestational age. Similar findings have been reported in almost all mammalian species studied (28, 33, 43, 49). The reduced contractile response of mammalian preterm DA to O2 and vasoconstrictors may reflect greater inhibition by locally released vasodilators (i.e., PGs) (43). However, several studies have demonstrated alterations of contractile pathways with advancing gestational age (47), suggesting a maturation of contractility rather than merely a reduced degree of inhibition by PGs (43).

Potassium-evoked depolarization induced significant contraction in the DA of 15-day embryos. This indicates that at this developmental stage DA smooth muscle cells are already equipped with contractile proteins and an excitation-contraction coupling. This receptor-independent contractile response increased, in the chicken DA, twofold between day 15 and day 19, whereas in chicken embryo carotid and femoral arteries depolarization-evoked contraction increased five- to sevenfold during the same incubation period (26). Moreover, the presence of responses to the thromboxane A2 mimetic U-46619 and ET-1, in the 15-day DA, indicates that pharmacomechanical coupling is also developed rather early in this vessel. Accordingly, several studies in mammals indicate that the smooth muscle cells in the DA are more differentiated than those in other fetal arteries (16, 25, 39). Expression of the adult-specific vascular smooth muscle myosin heavy chain isoform, SM2, is developmentally regulated and not detected, before birth, in the aorta or pulmonary artery of the rabbit, but expressed in the fetal DA (16, 25, 39). Moreover, advanced differentiation has also been reported in the human DA that highly expressed the actin binding proteins calponin and caldesmon, when compared with the fetal aorta (42).

Responsiveness to Catecholamines

Secretion of catecholamines plays an important role in several of the adaptations that characterize the transition from the pre- to the neonatal period (31). In the present study, we observed marked developmental differences in the response of chicken DA to adrenergic agonists. Thus, NE and Phe had no effects in 15-day DA but caused marked concentration-dependent contractions in 19- and 21-day DA. The lack of responses to NE and Phe in the 15-day DA seems to reflect a specific immaturity of the α-adrenergic pathway since both receptor-dependent [ET-1 and thromboxane A2 (TXA2)] and -independent contractions were present. Furthermore, developmental increase of responses to NE and Phe was observed in the DA but not in either pre- or postductal pulmonary arteries (present study and Ref. 52), indicating a specific maturation of
Fig. 5. Response of chicken DA to oxygen. The 21% O₂ challenge was performed by switching the bubbling gas from 95% N₂-5% CO₂ to 21% O₂-74% N₂-5% CO₂. A: representative traces demonstrating O₂-evoked contraction in 19- and 21-day but not in 15-day DA. B–C: representative traces of the effects of O₂ in 19- and 21-day DA precontracted with NE (1 μM; B) and in 21-day DA precontracted with 62.5 mM KCl (C). D: averaged contractile effects of O₂ over basal tone and in precontracted DA. *P < 0.05 for difference from 19-day. #P < 0.05 for difference from not precontracted DA. †P < 0.05 for difference from NE-precontracted DA. E: effects of the NO synthase inhibitor l-NAME (0.1 mM), the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 μM), and the peptidic ETA and ETB-receptor antagonist PD-142,893 (1 μM) on the response of 19-day DA to O₂. Results are expressed as the percentage of the response to a first O₂ challenge in the absence of the inhibitor. *P < 0.05 for difference from control. Each bar in D and E represents the mean and SD of the number of embryos.
α-adrenergic pathway in the DA. Interestingly, the time course of catecholamines in the plasma of chicken embryos is characterized by a maximum of epinephrine and NE at day 19, shortly before lung ventilation (i.e., internal pipping) is initiated (31, 54). In a previous study, developmental changes in the response of chicken embryo femoral and carotid arteries to adrenergic agents were also observed (26). Similarly to the DA, the carotid arteries of 15-day embryos did not respond to NE or Phe, but a contraction was observed at embryonic day 19. In contrast, the responses to NE and Phe were already present at day 15 in femoral arteries (26). Therefore, the presence of high catecholamine concentrations in the chicken embryo during transition to ex ovo life, together with the developmental increase of the responsiveness of DA and systemic arteries and the lack of pulmonary artery sensitivity to these neurohormones, suggest a relevant role of catecholamines on the circulatory transition (i.e., closure of the DA, increase in systemic vascular resistance, and fall of pulmonary vascular resistance) of the chicken fetus.

