Oxygen-induced Constriction of Rabbit Ductus Arteriosus Occurs via Inhibition of a 4-Aminopyridine-, Voltage-sensitive Potassium Channel

Martin Tristani-Firouzi,[‡] Helen L. Reeve,[§] Simona Tolarova,[‡] E. Kenneth Weir,* and Stephen L. Archer*
**Veteran's Affairs Medical Center, Minneapolis, Minnesota 55417; and [‡]Department of Pediatrics and [§]Department of Medicine, University of Minnesota, Minneapolis, Minnesota 55455*

Abstract

The ductus arteriosus is a vital fetal structure allowing blood ejected from the right ventricle to bypass the pulmonary circulation in utero. Closure of the ductus arteriosus at birth, essential for postnatal adaptation, is initiated by an increase in oxygen (O_2) tension. We recently demonstrated the presence of O_2-sensitive potassium channels in the fetal and adult pulmonary circulation which regulate vascular tone in response to changes in O_2 tension. In this study, we assessed the cellular mechanisms underlying O_2-induced constriction of the ductus arteriosus in late-gestation fetal rabbits. We report that O_2 reversibly inhibits a 58-pS voltage- and 4-aminopyridine-sensitive potassium channel, causing membrane depolarization, an increase in intracellular calcium through L-type voltage-gated calcium channels, and constriction of the ductus arteriosus. We conclude that the effector mechanism for O_2 sensing in the ductus arteriosus involves the coordinated action of delayed rectifier potassium channels and voltage-gated calcium channels. (*J. Clin. Invest.* 1996. 98:1959–1965.) Key words: oxygen-sensing • ion channels • fetal physiology • calcium • vascular tone

Introduction

The ductus arteriosus (DA)[1] is a vital fetal structure allowing blood ejected from the right ventricle to bypass the pulmonary circulation in utero. Closure of the DA at birth, essential for postnatal adaptation, is initiated by an increase in oxygen (O_2) tension (1–4). O_2 exerts a direct constrictor effect on DA vascular smooth muscle (4). Many agents, such as vasoconstrictor prostanoids (5) and endothelin-1 (6, 7), have been proposed to act as O_2 effectors. However, experimental evidence suggests that O_2-induced ductal constriction is not mediated by a single mediator. Despite extensive investigation, the cellular mechanisms whereby O_2 induces constriction of the DA remain elusive. We recently demonstrated the presence of O_2-sensitive potassium (K^+) channels in the fetal and adult pulmonary circulation which regulate vascular tone in response to changes in O_2 tension (8, 9). We hypothesized that O_2-induced DA constriction is mediated by inactivation of O_2-sensitive K^+ channels. Inhibition of these channels causes membrane depolarization, an increase in intracellular calcium ($[Ca^{2+}]_i$), and constriction of the DA. Using late-gestation fetal rabbit DA, we tested the effects of O_2 and K^+ channel antagonists on tension in isolated DA, on whole cell K^+ current (I_K) and membrane potential (E_m) in DA vascular smooth muscle (VSM) cells and on $[Ca^{2+}]_i$ levels.

Methods

The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1985) and was approved by the Minneapolis VA Medical Center Animal Studies Subcommittee and the University of Minnesota Animal Care Committee. Pregnant New Zealand White rabbits at 30 or 31 d of gestation (term = 31 d) were anesthetized with ketamine 75 mg and xylazine 20 mg intramuscularly and 50 mg pentobarbital intravenously. The fetal pups were delivered by cesarean section and a midline sternotomy was performed on the pups, before initiation of respiration. The heart, lungs, and great vessels were excised en bloc and placed in deoxygenated Earle's solution. The DA was carefully dissected free from adventitia under a dissecting microscope and severed distal to the takeoff of the left pulmonary artery and proximal to the insertion into the descending thoracic aorta.

Tension measurements in isolated DA rings. The isolated DA was placed between two stainless steel wires connected to strain gauge transducers in a 3-ml bath containing Earle's solution equilibrated with 0% O_2, 5% CO_2 (referred to as hypoxia, $pO_2 = 22 \pm 1$ Torr, $n = 15$) or 20% O_2, 5% CO_2 (normoxia, $pO_2 = 133 \pm 1$ Torr, $n = 15$) at an optimum tension of 400 mg. The optimum tension was determined by measuring the maximum contractile response to 60 mM KCl at varying basal tensions in hypoxia (200–1,000 mg tested in preliminary experiments). When desired, the endothelium was denuded by repeatedly passing a small wire through the lumen. The absence of endothelium was confirmed by the lack of relaxation to substance P (0.1 μM). Data were recorded using an analog-digital computer system (MacLab, AD Instruments Inc., Medford, MA).

After equilibration in hypoxia for 30 min, the DA was exposed to a normoxic solution and the effect of tension was recorded. The DA was returned to hypoxia and a dose–response was performed to one

Address correspondence to Stephen L. Archer, M.D., Division of Cardiology, 111C, Veteran's Affairs Medical Center, One Veteran's Drive, Minneapolis, MN 55417. Phone: 612-725-2000 x3653; FAX: 612-727-5668; E-mail: arche002@maroon.tc.umn.edu

Received for publication 22 July 1996 and accepted in revised form 3 September 1996.

1. *Abbreviations used in this paper:* 4-AP, 4-aminopyridine; $[Ca^{2+}]_i$, intracellular calcium; DA, ductus arteriosus; E_m, membrane potential; GLI, glibenclamide; I_K, whole cell K^+ current; K_{ATP}, ATP-sensitive K^+ channel; K_{Ca}, Ca^{2+}-activated K^+ channel; K_{DR}, delayed rectifier K^+ channel; NIS, nisoldipine; TEA, tetraethylammonium; VEH, vehicle; VSM, vascular smooth muscle.

