Oxygen-Induced Tension in the Sheep Ductus Arteriosus: Effects of Gestation on Potassium and Calcium Channel Regulation

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ABSTRACT: Compared with the full-term ductus arteriosus, the premature ductus is less likely to constrict when exposed to postnatal oxygen concentrations. We used isolated fetal sheep ductus arteriosus (pretreated with inhibitors of prostaglandin and nitric oxide production) to determine whether changes in K^+ - and Ca_L -channel activity could account for the developmental differences in oxygen-induced tension. In the mature ductus, K_V -channels appear to be the only K^+ -channels that oppose ductus tension. Oxygen concentrations between (2% and 15%) inhibit K_V -channel activity, which increases the Ca_L -channel-mediated increase in tension. Low oxygen concentrations have a direct inhibitory effect on Ca_L -channel activity in the immature ductus; this is not the case in the mature ductus. In the immature ductus, three different K^+ -channel activities (K_V , K_{Ca} , and K_{ATP}) oppose ductus tension and contribute to its decreased tone. Oxygen inhibits the activities of all three K^+ -channels. The inhibitory effects of the three K^+ -channel activities decline with advancing gestation. The decline in K^+ -channel activity is not due to decreased K^+ -channel expression. Super-physiologic oxygen concentrations ($\geq 30\%$ O_2) constrict the ductus by using calcium-dependent pathways that are independent of K^+ - and Ca_L -channel activities. Super-physiologic oxygen concentrations eliminate the difference in tensions between the two age groups. (*Pediatr Res* 65: 285–290, 2009)

The postnatal increase in arterial Pao_2 plays an important role in ductus arteriosus constriction after birth. In the late gestation fetus, the changes in tension that accompany changes in oxygen concentration (within the physiologic range) appear to be due to changes in calcium entry through the ductus smooth muscle Ca_L channels (1,2). The Ca_L channels are regulated by the smooth muscle's membrane potential; calcium enters the cells when the cells are depolarized. K^+ -channels and Na^+ - K^+ -ATPases are the main determinants of the smooth muscle's membrane potential. A decrease in K^+ -channel activity, due to K^+ -channel blockade and/or down-regulation of channel expression, leads to membrane de-

polarization. Oxygen appears to inhibit K^+ -channel activity, leading to smooth muscle depolarization and increased calcium entry through the voltage-dependent Ca_L -channels (1,3,4).

Most studies suggest that voltage-gated K^+ -channels (K_V channels) are responsible for oxygen's effect on membrane depolarization (3–5); ATP-sensitive K^+ -channels (K_{ATP} channels) and large conductance calcium-and-voltage sensitive K^+ -channels (K_{Ca} channels) have also been implicated as oxygen sensors (1,6). At the molecular level, K^+ -channels are composed of pore-forming α -subunits, which co-assemble with cytoplasmic regulatory/auxiliary β -subunits. Both K_V α -subunits (Kv1.2, Kv1.5, Kv2.1, Kv3.1, Kv4.2, Kv4.3, and Kv9.3) and K_V β -subunits have been shown to have oxygen-sensing capabilities (7). K_{ATP} channels [composed of K_{IR6} α -subunits and a sulfonylurea receptor (SUR2)] are responsive to hypoxic conditions and changes in oxygen concentration (8,9). K_{Ca} channels (composed of BK_{Ca} α -subunits and $\beta 1$ subunits) can be modulated by hypoxia and oxidant stress (6,9,10).

In contrast with the full-term ductus, the premature ductus is less likely to constrict when exposed to physiologic oxygen concentrations (11). Super-physiologic arterial oxygen concentrations, conversely, produce the same degree of constriction *in vivo* in the immature ductus as is observed in the mature ductus (11).

