The Role of Ion Channels in Hypoxic Pulmonary Vasoconstriction

E. Kenneth Weir, Jésus A. Cabrera, Saswati Mahapatra, Douglas A. Peterson, and Zhigang Hong

Abstract Hypoxic pulmonary vasoconstriction (HPV) is an important mechanism by which localized flow of blood in small resistance pulmonary arteries is matched to alveolar ventilation. This chapter discusses the role of several potassium and calcium channels in HPV, both in enhancing calcium influx into smooth muscle cells (SMCs) and in stimulating the release of calcium from the sarcoplasmic reticulum, thus increasing cytosolic calcium. The increase in calcium sensitivity caused by hypoxia is reviewed in Chapter 19. Particular attention is paid to the activity of the L-type calcium channels which increase calcium influx as a result of membrane depolarization and also increase calcium influx at any given membrane potential in response to hypoxia. In addition, activation of the L-type calcium channel may, in the absence of any calcium influx, cause calcium release from the sarcoplasmic reticulum. Many of these mechanisms have been reported to be involved in both HPV and in normoxic contraction of the ductus arteriosus.

Keywords Hypoxia • resistance pulmonary arteries • ductus arteriosus • voltage-gated potassium channels • L-type calcium channels • store-operated channels • sarcoplasmic reticulum

1 Introduction

Hypoxic pulmonary vasoconstriction (HPV) is one example of how the body senses a change in oxygen and, speaking teleologically, puts the information to good use. In the fetus, HPV helps to reduce blood flow through the unventilated lungs and consequently to direct blood through the ductus arteriosus (DA), which is open during the hypoxic, fetal stage of life. As a result of the onset of ventilation at birth and the associated increase in oxygen, the small, resistance pulmonary arteries (PAs) dilate, while

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the DA constricts. While the two diametrically opposite responses are both modulated by endothelial factors, the basic mechanisms of HPV and normoxic contraction of the DA reside in the smooth muscle cells (SMCs) of these vessels. In both cases, the "executive" mechanisms have two major components: an increase in cytosolic calcium and an increase in calcium sensitization. The former depends on ion channels and the sarcoplasmic reticulum and the latter on Rho/Rho kinase signaling.

2 Oxygen Sensing and Ion Channels in the Carotid Body

The role of ion channels in sensing hypoxia was first described in the carotid body, and this is still the best-understood model. Twenty years ago, López-Barneo et al. reported that hypoxia inhibited the outward potassium current (I_K) passing through voltage-gated potassium (K_v) channels in the plasmalemma of type 1 or glomus cells of the carotid body.[1] This in turn leads to membrane depolarization and calcium entry through L-type (voltage-gated) calcium channels down the calcium concentration gradient (2 m*M* extracellular, 100 n*M* intracellular). Subsequently, it has been demonstrated that other K^+ channels, calcium-sensitive (K_{Ca}) and Two-pore Domain Acid-Sensitive (TASK)-like, are also inhibited by hypoxia[2,3] and contribute to the depolarization. The essential part played by membrane depolarization in oxygen sensing is shown in an elegant experiment (Fig. 1.1),[4] in which membrane potential and cytosolic calcium are measured simultaneously in a type 1 cell. When the cell is made