The Journal of Clinical Investigation
Volume 98, Number 9, November 1996, 1959–1965

of the following K^+ channel antagonists: 4-aminopyridine (4-AP; 1, 5, 10 mM), glibenclamide (GLI; 1, 10 μM), or tetraethylammonium (TEA; 1, 5, 10 mM), preferential inhibitors of delayed rectifier (K_{DR}), ATP-sensitive (K_{ATP}), and Ca^{2+}-activated (K_{Ca}) K^+ channels, respectively. The DA was exposed to normoxia in the continued presence of K^+ channel inhibition to test any additive effect of O_2. Each DA was exposed to only one K^+ channel antagonist. The number of rings used with each K^+ channel blocker is given in the legend to Fig. 1.

In a separate group of DA rings, the role of L- and T-type Ca^{2+} channels in O_2-induced DA constriction was assessed. To eliminate the effects of prostanoids and nitric oxide, the endothelium was denuded as described above and rings were pretreated with indomethacin (3 μM) and L-N^G-nitro arginine methyl ester (L-NAME, 0.1 mM). After constriction with normoxia, the nonspecific Ca^{2+} channel antagonist lanthanum (La^{3+}; 5 mM, $n = 6$), L-type Ca^{2+} antagonist nisoldipine (NIS; 0.5 μM, $n = 4$), T-type antagonist RO40-5967 (0.5 μM, $n = 5$), or polyethylene glycol vehicle (VEH, $n = 2$) was administered and the effect on tension was recorded. The RO40-5967 dose was chosen to preferentially inhibit T-type Ca^{2+} channels (10).

Whole-cell patch–clamp technique. The effect of O_2 and K^+ channel blockade on whole-cell I_K and E_m was assessed using the amphotericin-perforated patch–clamp technique (11). Freshly dispersed DA VSM were obtained daily by enzymatic digestion with 0.5 mg/ml papain and 0.5 mg/ml collagenase. Cells were perfused (2 ml/min) with a solution containing (mM): NaCl 115, $NaHCO_3$ 25, KCl 4.2, $MgCl_2$ 0.5, $CaCl_2$ 1.5, glucose 10, and Hepes 10, pH 7.4, equilibrated with 0% O_2, 5% CO_2 (hypoxia, $pO_2 \sim 33$ Torr) or 20% O_2, 5% CO_2 (normoxia, $pO_2 \sim 130$ Torr) at 32°C. Pipette solutions contained (mM): KCl 140, $MgCl_2$ 1.0, EGTA 5, phosphocreatine 2, Hepes 10, and amphotericin B 120 μg/ml, pH 7.2. Cells were voltage clamped at a holding potential of −70 mV and currents evoked by 10-mV steps using test pulses of 200 ms in duration at a rate of 0.033–0.1 Hz. To record E_m, cells were held in current clamp at resting E_m. Data were recorded on-line and analyzed using pCLAMP 6.03 software (Axon Instruments, Foster City, CA) as described previously (9).

To determine the pharmacology of whole-cell currents and establish the effects of O_2 on the currents, cells were exposed to O_2 and K^+ channel antagonists. After equilibration in hypoxia, cells were exposed to normoxia ($n = 8$) for 2–4 min and returned to hypoxia to record recovery currents or E_m. Under hypoxic conditions, cells were exposed to 4-AP (1 mM, $n = 6$), GLI (1 μM, $n = 4$), or TEA (5 mM, $n = 3$) while recording I_K or E_m. In some cells, more than one K^+ channel antagonist was tested.

Cell-attached, single channel recordings. Cell-attached, single channel recordings were performed as described previously (9). Bath and pipette solutions were identical to those described above, except amphotericin was excluded from the pipette solution. Single channel recordings were performed in hypoxia, normoxia, and after return to hypoxia ($n = 3$). 4-AP (1 mM) or TEA (5 mM) was administered in hypoxia. Channel activities were expressed as NP_O, where N is the number of channels and P_O is the probability, measured at a pipette potential of +40 mV.

Measurement of $[Ca^{2+}]_i$. For analysis of $[Ca^{2+}]_i$, isolated DA rings were loaded with the Ca^{2+}-sensitive fluorophore Fura-2 AM (12 μM) at 37°C in room air for 2.5 h. After rinsing, the ring was placed between stainless steel stirrups connected to a force transducer, in a heated bath (37°C) atop an inverted microscope (Nikon Diaphot). Tension was measured exactly as in the previously described ring