In this study, we used fetal sheep, to determine whether changes in K^+ - and Ca_L channel function were responsible for the changes in oxygen-induced ductus tension that occur within the physiologic as well as super-physiologic range of oxygen concentrations. We also wanted to determine whether developmental differences in K^+ - and Ca_L channel function could account for the differences in tension between the immature and mature ductus. We hypothesized that decreased K^+ -channel expression would account for the differences in tension between the immature and mature ductus. We used a pharmacologic and quantitative PCR approach to examine the roles of K^+ -channels and Ca_L channels in maintaining ductus tensions.

METHODS

Fifty late-gestation, mature sheep fetuses (mixed Western breed: 137 ± 2 -d gestation, term = 145 d) and 47 immature fetuses (103 ± 2 d) were delivered by cesarean section and anesthetized with ketamine HCl (30 mg/kg

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IV) before rapid exsanguination. These procedures were approved by the Committee on Animal Research at the University of California, San Francisco.

The fetal sheep ductus arteriosus was divided into 1-mm-thick rings (two to three rings per animal) and isometric tension was measured in a Krebs-bicarbonate solution [$(\times 10^{-3}$ M): 118 NaCl, 4.7 KCl, 2.5 CaCl_2 , 0.9 MgSO_4 , 1 KH_2PO_4 , 11.1 glucose, 23 NaHCO_3 (pH 7.4)] containing indomethacin (5×10^{-6} M) and *N* ω -nitro-arginine methyl ester (L-NAME, 10^{-4} M), to inhibit endogenous prostaglandin and nitric oxide production, respectively (11). An oxygen electrode placed in the 10-mL organ bath, measured the oxygen concentration. The bath solution was equilibrated with gas mixtures containing 5% CO_2 and was changed every 20 min. Rings were stretched to initial lengths (preterm = 5.2 ± 0.4 mm; late-gestation = 6.7 ± 0.6 mm) that produce maximal contractile responses when exposed to K^+ -Krebs solution (containing 0.1 M KCl substituted for an equimolar amount of NaCl), equilibrated with 95% O_2 (11).

Once steady-state tension in 30% O_2 was achieved (~ 100 – 120 min), K^+ -Krebs solution (equilibrated with 95% O_2) was used to measure the maximal contraction that could be developed by the ductus. After returning the rings to the initial modified Krebs solution, equilibrated with 15% O_2 , the rings were sequentially exposed to five different oxygen conditions: 2, 6, 15, 30, and 95% O_2 . We refer to bath oxygen concentrations between 2 and 15% as the “physiologic range” because they produce ductus tissue oxygen concentrations similar to the physiologic extremes experienced *in vivo* (12) [15% bath O_2 concentration produces tissue oxygen concentrations similar to those achieved during the postnatal increase in arterial PO_2 ; the 2% bath O_2 concentration produces tissue oxygen concentrations similar to those needed to induce remodeling *in vivo* (11)]. Conversely, we considered the oxygen concentrations between 30 and 95% as the “super-physiologic range.”

After the oxygen dose-response curve, the rings were equilibrated with one of several experimental solutions before repeating the same oxygen dose-response curve. The solutions included: 10^{-5} M nifedipine (Ca_L -channel antagonist), 10^{-6} M BAY K8644 (Ca_L -channel agonist), calcium-free Krebs solution (containing 0.5×10^{-3} M EGTA without calcium), and K^+ -Krebs solution (to depolarize the muscle). Nifedipine (10^{-5} M) inhibits the effects of 10^{-6} M BAY K8644 (by $96 \pm 5\%$) and K^+ -Krebs solution (by $87 \pm 5\%$) when ductus rings are incubated in 15% O_2 (2). Neither 10^{-5} M nifedipine, 10^{-6} M BAY K8644, nor K^+ -Krebs solution have any effect in the absence of extracellular Ca^{++} (2).