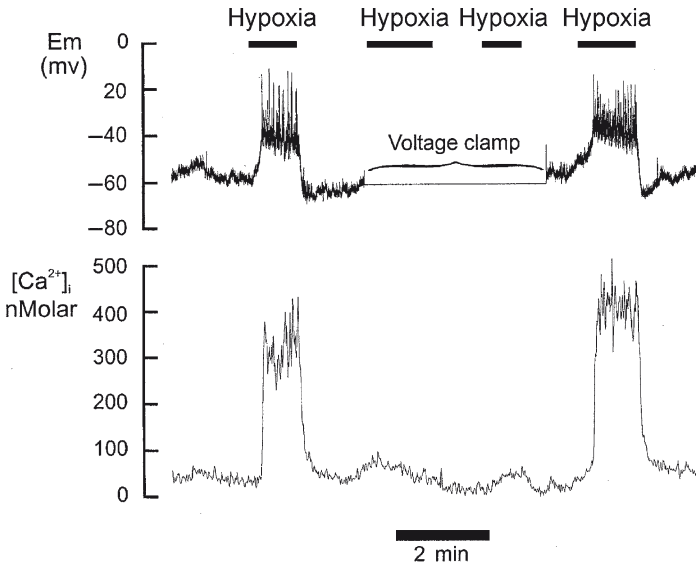

Fig. 1.1 The depolarization caused by hypoxia in the type 1 cell of the carotid body is associated with an increase in cytosolic calcium. If the membrane potential E_m is clamped at –60 mV, hypoxia does not increase the level of calcium. Adapted with permission[53]

hypoxic, the membrane depolarizes, and the calcium level increases. If, however, the membrane potential is artificially "clamped" at the approximate resting level of −60 mV, then another identical hypoxic challenge causes no increase in calcium. When the clamp is taken off, the next hypoxic challenge again stimulates an increase in cytosolic calcium. In the absence of external calcium or in the presence of an L-type calcium channel blocker, hypoxia causes little increase in cytosolic calcium.[4,5]

3 Hypoxic Pulmonary Vasoconstriction

In the adult animal, as first described in detail by von Euler and Liljestrand in 1946, acute alveolar hypoxia causes pulmonary vasoconstriction, which redistributes desaturated, mixed venous blood away from poorly ventilated areas of lung. Inhibition of HPV leads to a decrease in systemic arterial oxygen tension both in normal subjects and in patients with small airway disease or lung injury[6] because of a failure to match ventilation and perfusion. The executive mechanisms involving ion channels, which are responsible for hypoxic contraction of pulmonary artery smooth muscle cells (PASMCs), include inhibition of K^+ channels, membrane depolarization, and calcium entry through voltage-gated, L-type Ca^{2+} channels; an increase in Ca^{2+} influx through the L-type Ca^{2+} channels unrelated to depolarization; and the release of Ca^{2+} from the sarcoplasmic reticulum (SR), associated with repletion by Ca^{2+} influx through store-operated channels (SOCs).

4 K^+ Channels and HPV

In 1976, McMurtry et al. demonstrated that inhibitors of L-type calcium channels, such as verapamil, could ablate HPV in the isolated, perfused rat lung, while only moderately reducing the pressor response to angiotensin II or prostaglandin $F_{2\alpha}$.[7] This raised the possibility that hypoxia causes depolarization of the PASMCs. Hypoxia-induced depolarization in small PAs was subsequently reported by Harder et al. in 1985.[8] In the pancreatic islet cells, an increase in glucose metabolism yields adenosine triphosphate (ATP), which inhibits K_{ATP} channels, causing membrane depolarization, calcium entry, and insulin secretion.[9,10] Based on this observation, it was suggested that hypoxia might, through a change in redox status, reduce K^+ current I_K in PASMCs, again leading to calcium entry through L-type calcium channels.[11] The hypothesis was validated in part when it was shown that hypoxia inhibits I_K in PASMCs but not in SMCs from renal or mesenteric arteries.[12,13] Subsequently, a number of voltage-gated K^+ channels (K_v channels: $K_v1.2$, $K_v1.5$, $K_v2.1$, $K_v9.3$) have been found to be oxygen sensitive.[14,15] The evidence associating $K_v1.5$ with HPV is the best documented. Hypoxia inhibits $K_v1.5$ cloned from human PAs,[16] and HPV is diminished in mice in which $K_v1.5$ has been deleted.[17] The effect of hypoxia is apparently not directly on the channel protein

because, when the human $K_v1.5$ is overexpressed in mesenteric artery SMCs, hypoxia does not reduce I_K, but when it is overexpressed in PASMCs, hypoxia reduces I_K by 40%.[18] In the PASMC, studies using single-cell reverse-transcription polymerase chain reaction (PCR), have demonstrated that the level of expression of $K_v1.5$ correlates with the sensitivity of I_K, as a whole, in the individual SMCs to hypoxia.[19] This observation that different PASMCs have varying responses to hypoxia gave rise to the concept of pacemaker cells, which may initiate contraction and pass the signal to other PASMCs through gap junctions. This would be analogous to the calcium waves that have been described in pulmonary endothelial cells.[20]