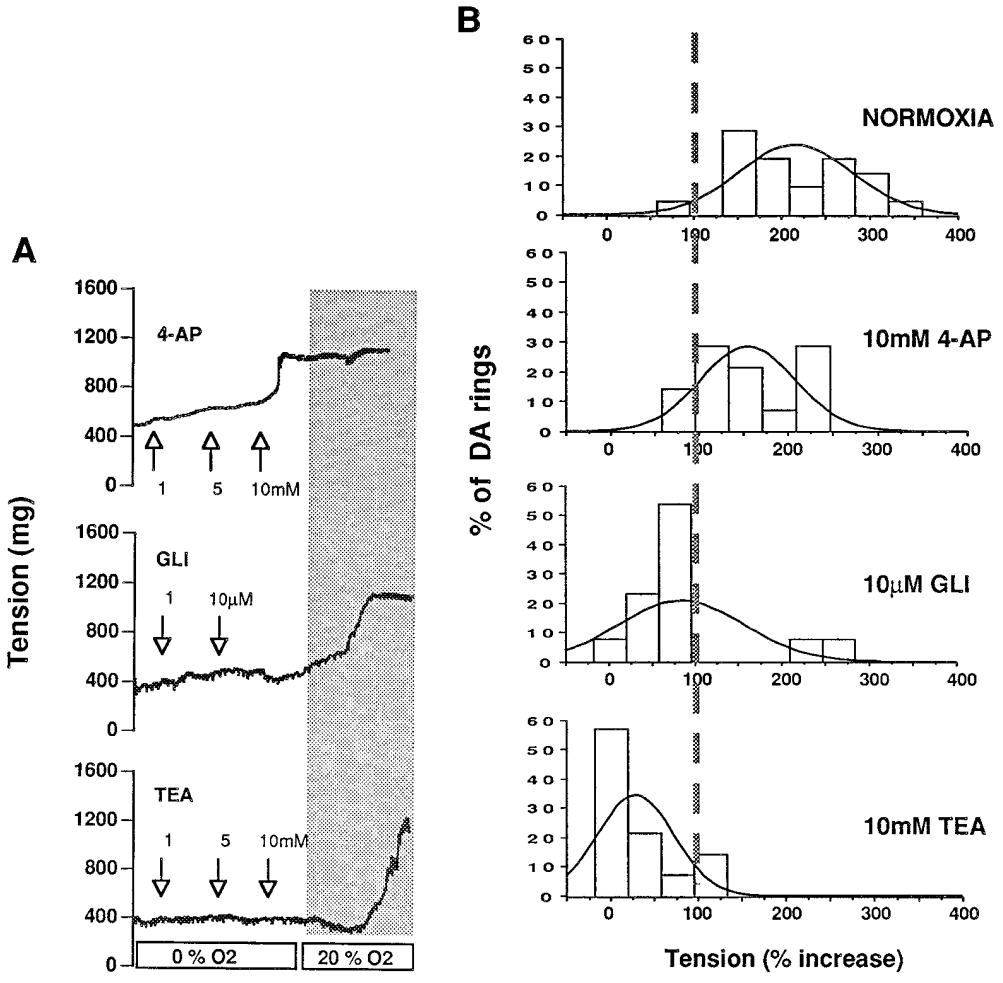

Figure 1. O_2 and the K^+ channel antagonist 4-AP constrict the isolated DA. (*A*) Representative tension tracings of dose–response to 4-AP (1, 5, 10 mM; $n = 15$), GLI (1, 10 μM; $n = 13$), and TEA (1, 5, 10 mM; $n = 13$) in endothelium-intact DA rings in hypoxia and with the superimposition of normoxia (*shaded panel*). (*B*) Frequency histograms in endothelium-intact DA rings, depicting the percentage of DA rings that constrict to the tension demonstrated on the horizontal axis. 4-AP (10 mM) caused a similar degree of constriction as normoxia in most rings, whereas the constriction induced by GLI (10 μM) and TEA (5 mM) was often absent and when it occurred was significantly less than that induced by normoxia (both $P < 0.05$). Similar results were obtained in endothelium-denuded rings (data not shown).

studies. Rings were stimulated with alternating 340- and 380-nm wavelengths at 60 Hz while collecting the 510-nm emission with an epifluorescence microscope and photomultiplier (Photon Technology Inc.) A 75 W xenon lamp served as the light source. $[Ca^{2+}]_i$ was calculated using the Grynkiewicz formula (12): $[Ca^{2+}]_i$ (nM) = $K_d \times R - R_{min})/(R_{max} - R)$(sf380/sb380), where R is the ratio of 340/380 under the experimental condition. R_{max} was obtained by permeabilizing the ring with 40 μM ionomycin, thus saturating Fura-2 with extracellular Ca^{2+}. R_{min} was determined by chelating all free Ca^{2+} with 10 mM EGTA. sf380 is the maximum 380-nm intensity obtained and sb380 is the minimum. The experimentally determined dissociation constant (K_d) for Fura-2 was 245 nM.

After equilibration in hypoxia, the ring was exposed to normoxia ($n = 4$), while measuring $[Ca^{2+}]_i$ and tension. The effect of 10 mM 4-AP was measured in hypoxia ($n = 3$).

Data analysis. The results are presented as the mean±SEM. Intergroup differences were analyzed using the factorial ANOVA with the Fisher least significant post-hoc test. I-V plots were analyzed using repeated measures ANOVA. $P < 0.05$ was required for significance. Statistical calculations were performed using Statview 4.1 for Macintosh (Abacus Concepts, Berkeley, CA).

Results

Tension measurements in isolated DA rings. To test the hypothesis that O_2 constricts the DA by inhibiting K^+ channels, we examined the effect of K^+ channel antagonists on tension in isolated DA rings. 4-AP caused a dose-dependent increase in DA tension in endothelium-intact (Fig. 1) and -denuded (Fig. 2) rings in hypoxia, with 10 mM constricting to the same degree as normoxia. Neither GLI nor TEA consistently increased tension in endothelium-intact (Fig. 1) or -denuded DA rings (data not shown). There was little additive constrictor effect of O_2 in the presence of 4-AP, whereas the addition of normoxia in the presence of GLI and TEA caused further constriction (Figs. 1 and 2). These data demonstrate that 4-AP, a preferential K_{DR} inhibitor, constricts the DA, acting independent of the endothelium, and that O_2 exerts no additional constrictor effect in the presence of 4-AP.