Ductus were also exposed to K^+ channel antagonists before the second oxygen dose-response curve: 3×10^{-3} M 4-Aminopyridine (4AP) (K_V -channel antagonist), 10^{-5} M glibenclamide (K_{ATP} channel antagonist), 50×10^{-9} M iberiotoxin (K_{Ca} -channel antagonist), 10^{-3} M tetraethylammonium ion (TEA) (K_{Ca} -channel antagonist), 10^{-6} M apamin (small conductance calcium-activated K^+ -channel antagonist), and 10^{-2} M TEA (which produces 100% blockade of K_{Ca} -channels plus 50% blockade of K_V and K_{ATP} channels). The specificities of the above K^+ channel antagonists have been reviewed in Ref. 13.

The ductus' contractile response to the oxygen dose-response curve does not change when it is repeatedly exposed in time matched control experiments (11). Sodium nitroprusside, 10^{-3} M, was added to each ring at the end of the experiment to determine its minimal tension.

The difference in tensions between any measured steady-state tension and the minimal tension produced by sodium nitroprusside was considered the Net active tension. The difference in tensions between the maximal contraction (produced by K^+ -Krebs/95% O_2) and the minimal tension (with sodium nitroprusside) was treated as the maximal active tension (MAT) capable of being developed by the rings.

Active tensions are expressed as a percentage of the maximal active tension. Maximal active tensions were 16 ± 2 g in immature fetuses and 20 ± 3 g in mature fetuses. The ductus wet weights were as follows: 30 ± 9 mg, immature fetuses; 36 ± 10 mg, mature fetuses. Chemicals were from Sigma Chemical Co. Chemical (St. Louis, MO).

We used the TaqMan Universal PCR master mix of PE Applied Biosystems (Foster City, CA) to quantify the expression of the K^+ -channel subunits as previously described (14). Total RNA was isolated from the ductus of immature and mature fetal sheep (Table 1) and compared with RNA from the ductus of immature (125-d gestation) and mature (175-d gestation) fetal baboons (*Papio* sp., full term = 185-d gestation), and from immature (14-d gestation) and mature (19-d gestation) fetal mice (CD-1, full term = 19-d gestation) that were delivered by cesarean section and euthanized before breathing.

Statistics. Statistical analyses were performed by the appropriate Mann-Whitney test, *t* test, or by analysis of variance. Scheffe's test was used for *post hoc* analysis. Values are expressed as mean $\pm$ SD.

RESULTS

Oxygen-induced tension in the mature ductus arteriosus.

The late gestation ductus arteriosus developed an active tension that was maintained by extracellular Ca^{++} -dependent and extracellular Ca^{++} -independent pathways (Fig. 1). Oxygen increased ductus tension through a mechanism that depended on the presence of extracellular calcium and could be blocked by high K^+ -containing Krebs solution (Fig. 1). After the removal of extracellular calcium, both oxygen and elevated K^+ had only a negligible effect on ductus tension (Fig. 1A).

Only a part of the oxygen-induced increase in ductus tension could be accounted for by changes in Ca_L -channel activity (Fig. 1B). The Ca_L -channel inhibitor, nifedipine, relaxed the ductus and nearly eliminated oxygen's effect on ductus tension in the physiologic range (2–15% O_2), but only partially inhibited oxygen's effects in the super-physiologic range ($\geq 30\%$ O_2). Similarly, BAY K8644, a Ca_L -channel opener, constricted the ductus and prevented oxygen from affecting ductus tension in the physiologic range, but only partially inhibited oxygen's effects in the super-physiologic range (Fig. 1C).