Lack of response to electrical field stimulation and glyoxylic acid-induced histofluorescence indicate the absence of catecholamine-containing nerves in chicken DA. Accordingly, immunohistochemical staining with anti-neurofilament antibodies did hardly reveal innervations of the DA in either mouse or chicken embryos (3, 30). In contrast, catecholamine containing nerves have been demonstrated in human (2), lamb (23), and guinea pig (6) DA, but nerve density appears very low compared with third and fourth pharyngeal arch-derived vessels, and there is no information on the extent to which innervation of the DA is involved in its peculiar reactivity (3, 43).

Responsiveness to Oxygen

The cellular pathways initiated by O2 and leading to mammalian DA constriction are still controversial. Several studies have suggested that changes in O2 tension are signaled by changes in redox status and that O2-induced contraction of the DA is, at least partly, induced by membrane depolarization, which then results in entry of calcium through L-type voltage-operated Ca2+ channels (32, 43, 49, 53). Nakanishi et al. (32) hypothesized that, in the rabbit DA, membrane depolarization is related to O2-induced closure of a KATP channel. In contrast, Tristani-Firouzi et al. (49) suggested that a KV channel was responsible for the O2-induced membrane depolarization of rabbit DA. Moreover, ex vivo transfer of the gene for Kv1.5 or Kv2.1 partially restores constriction to O2 in the preterm rabbit DA (47). Accordingly, regulation by a mitochondrial redox sensor of KV channels in human DA smooth muscle cells has been demonstrated by Michelakis et al. (28, 29). On the other hand, Coccoani et al. (11–14) suggested that cytochrome P-450 acts as O2 sensor and that its interaction with O2 might increase ET-1 production by the ductal smooth muscle cells, resulting in contraction. Therefore, O2 may, either directly or mediated by ET-1, cause membrane depolarization of the smooth muscle cells, and increase intracellular Ca2+ concentration through the increase of Ca2+ influx, or the induction of Ca2+ release from intracellular store sites, and finally cause DA contraction.

In the present work, we observed that O2-induced contraction of chicken DA was not affected or even augmented by NE pretreatment. However, in high K+ stimulated DA the response to O2 was reduced, suggesting that membrane depolar-
Oxygen-induced contraction of chicken DA was reduced by the presence of an ET-receptor blocker. Numerous findings implicate ET-1 as a regulator of mammalian DA tone and as a messenger in the constriction of the DA to O$_2$ (11–14, 34, 41, 43). Interference with ET-1 synthesis or action, whether achieved pharmacologically or through genetic manipulation, curtails the constrictor effect of O$_2$ due to an immaturity of this or another O$_2$ sensing system and not because its smooth muscle cells are deficient in KV channels.

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reduction of $O_2$-evoked contraction by ET-receptor antagonists is more marked in the lamb (12) and rat (41) than in the rabbit (41) or chicken (present work) DA. Whether maturational differences in the chicken DA response to $O_2$ and ET-1 are due to developmental changes in ET-1 receptor expression or in transductional pathways and the putative role of ET-1 in chicken DA closure warrant further investigation.

Compounds that inhibit NO synthesis constrict the DA of several mammalian species, providing indirect evidence of endogenous NO production and implying NO in DA regulation (37, 46). Moreover, endogenous NO appears to play a more relevant role in regulating the patency of the mammalian DA in earlier fetal stages than in the near-term fetus (37, 46, 47). In contrast, we observed that the constriction induced by the NO synthase inhibitor L-NAME was absent in the DA of 15-day chicken embryos but present at day 19. In addition, L-NAME and the soluble guanylate cyclase inhibitor ODQ produced a marked augmentation of $O_2$-evoked constriction in the 19-day DA, whereas the absence of response to $O_2$ of the 15-day DA was not modified by L-NAME. Interestingly, the DA of 15- and 19-day chicken embryos demonstrated a similar relaxation to endothelium-derived NO and to NO donors (1), indicating that the mechanisms of NO production and relaxation are already active in the 15-day DA.

An increase in $O_2$ tension produces not only constriction of the DA but also has a profound modulatory effect on other vasoactive systems (23, 43, 44). We have observed that the efficacy (but not the potency) of NE increased when 19-day DA were bubbled with high $O_2$ concentrations. In addition, when 21-day DA was precontracted with NE, the $O_2$-evoked response was larger than under basal conditions. Therefore, regarding our results, one might speculate that the presence of higher $O_2$ tension, higher catecholamines concentrations, the positive interactions between $O_2$- and NE-induced contractions, and a developmental increase in the sensitivity of DA to these vasoactive mediators are critical factors involved in the closure of chicken DA during transition to ex ovo life.