In addition to the inhibition of I_K by acute hypoxia, the expression of K_v channels is reduced quite rapidly in chronic hypoxia. Thus, single PASMCs dispersed from the small PAs of rats maintained in 10% oxygen for 4 weeks had a resting membrane potential of −43.5 ± 2 mV compared to –54.3 ± 2 mV in cells from normoxic animals.[21] Subsequent work has shown that there is decreased messenger RNA (mRNA) and protein for several oxygen-sensitive K^+ channels in the chronically hypoxic cells.[22] In the case of $K_v1.2$, $K_v1.5$, and $K_v2.1$, the decrease in mRNA can occur within 6 h of the start of hypoxia.[23] In concordance with this observation, acute HPV is reduced in the isolated perfused lungs of rats that have previously been exposed to chronic hypoxia[24] and can be restored by the transfection of human $K_v1.5$ using an aerosol.[25] This experiment illustrated the importance of $K_v1.5$ in the mechanism of HPV, which is further reinforced by the finding that $K_v1.5$ protein is most abundant in the SMCs of resistance PAs, even though the mRNA for many K_v channels is present.[16] These experiments demonstrated that if oxygen-sensitive K_v channels are not expressed, there is membrane depolarization in PASMCs and loss of acute HPV, which can be restored by transfection of specific K_v channels.

It will be clear from the subsequent discussion that oxygen-sensitive K_v channels are not the sole executive mechanism of HPV but they play an important part. One question that arises concerning the role of K_v channels is the membrane potential at which they are active under normoxic conditions. As K_v channels are inactivated by depolarization, it may be that patch-clamp protocols that involve depolarization to positive membrane potentials (greater than 0 mV) inactivate some K_v channels that would otherwise be active at −60 mV.[16] Other K^+ channels that are known to be open at physiologic resting membrane potentials are KCNQ[26] (K_v 7) and the two-pore domain acid-sensitive potassium channel, TASK-1.[27] Consequently, it is possible that hypoxic inhibition of these channels might help to depolarize membrane potentials of PASMCs into the range where K_v channels are more active.[27,28]

5 L-Type Calcium Channels and HPV

Much attention has been paid to the effect of hypoxia on K^+ channels and the subsequent membrane depolarization. However, it is also important to note the effect of hypoxia on calcium influx through L-type channels into PASMCs at a specific fixed membrane potential. Just as there is a difference in the K^+ channels expressed in SMCs from conduit and resistance PAs,[29] so there is a difference in the

expression and behavior of L-type/Ca^{2+} channels.[30] In the PASMCs from resistance arteries, hypoxia increases $I_{Ca}{}^{2+}$ at membrane potentials below 0 mV, while inhibiting the calcium entry at more positive potentials. Interestingly, in the PASMCs from proximal or conduit arteries, hypoxia causes only inhibition. The differences in K^+ and Ca^{2+} channel expression and gating in response to hypoxia may help to explain HPV in the resistance arteries and hypoxic relaxation in the conduit arteries. The importance of L-type calcium channels in HPV is emphasized by the marked reduction of HPV by verapamil in the isolated rat lung mentioned earlier and by nifedipine and nisoldipine in the intact dog.[31]

6 Intracellular Calcium Release and HPV

More than 20 years ago, Hoshino et al. reported that a switch from hyperoxia to hypoxia caused contraction of human PA strips, in the presence of either histamine or KCL and the calcium ionophore A23187.[32] They considered that this hypoxic condition involved both calcium entry and calcium release from intracellular stores. However, the inhibitor of calcium release, HA 1004, also reduced the histamine preconstriction by half, so it is not clear whether the decrease in HPV was really an effect on the mechanism triggered by hypoxia or on the preconstriction. The same caveat applies to all interventions on HPV conducted in the setting of preconstriction.