We used a combination of K^+ -channel inhibitors (4AP/TEA/glibenclamide), at concentrations that block K_V , K_{Ca} , and K_{ATP} channel activities (13), to determine whether alterations in K^+ -channel activity could account for the oxygen-induced changes in ductus tension. Inhibition of K^+ -channel activity had the same effect on ductus contractility as the Ca_L -channel opener, BAY K8644 (Fig. 2A). The K^+ -channel inhibitors blunted the ability of oxygen to affect ductus tension in the physiologic range (2–15% O_2), but only partially inhibited its effects in the super-physiologic range. The selective K_{Ca} and K_{ATP} inhibitors, iberiotoxin and glibenclamide, had no effect on tension. Only the K_V inhibitor, 4AP, increased tension and blunted the oxygen-induced response (Fig. 2B, C, and D). These findings are consistent with the hypothesis that, in the mature ductus, most of oxygen's contractile effects (in the physiologic range of oxygen concentrations) can be attributed to decreased K_V -channels activity, which results in subsequent calcium entry through voltage-gated Ca_L -channels; conversely, at super-physiologic oxygen concentrations, oxygen appears to use additional mechanisms that depend on extracellular calcium entry but are independent of K_V -channel and Ca_L -channel activities.

Oxygen-induced tension in the immature ductus arteriosus.

The immature ductus also develops an active tension that is maintained by extracellular Ca^{++} -dependent and Ca^{++} -independent pathways. Extracellular Ca^{++} -dependent pathways appear to play a smaller role in maintaining tension in the physiologic range of oxygen concentrations, in the immature ductus, than they do in the mature ductus (compare Figs. 1A and 3A). As the immature ductus becomes hypoxic, pathways that depend on extracellular Ca^{++} no longer appear to play a significant role in maintaining ductus tension. This appears to be due to a direct effect of low oxygen concentrations on Ca_L -channel activity. Conditions that usually open or close Ca_L -channels have either no effect (BAY K8644 and nifedipine) or a blunted effect (K^+ -Krebs solution) on tone at 2% O_2 (Fig. 3). This contrasts with the mature ductus, where

Table 1. Expression of K-channel subunits in the mature and immature ductus arteriosis

Gene	Gestation	Sheep ΔCT(MDH-gene)		Baboon ΔCT(MDH-gene)		Mouse ΔCT(MDH-gene)	
		Mean	(Sd)	Mean	(sd)	Mean	(sd)
Kv1.2	Immature	-8.0	(0.8)	-3.3	(1.3)	-3.9	(0.4)
	Mature	-5.6	(0.9)	-1.1	(0.9)	-4.8	(0.4)
	p<0.05	↑		↑		↓	
Kv1.5	Immature	-7.6	(1.5)	1.2	(0.2)	-3.6	(0.2)
	Mature	-4.5	(0.5)	0.7	(0.2)	-3.2	(0.3)
	p<0.05	↑		↓		↑	
Kv2.1	Immature	-4.0	(0.6)	-3.8	(0.8)	-4.7	(0.3)
	Mature	-3.0	(0.2)	-3.0	(0.5)	-3.6	(0.8)
	p<0.05	↑		↑		↑	
Kv9.3	Immature	-6.6	(1.4)	-6.5	(1.6)	-1.5	(0.3)
	Mature	-4.2	(0.9)	-4.7	(1.0)	-1.6	(0.4)
	p<0.05	↑		↑		—	
Kvβ1.2	Immature	-11	(0.5)	-5.1	(1.2)	-3.9	(0.3)
	Mature	-11	(1.0)	-3.3	(0.8)	-3.9	(0.6)
	p<0.05	—		↑		—	
Kvβ1.3	Immature	-2.6	(0.5)	-3.0	(1.2)	-1.5	(0.2)
	Mature	-1.3	(0.3)	-1.2	(0.8)	-2.0	(0.3)
	p<0.05	↑		↑		↓	
K _{IR} 6.1	Immature	-6.3	(0.3)	-1.7	(1.0)	-4.3	(0.4)
	Mature	-5.7	(0.3)	-2.0	(1.0)	-2.4	(0.7)
	p<0.05	↑		—		↑	
SUR2	Immature	-4.0	(0.2)	-0.8	(0.2)	-6.5	(0.5)
	Mature	-3.3	(0.3)	-1.2	(0.2)	-3.2	(0.4)
	p<0.05	↑		↓		↑	
BK _{Ca}	Immature	-9.0	(0.3)	-1.8	(0.3)	-10.6	(0.6)
	Mature	-8.6	(0.4)	-1.8	(0.3)	-9.5	(0.9)
	p<0.05	—		—		—	
BK _{Ca} β1	Immature	-2.6	(0.3)	-0.5	(0.3)	-4.8	(0.6)
	Mature	-1.2	(0.4)	0.1	(0.3)	-3.7	(0.9)
	p<0.05	↑		↑		↑	