Whole-cell patch–clamp technique. The effect of O_2 on whole-cell I_K was tested using the amphotericin-perforated patch–clamp technique in DA VSM (Fig. 3). Normoxia reversibly suppressed I_K by 22.5±2.7% (+40 mV, $n = 9$). 4-AP (1 mM) and TEA (5 mM) administered in hypoxia inhibited I_K 65.3±6.3% and 50.3±9.0% (+40 mV), respectively, whereas GLI (10 μM) was without effect. TEA suppressed I_K at test potentials positive to resting E_m, whereas O_2 suppressed I_K at potentials near resting E_m (Fig. 3 *B*, *inset*). This explains why O_2 causes E_m depolarization and DA constriction, while TEA causes neither depolarization nor vasoconstriction. The effects of O_2 and K^+ channel blockade on E_m are demonstrated in Fig. 3 *C*. Resting E_m in hypoxic DA VSM was −34.3±2.1 mV ($n = 13$) and depolarized to −19.8±5.2 mV within 2 min of normoxia ($n = 4$, $P < 0.05$). 4-AP in hypoxia depolarized E_m to a similar degree as normoxia. GLI and TEA did not alter E_m. The addition of normoxia in the presence of 4-AP did not cause further E_m depolarization (data not shown), implying that O_2 and 4-AP may act through a similar mechanism.

Cell-attached, single channel recordings. To further characterize the K^+ channel inhibited by O_2, cell-attached, single channel recordings were performed (Fig. 4). Normoxia inhibited a K^+ channel with a calculated conductance of 58±1 pS ($n = 3$), with an associated decrease in NP_O of 74%. This channel was inhibited by O_2 and 4-AP, but not TEA. These data suggest that O_2-induced DA constriction is mediated through inhibition of a specific K_{DR} channel, resulting in membrane depolarization. Normoxia did not inhibit a 150-pS channel, which was blocked by TEA.

Measurement of $[Ca^{2+}]_i$ and role of Ca^{2+} channels. The effect of O_2 on $[Ca^{2+}]_i$ in isolated DA rings is demonstrated in Fig. 5 *A*. Normoxia increased $[Ca^{2+}]_i$ 258±90%, while simultaneously increasing tension 180±34%. The role of extracellular Ca^{2+} and voltage-gated Ca^{2+} channels in O_2-induced DA tone is demonstrated in Fig. 5 *B*. La^{3+} and NIS produced profound vasodilation in O_2-constricted DA rings, while RO40-5967 and VEH were without significant effect. These data suggest that O_2-induced DA constriction is primarily dependent upon Ca^{2+} influx through L-type Ca^{2+} channels.

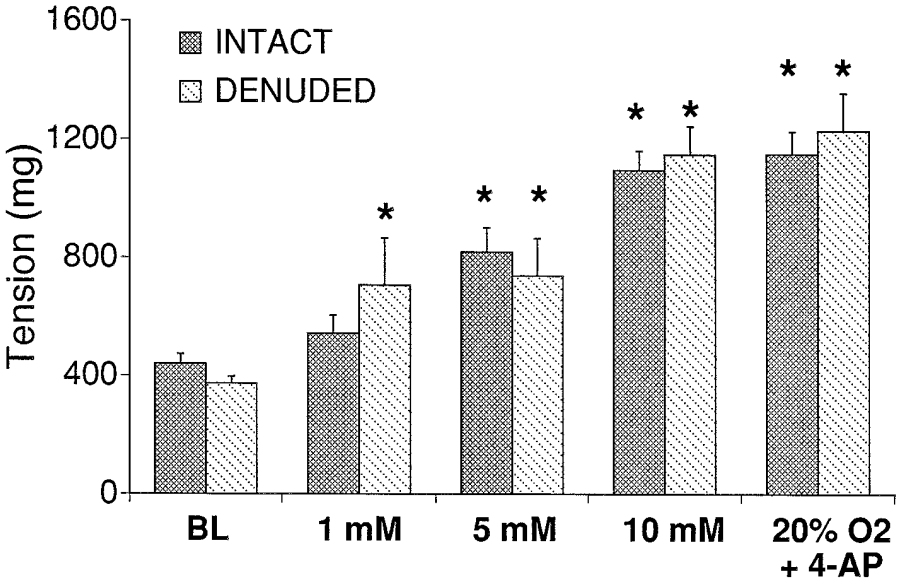

Figure 2. 4-AP increases tension in endothelium-intact and -denuded DA rings. 4-AP caused a dose-dependent increase in tension under hypoxic conditions in both endothelium-intact ($n = 15$) and -denuded rings ($n = 5$). The addition of normoxia did not increase tone beyond that observed with 10 mM 4-AP. *$P < 0.05$ compared with baseline (*BL*).