A subsequent study demonstrated that release of calcium from the SR by caffeine or thapsigargin would reduce the increase in cytosolic calcium in cultured PASMCs caused by "hypoxia."[33] In this instance, concern arises because the cells were cultured from conduit PA, not resistance PA, and sodium dithionite was used to induce hypoxia. Sodium dithionite leads to hypoxia by generating reactive oxygen species (ROS).[34] Authentic hypoxia was used in an experiment looking at the effect of hypoxia on cytosolic calcium in PASMCs from resistance and conduit arteries and in SMCs from cerebral arteries.[35] Calcium went up with hypoxia in resistance PASMCs but down in SMCs from the other two arteries. Pretreatment of the resistance PASMCs with ryanodine, to release calcium from the SR, diminished the hypoxic increase in calcium. The importance of extracellular calcium influx was shown by the observation that chelation of extracellular calcium with EGTA rapidly ablated the elevated cytosolic calcium induced by hypoxia. A potential role for a hypoxic increase in calcium sensitivity was also raised in this article.[35]

In subsequent work using rings of canine resistance PA pretreated with phenylephrine, it was found that switching from hyperoxia to hypoxia caused contraction that could be reduced by ryanodine and caffeine or enhanced by cyclopiazonic acid (CPA).[36] These data suggest that hypoxia may release calcium from a ryanodine- and caffeine-sensitive store, and that the contraction may be modulated by calcium uptake into a distinct $InsP_3$ (inositol 1,4,5-trisphosphate) store, which can be blocked by CPA. HPV in this ring model did not require the presence of endothelium. The authors raised the possibility that some of the calcium entered the PASMC through a capacitative calcium entry (CCE) pathway activated by store depletion.[36]

7 Store-Operated Calcium Channels

HPV does not occur in the absence of external calcium in the isolated PA ring[37] or in the isolated perfused rat lung.[38] In the ring model, much of the calcium entry stimulated by hypoxia occurs through store-operated channels (SOCs).[37] More recent studies confirmed, in cultured rat PASMCs, that CCE induced by CPA is increased by hypoxia and inhibited by the SOC blockers SKF-96365, nickel, and lanthanum.[39] Incidentally, these workers reported that nifedipine (5 μ*M*) inhibited about 50% of the increase in calcium caused by hypoxia. Similar results have been described in canine PASMCs[36,40] in which the component of the hypoxia-induced increase in cytosolic calcium that was insensitive to nisoldipine (10 μ*M*) could be inhibited by SKF-96365 or nickel. In the isolated perfused rat lung, the same two SOC blockers prevented HPV at concentrations that did not alter the pressor response to KCl but did inhibit the pressor responses to angiotensin II that was used to "prime" the lungs prior to HPV.[38] Nifedipine (5 μ*M*) completely reversed HPV in this model. These observations reinforce the view that HPV requires calcium entry through both the SOC and voltage-gated calcium channels.

A recent article provided evidence that may help to link both elements.[41] Using mice with homozygous and heterozygous gene deletion of the ryanodine receptor 1, it was observed that in PASMCs from these mice there was a marked decrease in the ability of hypoxia to cause a rise in cytosolic calcium. Hypoxic vasoconstriction was diminished in the heterozygous mouse PAs (Fig. 1.2). This finding, along with similar data on the effects of homozygous RyR3 gene deletion,[42] confirmed the importance of calcium release from the ryanodine receptor in HPV. Interestingly, the increase in PASMC cytosolic calcium and in PA contraction stimulated by the addition of KCl is also less in the RyR1 heterozygous gene deletion mice.[41] This might point to the effect of calcium entering through the L-type voltage-dependent calcium channels, leading to calcium-induced calcium release (CICR). However, a reduction in KCl-induced calcium in $RyR1^{-/-}$ PASMCs and in contraction in the $RyR1^{+/-}$ PA rings was also reported in the absence of calcium in the perfusing solution. This observation suggests that membrane depolarization can lead to release of calcium from the ryanodine receptor even in the absence of calcium entry. Such a mechanism has been demonstrated.