Real Time polymerase chain reaction (PCR) measurements of K⁺-channel subunits in the mature and immature ductus arteriosus of sheep, baboons and mice. ΔCT(MDH-gene) represents the difference in cycle threshold (CT) between the expression of the housekeeping gene Malate dehydrogenase (MDH) and the gene of interest. Each unit of ΔCT(MDH-gene) represents a 2-fold increase in a gene's mRNA. The more negative the ΔCT(MDH-gene), the fewer the number of starting copies of a gene (mRNA). Number of separate animals used: sheep (immature = 7, mature = 7); baboon (immature = 10, mature = 10); mouse litters (immature = 10, mature = 9). From each litter of mice we obtained between 10–12 ductus. ↑ *p* < 0.05, mature significantly greater than immature; ↓ *p* < 0.05, mature significantly less than immature; -*p* > 0.05, mature not significantly different from immature.

Figure 1. Effects of oxygen on active tension in the mature ductus arteriosus. Values represent active tension (mean ± SD) as a percent of maximal active tension. *n* = number of ductus rings from separate animals. Ductus were studied in the presence or absence of *Panel A*: Krebs solution (Control, □, *n* = 10), High K⁺-Krebs solution (■, *n* = 8), calcium-free Krebs solution (△, *n* = 10), or calcium-free/High K⁺-Krebs solution (▼, *n* = 8); *Panel B*: 10⁻⁵ M nifedipine (○, *n* = 10); *Panel C*: 10⁻⁶ M BAY K8644 (●, *n* = 7). **p* < 0.05, experimental condition vs Control (□). Ductus tension at 6, 15, 30, and 95% O₂ differed significantly (*p* < 0.05) from the tension at 2% O₂, except for rings incubated in High K⁺-Krebs solution where tensions at 6, 15, 30, and 95% O₂ were not significantly different from those at 2% O₂; and in calcium-free Krebs, in calcium-free/High K⁺-Krebs solution, in nifedipine, and in BAY K8644 where tensions at 6% O₂ were not significantly different from those at 2% O₂.

a significant proportion of the tension in 2% O₂ is maintained by pathways that depend on the presence of extracellular Ca⁺⁺, and the effects of nifedipine, BAY K8644 and High-K⁺ solution are minimally affected by changes between 2 and 30% O₂ (Fig. 1).

We next examined the extent to which K⁺-channel activity affected tension in the immature and mature ductus. To account for the effect of low oxygen concentrations on the maximal tension that can be produced by K⁺-Krebs solution in the immature ductus (Fig. 3A), we expressed the net active tension (at a

Figure 2. Effects of oxygen on active tension in the mature ductus arteriosus. Ductus were studied in the presence or absence of *Panel A:* 10^{-6} M BAY K8644 (●) or (3×10^{-3} M 4AP + 10×10^{-3} M TEA + 10^{-5} M glibenclamide) (4AP/TEA/Glib) (▲, $n = 7$); *Panel B:* 3×10^{-3} M 4AP (■, $n = 7$); *Panel C:* 50×10^{-9} M iberiotoxin (○, $n = 5$); *Panel D:* 10^{-5} M glibenclamide (△, $n = 6$). * $p < 0.05$, experimental condition vs Control (□). Ductus tension at 6, 15, 30, and 95% O_2 differed significantly ($p < 0.05$) from the tension at 2% O_2 , except for rings incubated with BAY K8644 and rings incubated with 4AP/TEA/glibenclamide, where tensions at 6% O_2 were not significantly different from those at 2% O_2 .

particular oxygen concentration) as a percentage of the net active tension produced by K^+ -Krebs solution at that particular oxygen concentration (Fig. 4).