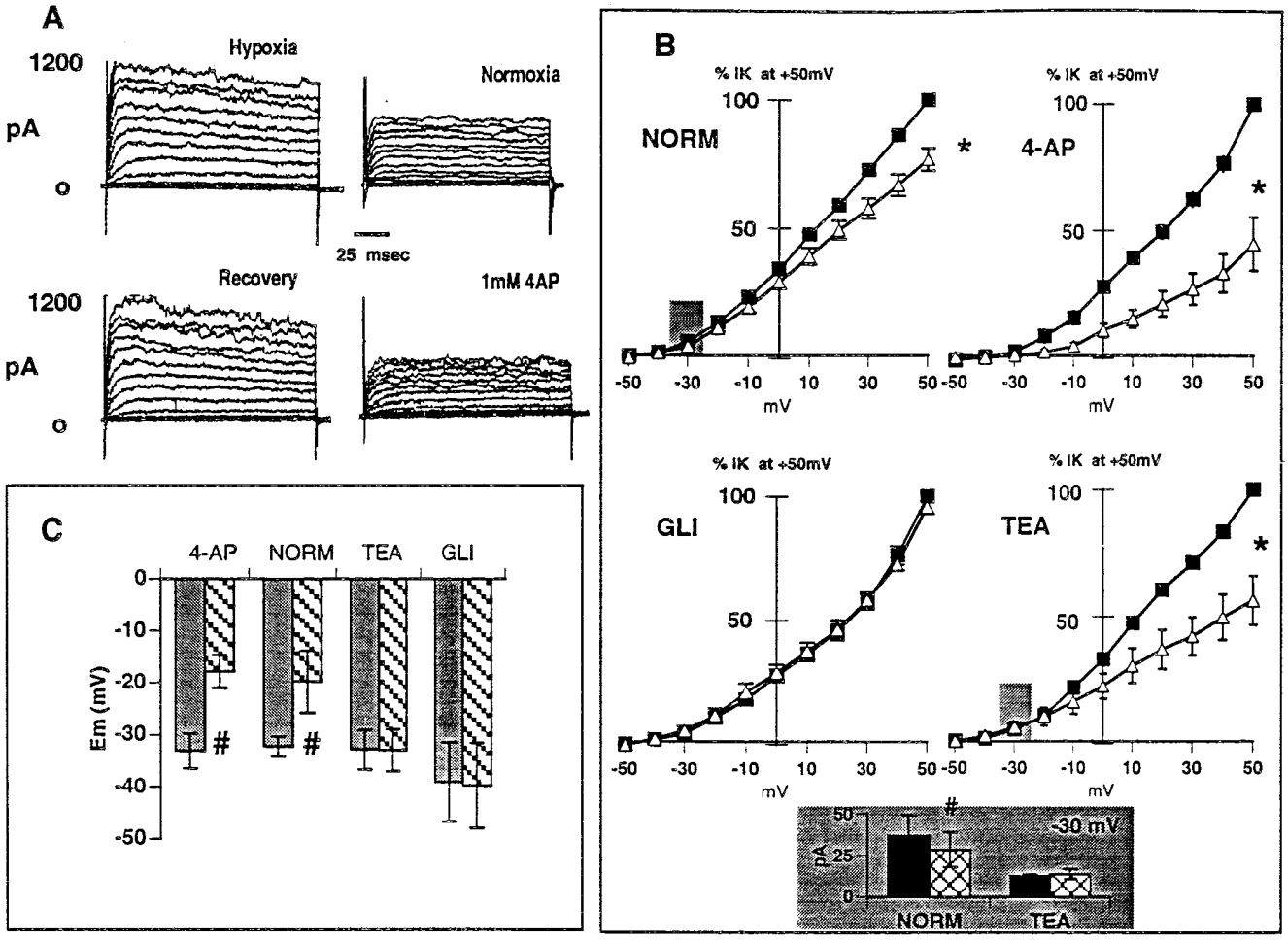

Figure 3. Effect of O_2 and K^+ channel blockade on whole-cell I_K and E_m. (*A*) Representative current traces of I_K recorded in hypoxia, normoxia, before return to hypoxia (recovery), and after 1 mM 4-AP added in hypoxia. (*B*) Average current voltage (I–V) relationships for O_2 and K^+ channel antagonists. Current was normalized to peak I_K at +50 mV. O_2 ($n = 8$), 4-AP (1 mM, $n = 6$), and TEA (5 mM, $n = 3$) suppressed I_K, while GLI (10 μM, $n = 4$) was without effect. (*Shaded inset*) Average raw currents evoked at −30 mV test potential in hypoxic control (*solid bar*) and during normoxia or TEA (*cross-hatched bar*). Normoxia, but not TEA, suppressed I_K at test potentials near resting E_m. (*C*) O_2 ($n = 5$) and 4-AP ($n = 5$) depolarized E_m, while GLI ($n = 3$) and TEA ($n = 3$) were without effect. $^*P < 0.05$ compared with control I–V curve, using repeated measures ANOVA. $^\#P < 0.05$ compared with hypoxic control. K^+ channel antagonists were tested in continued hypoxia.

Discussion

Constriction of the DA at birth routes blood flow through the pulmonary circulation, where postnatal gas exchange occurs. Failure of the DA to constrict (patent DA) is a cause of significant morbidity and mortality in premature infants. The increase in O_2 tension at birth exerts a direct effect on DA VSM to initiate DA constriction (4). However, the precise mechanism of O_2-induced DA constriction remains unknown. This study shows that O_2 inhibits a 58-pS, K_{DR} channel in DA VSM, resulting in membrane depolarization, activation of L-type Ca^{2+} channels, an increase in $[Ca^{2+}]_i$, and DA constriction.

This is the first complete characterization of the cellular pathway initiated by O_2 and leading to DA constriction. O_2-sensitive K^+ channels, initially described in carotid body chemoreceptors (13), also regulate vascular tone in response to changes in pO_2 in pulmonary (8, 9, 14), coronary (15), and cerebral (16) arteries. In fetal resistance pulmonary artery VSM, hypoxia inhibits K_{Ca} channels which causes membrane depolarization, activation of voltage-operated Ca^{2+} channels, and pulmonary vasoconstriction (8, 17). In the adult pulmonary circulation, hypoxia inhibits a K_{DR} channel and initiates vasoconstriction (9, 18). In the DA, hypoxia activates rather than inhibits K_{DR} channels. It is intriguing that subsets of the same class of K^+ channel (delayed rectifier) respond in an opposite fashion when faced with similar O_2 tensions. Although the two channels appear different based on their different conductances, a molecular approach is necessary to definitively identify the K_v channels in each of these O_2-sensitive tissues. The K_{DR} antagonist 4-AP constricts both the pulmonary artery and the DA, suggesting that the O_2 sensor mechanism is proximal to the channel; that is, the channel itself is not the sensor, but perhaps the sensor is an associated subunit which regulates gating of the channel in response to changes in pO_2. In the pulmonary artery, the sensor appears to be redox modulated, in that hypoxia and reductants close, while normoxia and oxidants open, K^+ channels (19, 20). The opposing effects of O_2 on the K_{DR} channel may be explained by differential modula-