8 Depolarization and Calcium Release

In addition to CICR, there is another sequence (described in basilar artery SMCs) in which the L-type calcium channels sense membrane depolarization and activate G proteins and the phospholipase C-$InsP_3$ pathway.[43] Subsequently, $InsP_3$-induced release of calcium leads to further release from the ryanodine-sensitive compartment of the SR. This occurs without influx of calcium through the L-type channel, for instance, in the absence of external calcium. However, the calcium release induced by calcium channel activation can be blocked by diltiazem or

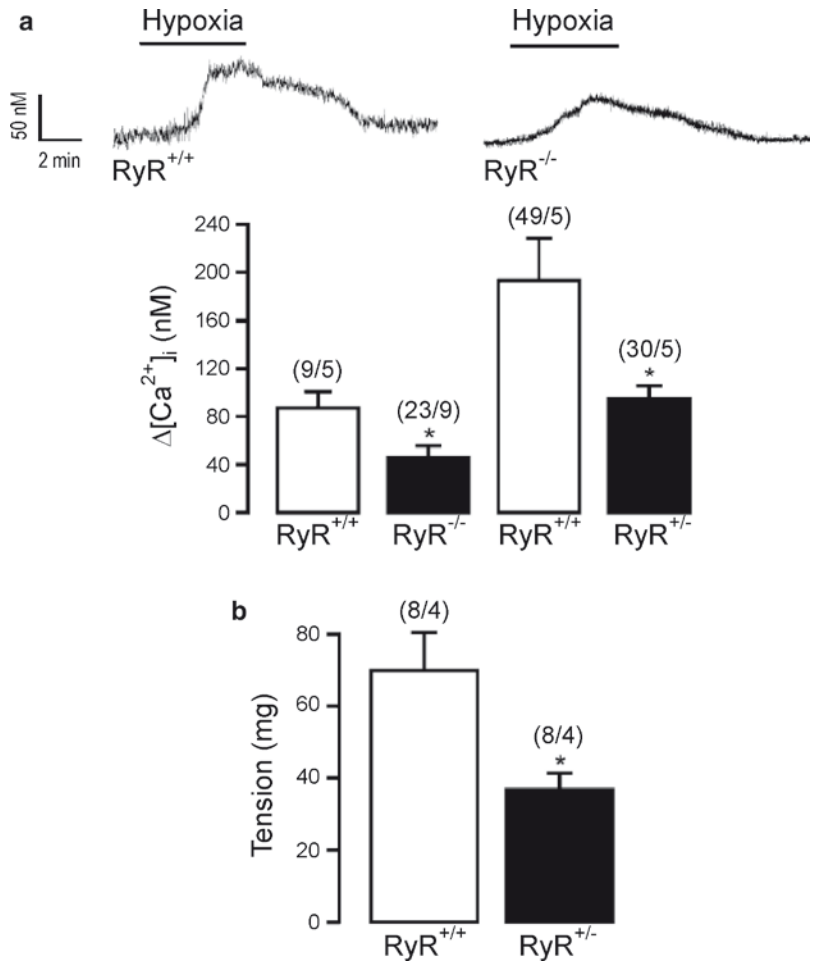

Fig. 1.2 The hypoxic increase in cytosolic calcium in PASMCs (**a**) and contraction in pulmonary artery rings (**b**) is reduced in mice that have homozygous ($RyR1^{-/-}$) or heterozygous ($RyR1^{+/-}$) gene deletion of the ryanodine receptor 1. Reproduced with permission[41]