Tensions in the immature ductus (incubated under Control conditions, at oxygen concentrations between 2 and 15% O_2) were significantly less than those in the mature ductus (Fig. 4A). Inhibition of K^+ -channel activity, with 4AP/TEA/glibenclamide, increased tension in both the mature and immature ductus and eliminated the difference in tension between the two age groups (Fig. 4A).

In contrast with the mature ductus, where only the K_V channel inhibited tension (Fig. 2B, C, and D), tension in the immature ductus was inhibited by three types of K^+ -channels (K_V , K_{Ca} , and K_{ATP} channels). In the immature ductus, K_V , K_{Ca} , and K_{ATP} channel inhibitors blunted the effects of physiologic oxygen concentrations on ductus contractility (Fig. 4A–D). The K^+ channel inhibitors did not prevent oxygen from constricting the immature ductus at super-physiologic oxygen concentrations (Fig. 4A). Apamin had no effect on either the immature or mature ductus (data not shown).

The inhibitory effects of K_V , K_{Ca} , and K_{ATP} channels on contractility appeared to decline with advancing gestation because their selective antagonists were less effective in the mature compared with the immature ductus (Figs. 2 and 4). Their declining role was not due to decreased expression of K^+ -channel α - and β - subunits because their expression was either the same (BKCa) or higher (Kv1.2, Kv1.5, Kv 2.1, Kv 9.3, K_{IR} 6.1, Kv β 1.3, SUR2, and BKCa β 1) in the mature sheep ductus (Table 1).

Developmental changes in K^+ -channel α - and β -subunit expression also occurred in the baboon and mouse ductus; however, there was no consistent pattern of change in K^+ -

channel expression across the species (Table 1). The Kv2.1 and the BKCa β 1 subunits were the only channel subunits that changed their expression in the same direction (increase) with advancing gestation (Table 1).

DISCUSSION

Effects of physiologic and super-physiologic oxygen concentrations on the mature ductus. The ductus arteriosus develops an active tension that consists of both extracellular Ca^{++} -dependent and extracellular Ca^{++} -independent components. Recent reports suggest that the extracellular Ca^{++} -independent component of ductus tension may be due to rho kinase or tyrosine kinase-mediated Ca^{++} sensitization: a process whereby constriction occurs, independent of ongoing increases in cytosolic Ca^{++} (2,15–17). However, the changes in ductus tension that occur with changes in oxygen concentration appear to be mediated by pathways that regulate the entry of extracellular calcium into the ductus wall; removal of extracellular Ca^{++} eliminates the ability of oxygen to alter ductus tension (Fig. 1A).

In the late gestation fetus (in the absence of prostaglandin and nitric oxide production), changes in Ca_L -channel activity appear to be responsible for the changes in ductus tension that occur when oxygen concentrations are varied between 2 and 15% O_2 (the physiologic range) (Fig. 1). The effects of physiologic oxygen concentrations on ductus tension are blunted by the removal of extracellular calcium, or the presence of Ca_L -channel inhibitors or activators (Fig. 1). In addition, the effects of physiologic oxygen concentrations on ductus tension appear to be because of their ability to inhibit K^+ -channel activity (Fig. 2A). Our observations are consistent with prior studies that found that oxygen increased Ca^{++} entry through voltage-dependent Ca_L -channels, by inhibiting K^+ channel activity and depolarizing ductus smooth muscle (1,3,4,18,19).