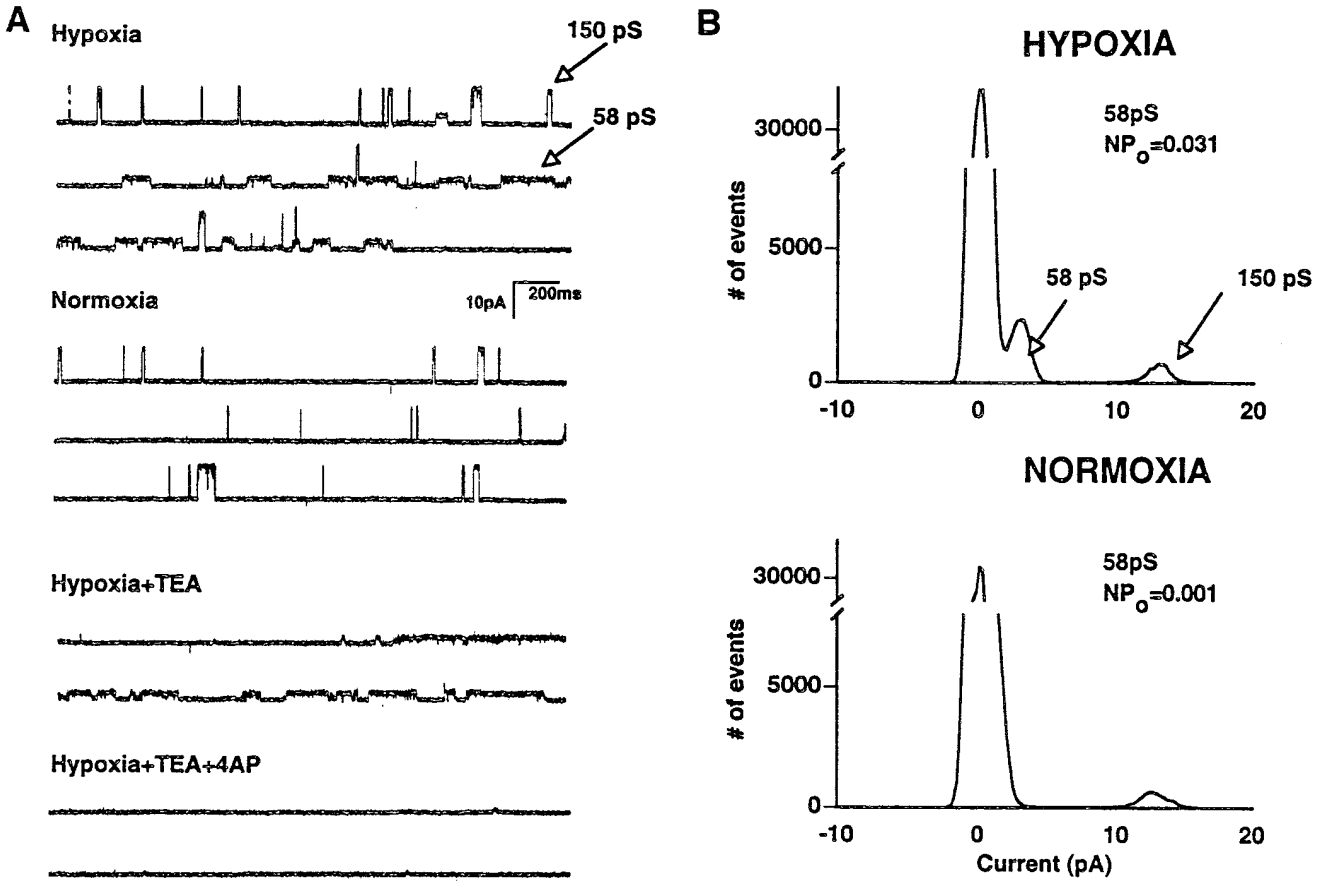

Figure 4. The effect of O_2 and K^+ channel antagonists on single channels. (*A*) Recordings from a cell-attached patch (+40 mV) demonstrating the presence of two distinct channels, a 58-pS channel, sensitive to 4-AP (1 mM), and a 150-pS channel, sensitive to TEA (5 mM). Normoxia decreased the NP_O of the 58-pS channel, while the 150-pS channel was not inhibited by changes in pO_2. (*B*) Events histograms showing normoxic inhibition of the 58-pS channel.

tion of the channel by cellular or membrane redox state, by differential responses of the pulmonary artery and DA to NAD(P)H oxidase or O_2 radicals. We also report the presence of a large conductance, TEA-sensitive channel in the DA which is not inhibited by changes in pO_2. The role of this channel was not investigated.

Similar to resistance pulmonary arteries (9), this study suggests that basal E_m in the DA is largely controlled by K_{DR} activity. 4-AP depolarized E_m and constricted the isolated DA. O_2-induced E_m depolarization was first reported in 1981 by Roulet and Coburn (21), using microelectrode puncture studies in isolated DA strips. The present study demonstrates that O_2 depolarizes E_m by suppressing whole-cell I_K and specifically, by decreasing the NP_O of K_{DR} channels. As gating of Ca^{2+} channels is strongly dependent upon E_m, depolarization activates Ca^{2+} channels, causing an influx of Ca^{2+} and vasoconstriction (22). Nakanishi et al. (23) found that O_2 only increased $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}, underscoring the importance of extracellular Ca^{2+} in O_2-induced DA constriction. We confirm this observation and show that the Ca^{2+} influx initiated by O_2 is carried almost exclusively through L-type Ca^{2+} channels.