D600. This phenomenon has been observed in PASMCs[44] and might explain the many references mentioned here that described the efficacy of calcium channel blockers in inhibiting HPV. If the blockers prevent activation of the L-type channels, they may not only inhibit calcium entry through these channels but also reduce the release of calcium from the SR that is initiated by the activation but unrelated to the calcium influx. Interestingly, in coronary artery SMCs, hypoxia inhibits the calcium release initiated by L-type calcium channel activation.[45] This mechanism may help us to understand the interaction of depolarization induced by K^+ channel inhibition and the increase in calcium observed in HPV as well as the action of calcium channel blockers.

9 TRPCs and HPV

How could the signal of calcium depletion in the SR be translated into calcium entry? SOC may be heterotetrameric assemblies of canonical transient receptor potential (TRPC) proteins. Many TRPC isoforms have been identified in the PA smooth muscle. By both mRNA and protein measurements, the expression of TRPC1, TRPC4, and TRPC6 is greater in distal resistance PAs than in more proximal conduit arteries.[46] This corresponds with evidence for greater calcium entry through the SOC, stimulated by hypoxia, in PASMCs from resistance arteries. Stromal interacting molecule 1 (STIM1) plays a role in translating the signal of calcium depletion in the SR so that calcium entry through the SOC is increased. Interestingly, the expression of STIM1 is also greater in the resistance PAs.[46]

Additional evidence for the involvement of TRPC-related channels comes from the study of mice lacking TRPC6. The isolated lungs of wild-type mice had an acute pressor response to hypoxia of 1.2 mmHg. This relatively small degree of HPV was absent in the TRPC6$^{-/-}$ mice, although the vasoconstrictor response to U46619 was the same in both groups.[47] HPV occurring later, after 60 min of continued hypoxia, was also the same in both groups. The increase in calcium stimulated by hypoxia, after "priming" with endothelin 1 or AII, seen in the PASMCs of the wild-type mice, was not present in TRPC6$^{-/-}$ PASMCs. Thus, SOCs/TRPCs are likely to be important in the CCE component of HPV. The authors of this paper also reported that nicardipine almost completely inhibited acute HPV in the isolated lungs and the calcium influx caused by hypoxia in the wild-type PASMCs.[47] They speculated that Na^+ influx through the TRPC6 leads to membrane depolarization and activation of voltage-gated calcium channels.

10 Normoxic Contraction of the Ductus Arteriosus

The mechanisms relating normoxic contraction of the DA to ion channels and calcium flux are a mirror image of those discussed in HPV and may help to provide insight into the signaling of changes in oxygen tension. Instead of acute hypoxia that inhibits K^+ channels in PASMCs, it is the transition from hypoxia to normoxia that inhibits K^+ channels in ductus arteriosus smooth muscle cells (DASMCs).[48] Normoxia causes the same depolarization in DASMCs as the K_v channel blocker 4-aminopyridine (4-AP) (1 m*M*). As 4-AP also causes PA depolarization and contraction, the difference in oxygen sensing must be proximal to the channel protein. Normoxic contraction of the DA can be markedly reduced by nisoldipine (0.5 μ*M*), indicating an important role for the L-type calcium channel.[48] As described in the PA, calcium entry through the L-type channel occurs not only because of membrane depolarization but also because the change in oxygen tension (in the case of DASMCs, the shift to normoxia) increases the influx of calcium at any given membrane potential.[49] This mechanism only appears in DASMCs near to term and thus may prevent premature contraction of the DA.