Oxygen concentrations above the physiologic range ($\geq 30\%$ O_2 , the super-physiologic range) appear to inhibit most of the ductus' K^+ -channel activity because K^+ -channel inhibitors no longer affect ductus tension (Fig. 2). Although oxygen concentrations in the super-physiologic range eliminate the ability of K^+ -channel inhibitors to affect ductus tension, the converse is not the case; K^+ -channel inhibitors only partially inhibit oxygen's contractile effects in the super-physiologic range. Similarly, the Ca_L -channel opener, BAY K8644, and the Ca_L -channel blocker, nifedipine, only partially inhibit oxygen's effects in the super-physiologic range (Fig. 1). Therefore, additional extracellular Ca^{++} -dependent mechanisms that are independent of K^+ - and Ca_L -channel activities, must affect ductus tension when oxygen concentrations increase above 30% O_2 . We speculate that these may involve other depolarization-sensitive calcium channels (like T-type channels) rather than mechanisms that alter Ca^{++} sensitivity: both high K^+ and low Ca^{++} containing solutions block the effects of super-physiologic oxygen concentrations on ductus tension, whereas inhibitors of rho kinase and tyrosine kinase do not prevent super-physiologic oxygen concentrations from contracting the ductus (data not shown).

Figure 3. Effects of oxygen on active tension in the immature ductus arteriosus. Ductus were studied in the presence or absence of *Panel A*: Krebs solution (Control, □, $n = 13$), K⁺-Krebs solution (■, $n = 13$), calcium-free Krebs solution (△, $n = 13$), or calcium-free/High K⁺-Krebs solution (▼, $n = 13$); *Panel B*: nifedipine (○, $n = 12$); *Panel C*: BAY K8644 (●, $n = 12$). * $p < 0.05$, experimental condition vs Control (□). Ductus tension at 6, 15, 30, and 95% O₂ differed significantly ($p < 0.05$) from the tension at 2% O₂, except for rings incubated with calcium-free Krebs solution, in calcium-free/High K⁺-Krebs solution, and in nifedipine where tensions at 6% O₂ were not significantly different from those at 2% O₂.

Figure 4. Effects of oxygen and K⁺-channel inhibitors in the mature and immature ductus arteriosus. Values represent ductus tension (mean $\pm$ SD) (at a particular oxygen concentration) expressed as a percent of the net active tension produced by K⁺-Krebs solution at that particular oxygen concentration (Max Tension). *Panel A*: Ductus from mature ($n = 7$) and immature ($n = 9$) fetuses were studied in the presence or absence of 4AP/TEA/glibenclamide (Glib). Tensions in immature ductus, under Control (○) conditions (at 2 and 6% O₂) differed significantly ($p < 0.05$) from tensions in mature ductus under Control (□) conditions; the tensions at higher oxygen concentrations (15, 30, and 95% O₂) were not significantly different between the immature and mature ductus under Control conditions. When the rings were incubated in 4AP/TEA/glibenclamide, immature ductus (●) developed the same tensions as mature ductus (■). *Panel B*: Immature ductus were studied in the absence (○) or presence (◆) of 4AP ($n = 7$); *Panel C*: Immature ductus were studied in the absence (○) or presence (◆) of iberiotoxin ($n = 6$); *Panel D*: Immature ductus were studied in the absence (○) or presence (△) of glibenclamide (Glib, $n = 6$). * $p < 0.05$, experimental condition vs Immature Control. Ductus tension at 6, 15, 30, and 95% O₂ differed significantly ($p < 0.05$) from the tension at 2% O₂, except for rings incubated in 4AP/TEA/glibenclamide, where tensions at 6% O₂ were not significantly different from those at 2% O₂.

Effects of physiologic oxygen concentrations on the immature ductus. When oxygen concentrations are in the physiologic range, the tensions developed by the immature ductus are significantly less than those developed by the mature ductus (Figs. 1A and 3A) (2,11). Our studies suggest that the major factor responsible for the difference in tensions between the immature and mature ductus is the way the immature ductus processes extracellular Ca⁺⁺. In the absence of extracellular Ca⁺⁺, the tension in the mature ductus drops to the same level as the immature ductus ($32 \pm 6\%$ MAT) (Figs. 1A and 3A).