Contrary to our findings, Nakanishi et al. (23) reported that 4 μM GLI produced maximal constriction of the isolated DA. They proposed that hypoxia activates K_{ATP} channels, promoting vasorelaxation and that normoxia inhibits K_{ATP} channels to cause vasoconstriction, although the patch–clamp technique was not used to confirm this hypothesis. A similar mechanism, involving K_{ATP} channels, is postulated to mediate hypoxia-induced coronary artery vasodilation (15). In our study, 10 mM 4-AP, but not 10 μM GLI, caused maximal constriction in hypoxia in the majority of rings tested. Furthermore, GLI did not depolarize resting E_m and did not suppress I_K in DA VSM. In support of the K_{DR} channel as the O_2-sensitive K^+ channel subtype, 4-AP depolarized basal E_m and suppressed outward I_K, as did normoxia. Finally, as O_2 inhibits a 4-AP-sensitive, 58-pS channel, this suggests that the O_2-sensitive K^+ channel belongs to the K_{DR} class.

How do the various mechanisms controlling tone in the DA interact? DA tone is determined by a balance between vasodilator and vasoconstrictor mechanisms. In utero, DA patency is primarily dependent upon production of prostaglandin E2 (24) with a minor contribution by nitric oxide (25). Although there is a decrease in the production and potency of prostaglandin E2 at birth (26), closure of the DA is largely the result of an independent, vasoconstrictor mechanism. The proposed mechanisms by which this vasoconstriction occurs involve O_2-induced increases in the production of endothelin-1 (7) or K^+ channel inhibition.

Endothelin-1 is a potent constrictor of DA rings (although

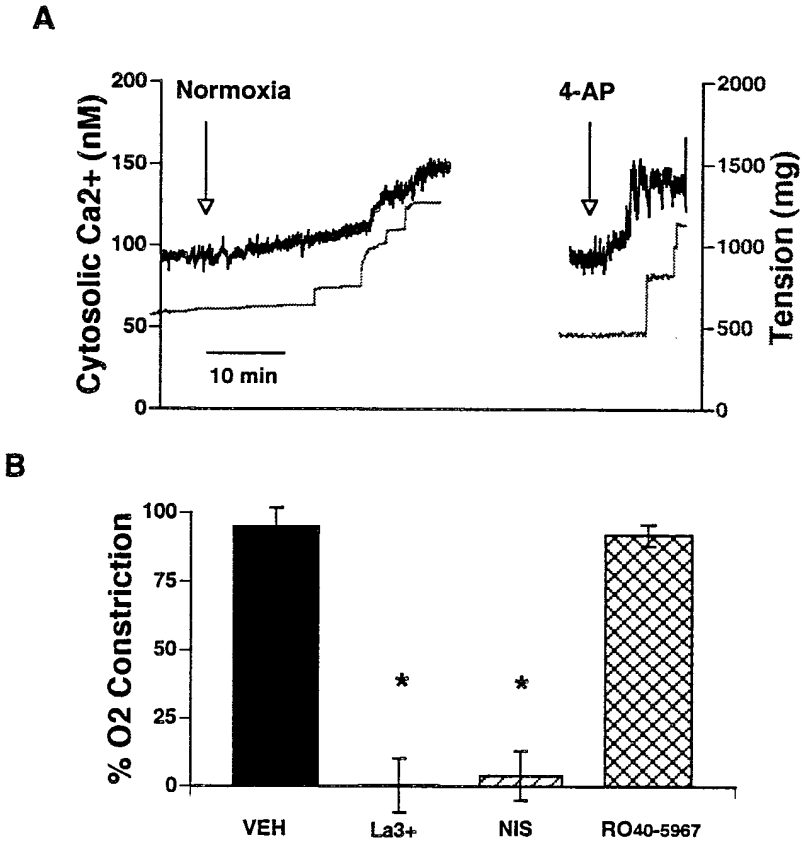

Figure 5. Essential role of extracellular Ca^{2+} in O_2-induced DA constriction. (*A*) Representative tracings of simultaneous tension and $[Ca^{2+}]_i$ measurements in response to O_2 and 4-AP (10 mM). (*B*) Effect of inhibitors of Ca^{2+} entry on O_2-induced DA tone. La^{3+} (5 mM) and NIS (0.5 μM) completely relaxed O_2-constricted DA rings, while RO40-5967 (0.5 μM) and VEH had no effect. $*P < 0.001$ compared with VEH.

it may not constrict the ductus in vivo (6). Furthermore, endothelin-1 is produced in the smooth muscle layer of the DA in response to increased O_2 levels (7). It does appear, however, that the time course of production of endothelin-1 is slower than the ductal constriction to O_2. Nonetheless, an endothelin-1 receptor antagonist (BQ123) does attenuate O_2-induced DA constriction in lamb (6). Thus, it is likely that the endothelin-1 mechanism is important in closure of the DA (6). How does the endothelin-1 mechanism relate to the K^+ channel mechanism? There are data to support the possibility that the two mechanisms are additive. Although Coceani et al. (6) found that BQ123 attenuates O_2-induced DA constriction, there was still significant residual constriction to O_2, despite administration of an endothelin-1 antagonist. On exposure to O_2, particularly at 95% O_2, DA rings constricted to roughly 50% of control levels despite the presence of BQ123 (1 μM) (6).

We conclude that O_2-induced DA constriction is initiated by inhibition of a 58-pS K_{DR} channel by O_2, which causes E_m depolarization, activation of L-type Ca^{2+} channels, and an increase in $[Ca^{2+}]_i$. Identification of the specific K^+ channel responsible for initiating normoxic ductal closure may facilitate development of therapy for patent DA.

Acknowledgments

We thank Daniel P. Nelson for expert and dedicated technical assistance.

This study was supported by the American Heart Association-Minnesota Affiliate Research Fellowship Award (M. Tristani-Firouzi), US Department of Veteran's Affairs, Minnesota Medical Foundation, and National Institutes of Health grant HL-45735 (S.L. Archer).