11 Store-Operated Channels and Normoxic Contraction of the DA

Nifedipine (1 μ*M*) is sufficient to completely inhibit the contraction of a DA ring caused by KCl (80 m*M*). However, approximately half of the normoxic contraction remains after nifedipine addition, suggesting that an alternative mechanism exists.[50] Much of the normoxic contraction can be blocked by the SOC inhibitors 2-APB (2-Aminoethyl Diphenylborinate) (30 μ*M*) or SKF-96365 (10 μ*M*), indicating a role for SOC. Similarly, in the presence of nifedipine, switching from 0 to 2 m*M* calcium in the bath solution causes a much stronger contraction of the DA ring in normoxia than in hypoxia (Fig. 1.3). These observations and the identification of TRPC1 and TRPC4 protein in the DA make it likely that SOCs play a significant part in the normoxic contraction of the DA.

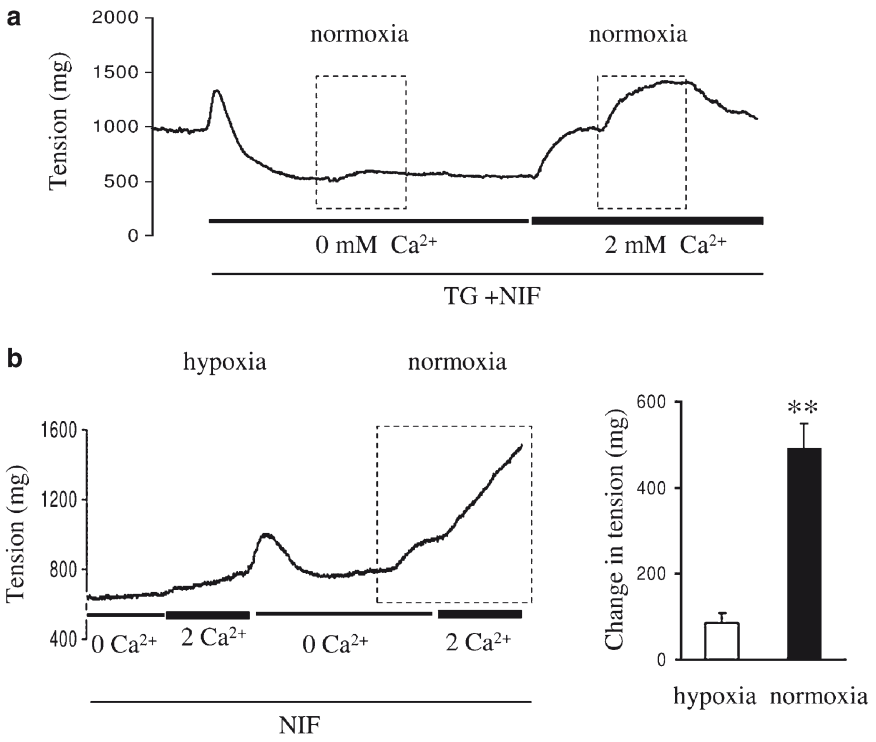

Fig. 1.3 Ductus arteriosus ring tension traces. (**a**) In the presence of thapsigargin (TG) and nifedipine (NIF), normoxia causes only a small contraction with no calcium in the bath solution but significant contraction in the presence of 2 m*M* calcium. (**b**) Switching the bath calcium from 0 to 2 m*M* causes greater contraction under normoxic than under hypoxic conditions. $^{**}p < 0.01$ versus hypoxia ($n = 14$). Reproduced with permission[50]

12 Conclusion

Much of the work reviewed in this chapter illustrates an expanded role for the L-type calcium channel in the response to hypoxia in the PA or to normoxia in the DA. In addition to responding to membrane depolarization by increased calcium influx in the traditional manner, hypoxia in the PA or normoxia in the DA increases calcium influx at any given membrane potential. Depolarization may also stimulate the L-type channel to signal the release of calcium from the SR. Clearly, hypoxia in the PA and normoxia in the DA also increase calcium entry through SOCs and enhance calcium sensitivity. Our knowledge of the role of ion channels in HPV and in normoxic contraction of the ductus is increasing, but we still do not have a good understanding of why these vessels behave in an opposite manner to a decrease in oxygen tension, and this continues to be debated.[51,52]

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