In the physiologic range of oxygen concentrations, oxygen affects the immature ductus' tension both by affecting the

influx of extracellular calcium (Fig. 3) and by altering K⁺-channel activity (Fig. 4). The inhibitory effects of low oxygen concentrations on calcium entry appear to be limited to their effects on Ca_L-channels; low oxygen concentrations do not alter the contractile effects of agonists that increase intracellular calcium through pathways that are independent of Ca_L-channels (2).

The differences in tensions between the immature and mature ductus also appear to be due to differences in K⁺ channel activity. Inhibition of K⁺-channel activity, with 4AP/TEA/glibenclamide, increased ductus tension in both the mature and immature ductus and eliminated the difference in tensions between the two age groups (Fig. 4A). Three K⁺-channels (K_V, K_{Ca}, and K_{ATP}) appear to play a role in inhibiting tension in the immature ductus (Fig. 4B, C, and D). The inhibitory effects of the K_{Ca} and K_{ATP} channels appear to decline with advancing gestation, because their selective antagonists have less of an effect on ductus tension as the fetus matures (compare Figs. 2B–D and 4B–D). The declining effects of K⁺-channel activity on ductus contractility is not due to decreased expression of K⁺-channel α - or β -subunits because their expression is increased, if anything, with advancing gestation (Table 1).

Effects of super-physiologic oxygen concentrations on the immature ductus. When oxygen concentrations increase into the super-physiologic range, in the immature ductus, the inhibitory effects of the K_V, K_{Ca}, and K_{ATP} channels appear to be blocked because their respective inhibitors (4AP, iberiotoxin, and glibenclamide) no longer have any effect on ductus contractility (Fig. 4). Whether the effects of super-physiologic oxygen concentrations are mediated through changes in the K⁺-channels themselves or through changes in commonly shared downstream pathways will have to await the results of future electrophysiological studies.

Super-physiologic oxygen concentrations eliminate the difference in tensions between the mature and immature ductus (Fig. 4A). These results may explain why super-physiologic arterial oxygen concentrations are able to constrict the immature ductus to the same degree as the mature ductus, *in vivo* (11).

Oxygen responsiveness in the immature ductus and Kv channel expression. Prior studies, in rabbits, suggest that reduced expression of the oxygen-sensitive K_V-channel α -subunits, Kv1.5 and Kv2.1, lead to impaired oxygen-induced constriction in the immature ductus (4). In these

studies, ex vivo transfer of the genes for Kv1.5 or Kv2.1 into the immature ductus partially rescued the contractile response to oxygen (4). In contrast, a recent study, performed in rats, found exactly the opposite results [*i.e.* expression of K_V α -subunits was greater in the immature than the mature ductus (20)]. We examined the developmental expression of several K_V-channel α -subunits in sheep, baboon, and mouse ductus to determine whether decreased K_V-channel α -subunit expression could lead to impaired oxygen-induced constriction in other species. The expression of several of the K_V α -subunits was reduced in the immature ductus in each of the species; however, only the Kv2.1 α -subunit was consistently reduced in all three species (Table 1).

Although decreased K_V α -subunit expression may lead to decreased oxygen-induced constriction in the immature rabbit ductus (4), it did not lead to a decreased contractile response to oxygen in the immature sheep ductus. In fact, changes in oxygen concentration (in the physiologic range) produced larger changes in tension in the immature ductus than in the mature ductus (Fig. 4A: Immature Control *versus* Mature Control). In addition, K⁺-channel inhibition did not prevent oxygen from constricting the immature sheep ductus at super-physiologic oxygen concentrations (Fig. 4A). Therefore, we speculate that other mechanisms may play a more significant role in the diminished responsiveness of the immature ductus to oxygen. These may include increased K_{Ca} and K_{ATP} activity (Fig. 4), impaired calcium [Ca_L (2,21) or store-operated (17)] channel function, decreased rho kinase activity (2,15–17,21), decreased endothelin production (22), and/or increased sensitivity to prostaglandins and nitric oxide (14,23,24).

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