References

1. Kennedy, J.A., and S.L. Clark. 1941. Observations on the ductus arteriosus of the guinea pig in relation to its method of closure. *Anat. Rec.* 79:349–371.

2. Born, G.V.R., G.S. Dawes, J.C. Mott, and B.R. Rennick. 1956. The constriction of the ductus arteriosus caused by oxygen and by asphyxia in newborn lambs. *J. Physiol.* 132:304–342.

3. Kovalcik, V. 1963. The response of the isolated ductus arteriosus to oxygen and anoxia. *J. Physiol.* 169:185–197.

4. Fay, F.S. 1971. Guinea pig ductus arteriosus. I. Cellular and metabolic basis for oxygen sensitivity. *Am. J. Physiol.* 221:470–479.

5. Coceani, F., C.A. Breen, J.G. Lees, F.J.R., and P.M. Olley. 1988. Further evidence implicating a role for cytochrome P450-mediated reaction in the contractile tension of the lamb ductus arteriosus. *Circ. Res.* 62:471–477.

6. Coceani, F., L. Kelsey, and E. Seidlitz. 1992. Evidence for an effector role of endothelin in closure of the ductus arteriosus at birth. *Can. J. Physiol. Pharmacol.* 70:1061–1064.

7. Coceani, F., and L. Kelsey, 1991. Endothelin-1 release from lamb ductus arteriosus: relevance to postnatal closure of the vessel. *Can. J. Physiol. Pharmacol.* 69:218–221.

8. Cornfield, D.N., H.L. Reeve, S. Tolarova, E.K. Weir, and S.L. Archer. 1996. Oxygen causes fetal pulmonary vasodilation through activation of an oxygen sensitive potassium channel. *Proc. Natl. Acad. Sci. USA.* 93:8089–8094.

9. Archer, S.L., J.M.C. Huang, H.L. Reeve, V. Hampl, S. Tolarova, E. Michelakis, and E.K. Weir. 1996. The differential distribution of electrophysiologically distinct myocytes in conduit and resistance arteries determines their response to nitric oxide and hypoxia. *Circ. Res.* 78:431–442.

10. Mishra, S.K., and K. Hermsmeyer. 1994. Selective inhibition of T-type Ca^{2+} channels by Ro 40-5967. *Circ. Res.* 75:144–148.

11. Rae, J., K. Cooper, G. Gates, and M. Watsky. 1991. Low access resistance perforated patch recordings using amphotericin B. *J. Neurosci. Methods.* 37:15–26.

12. Grynkiewicz, G., M. Poenie, and R.Y. Tsien. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440–3450.

13. López-Barneo, J., J.R. López-López, J. Ureña, and C. González. 1988. Chemotransduction in the carotid body: K+ current modulated by PO2 in type I chemoreceptor cells. *Science (Wash. DC).* 241:580–582.

14. Post, J. M., J.R. Hume, S.L. Archer, and E.K. Weir. 1992. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. *Am. J. Physiol*. 262:C882–C890.

15. Daut, J., W. Maier-Rudolph, N. von Beckerath, G. Mehrke, K. Gunther, and L. Goedel-Meinen. 1990. Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science (Wash. DC).* 247:1341–1344.

16. Gebremedhin, D., P. Bonnet, A.S. Greene, S.K. England, N.J. Rusch, J.H. Lombard, and D.R. Harder. 1994. Hypoxia increases the activity of Ca^{2+}-sensitive K^+ channels in cat cerebral arterial muscle cell membranes. *Pflügers Arch*. 428:621–630.

17. Cornfield, D.N., T. Stevens, I.F. McMurtry, S.H. Abman, and D.M. Rodman. 1993. Acute hypoxia increases cytosolic calcium in fetal pulmonary artery smooth muscle cells. *Am. J. Physiol.* 265 (*Lung Cell. and Mol. Physiol.* 9): L53-L56.

18. Weir, E.K., and S.L. Archer. 1995. The mechanism of acute hypoxic pulmonary vasoconstriction: the tale of two channels. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 9:183–189.

19. Archer, S.L., J.A. Will, and E.K. Weir. 1986. Redox status in the control of pulmonary vascular tone. *Herz.* 11:127–141.

20. Reeve, H.L., E.K. Weir, D.P. Nelson, D.A. Peterson, and S.L. Archer. 1995. Opposing effects of oxidants and antioxidants on K+ channel activity and tone in rat vascular tissue. *Exp. Physiol.* 80:825–834.

21. Roulet, M.J., and R.F. Coburn. 1981. Oxygen-induced contraction in guinea pig neonatal ductus arteriosus. *Circ. Res.* 49:997–1002.

22. Nelson, M.T., J.B. Patlak, J.F. Worley, and N.B. Standen. 1990. Calcium channels, potassium channels, and voltage-dependence of arterial smooth muscle tone. *Am. J. Physiol*. 259:C3–C18.

23. Nakanishi, T., H. Gu, N. Hagiwara, and K. Momma. 1993. Mechanisms of oxygen-induced contraction of ductus arteriosus isolated from the fetal rabbit. *Circ. Res*. 72:1218–1228.

24. Coceani, F., and P.-M Olley. 1973. The response of the ductus arteriosus to prostaglandins. *Can. J. Physiol*. 51:220–223.

25. Coceani, F., L. Kelsey, and E. Seidlitz. 1994. Occurrence of endothelium-derived relaxing factor-nitric oxide in the lamb ductus arteriosus. *Can. J. Physiol. Pharmacol*. 72:82–88.

26. Clyman, R.I., F. Mauray, C. Roman, M.A. Heymann, and B. Payne. 1983. Effect of gestational age on ductua arteriosus response to circulating prostaglandin E2. *J. Pediatr.* 102:907–911.