Lack of Responsiveness to COX Inhibitors

In mammals, prenatal patency of the DA is critically dependent on PGs, with ample evidence implicating PGE$_2$, both locally formed and blood-borne, as the prime effector (8, 24). This PGE$_2$-based relaxing mechanism develops early in gestation and in the premature embryo is potent enough to override the constrictor action of $O_2$ (10, 43). The only mammalian species studied where PGs have not been shown to exert a significant dilator effect is the guinea pig (6, 43). The initial reaction in the synthesis of all PGs is catalyzed by COX, two isoforms of which (COX-1 and COX-2) have been identified (8, 9). Both COX-1 and COX-2 are present in the chicken and expressed in the vascular endothelium (27, 35). Lamb, mouse, rat, rabbit, and piglet ductus preparations contracted upon treatment with indomethacin and other COX inhibitors (15, 19, 20, 40, 43, 45). In contrast, we observed that, in the chicken DA, COX inhibitors did not produce significant contractile effects. These results indicate that locally derived PGs do not exert a tonic relaxant effect on chicken DA but do not exclude that blood-borne PGs might regulate chicken DA tone in vivo.

Heterogeneity of Contractile Responses Along the Chicken DA

In the chicken, the connection between the pulmonary artery and the aorta (i.e., the anatomical DA) is a bilateral structure that is proportionally longer than the unilateral DA of mammals. Along the chicken DA, morphological heterogeneity can be directly observed under the dissection microscope (see Fig. 1). This heterogeneity have been extensively studied by Bergwerff et al. (4), who characterized three morphological segments in the sixth pharyngeal arch artery of the chicken by the time of hatching. The first proximal segment displays an elastic morphology, similar to that of the proximal aorta, until it bifurcates into two typically muscular arteries: the DA and the postductal pulmonary artery that show strong actin expression in a small number of cell layers. Downstream from the muscular DA portion, a third elastic segment can be identified (4). It has been suggested that this third segment results from the incorporation of dorsal aorta tissue into the sixth arch artery (4, 21, 22). Transition between the muscular (proximal) and the elastic (distal) segment of chicken DA can be easily identified because the first one narrows considerably during the last week of in ovo development (4, 21).

In the present work, we confirmed the histological observations of Bergwerff et al. (3, 4) and we found an interesting functional correlation. Oxygen-induced contraction was located in the proximal segment of the DA, whereas the distal segment responded with relaxation. Moreover, a markedly higher contractile efficacy of NE was observed in the pulmonary side of the chicken DA. Similarly, higher contractile effects of catecholamines have been observed in the pulmonary end of human DA (48). In the majority of mammals, normal DA closure takes place from the pulmonary artery to the aortic end (17, 18), but the presence of areas with so marked differences in reactivity has not been described. Development of a specific phenotype compared with surrounding arteries provides the structural and biomechanical basis for DA constriction after birth (36). In the case of the chicken DA, abrupt changes in morphological and functional vascular phenotypes are present along the vessel, indicating a genetic basis for its unique status (3–5) and suggesting local differences in the mechanisms leading to its closure. Our results support the idea that vascular smooth muscle cells from different embryological origins and with a differential contribution of neural crest derivatives reside within the wall of chicken DA (3–5).

Perspectives

There is currently tremendous interest in understanding how the physiology of individual animals changes and develops during ontogeny and to what extent the timing of the onset of a particular physiological regulatory system can be altered (7). Because avian embryos developed in a self-contained egg, they have long been favored animals for investigating how vertebrate cardiovascular physiological regulation unfolds during development (7). In the present work, we describe the developmental changes of the responses to $O_2$ and other vasoconstrictors in the chicken DA. Our data support the concept that increasing $O_2$ tension plays a critical role in the closure of DA also in nonmammalian species. In addition, we document how the chicken DA preparation for its specific task of postnatal closure is reflected in critical maturational changes in reactiv-
ity. A patent DA complicates the clinical course of preterm infants, increasing their risks of developing chronic lung disease, necrotizing enterocolitis, and intraventricular hemorrhage (36, 43). Understanding the basic mechanisms of either normal or altered functional and structural development of the DA, as well as inter-species differences in DA homeostasis, may provide insights into human patent DA pathophysiology and treatment.

REFERENCES


