Role of Prostaglandins in Mediating Differences in Human Internal Mammary and Radial Artery Relaxation Elicited by Hypoxia

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Running Title: Low pO₂ dilates human arteries.

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Abstract

The effects of hypoxia-reoxygenation on internal mammary (IMA) and radial (RA) arteries used for coronary artery bypass grafting (CABG) were examined to identify mechanisms regulating contractile function and differences that could contribute to vasospasm. Isolated endothelium-intact IMA and RA rings precontracted with KCl (30 mM) rapidly dilated to hypoxia (95% N₂-5% CO₂), with a greater relaxation in RA than IMA. Inhibitors of cyclooxygenase (10 µM indomethacin) and the TxA₂ receptor (1 µM SQ-29548) potentiated the relaxation to hypoxia in IMA, but not RA, a response associated with increases in TxA₂. Relaxation of IMA and RA to hypoxia appears to involve a calcium-reuptake mechanism inhibited by cyclopiazonic acid (0.2 mM), and it was not attenuated by a blocker of potassium channels (10 mM TEA). The recovery of force generation of IMA, but not RA, upon reoxygenation after 30 min of hypoxia was significantly reduced in the initial phase of reoxygenation by indomethacin and SQ-29548, and by endothelin receptor blocker BQ-123. Thus hypoxia relaxes IMA and RA by a postaglandin-independent mechanism potentially involving enhanced intracellular calcium-reuptake. The prostaglandin-mediated alterations of responses to hypoxia-reoxygenation seen in IMA, but not in RA, may predispose IMA to vasospasm-related complications of CABG.
The internal mammary artery (IMA) has become the conduit of choice in coronary artery bypass grafting (CABG), because of its superior long-term patency rate. Now, radial artery (RA) is increasingly used as the second arterial conduit for myocardial revascularization. A significant problem with the arterial grafts is that they have a small lumen and a greater tendency to go into vasospasm. Vasospasm of IMA following CABG is a potential cause of peri- and post-operative morbidity and mortality (Sarabu et al., 1987; Loop and Thomas, 1993). Potential causes of vasospasm include physical (e.g. mechanical or temperature changes), pharmacological (e.g. neuronal activators or vasoconstrictor substances), or physiological (e.g. changes in PO2) stimuli. Studies have shown that endothelium is more active in IMA as compared to RA, since IMA has been shown to produce more endothelium-derived nitric oxide (NO), hyperpolarizing factor, and prostacyclin (PGI2) (Chardigny et al., 1993; Liu et al., 2000). In addition, although vascular smooth muscle cells of RA have been suggested to show a greater endothelin-1 receptor density, an endothelin-1-PKC signaling cascade appears to be proactive in IMA (Woods et al., 1999). Despite improvements in cardiopulmonary bypass surgery techniques, hypoxemia (indicated by low PaO2-FIO2 ratios) caused by many conditions including hypoventilation, arrhythmia etc., is often seen in patients immediately after CABG surgery (Weiss et al., 2000). Therefore, this study investigated the effects of vasoconstrictor substances and hypoxia-reoxygenation on the vasomotor function of IMA and RA.
Hypoxia elicits a complex set of physiologic responses that regulate vasomotor tone. In vivo studies have shown that hypoxia causes vasodilation of the systemic arteries to increase blood supply to stressed organs to meet the metabolic demand (Blitzer et al., 1996). In contrast, studies on the effect of hypoxia on human arteries have demonstrated that exposure of isolated IMA to hypoxia elicits a small transient relaxation followed by contraction, with the relaxation potentially mediated by increased PGI₂ and the contraction by generation of TxA₂, in addition to the inhibition of basal production of endothelium-derived NO (Pearson et al., 1993). These were shown to be endothelium-dependent responses, which functioned to prevent a vascular smooth muscle (VSM) relaxation to hypoxia. Previous studies have reported that the relaxation of isolated coronary arteries to hypoxia-reoxygenation is prostaglandin and NO dependent (Kalsner, 1977; Jiang and Collins, 1994). In contrast, the primary response of isolated bovine calf and porcine coronary arteries to hypoxia-reoxygenation is independent of prostaglandins, NO, and reactive O₂ species (Close et al., 1994; Mohazzab et al., 1996). Although the mechanisms underlying hypoxic relaxation are poorly understood, studies have indicated that inhibition of L-type Ca²⁺ channels (Franco-Obregon et al., 1995; Herrera and Walker, 1998), opening of ATP-dependent K⁺ channels (Daut et al., 1990; Kalsner, 1995), or accelerated sequestration of Ca²⁺ by sarcoplasmic reticulum (SR) (Close et al., 1994; Shimizu et al., 2000), potentially mediate relaxation of coronary arteries to hypoxia. Hyperpolarization of VSM by activation of K⁺ channels is one of the mechanisms of dilation of RA (Hamilton et
al., 2001). Responses of RA to hypoxia, and IMA and RA to post hypoxic reoxygenation have not been previously studied. Thus, the focus of this investigation was to investigate mechanisms regulating contractile function in responses of RA and IMA to hypoxia-reoxygenation, and to examine if alterations in the influence of pO$_2$-sensitive mechanisms (Gellai et al., 1973); including signaling mechanisms such as protein kinase C (PKC) involved in the generation of force to contractile agents, endothelium-derived mediators, prostaglandins, and endothelin contribute to differences in the responses that are observed.
Materials and Methods:

Drugs:

Indomethacin, phenylephrine, cyclopiazonic acid (CPA), and phorbol 12, 13-dibutyrate (PDBu) were obtained from Sigma Chemical Company (St. Louis, MO). U46619 and SQ-29548 were obtained from Cayman Chemical Company (Ann Arbor, MI). BQ-123 was from Novabiochem, Schwalbach/Ts, Germany. Other salts were reagent grade and obtained from J. T. Baker Chemicals (Phillipsburg, NJ).

Collection of human arteries:

Human IMA and RA were collected based on our institution’s policies for the use of human tissues from patients undergoing elective CABG surgery. IMA and RA was harvested by routine protocols published earlier (Sarabu et al., 1987). Segments from distal sections of IMA and RA surgical discards were obtained immediately after harvesting, but without treating with routinely used pharmacological agents employed to preserve and dilate these conduits prior to grafting to the aorta and coronary artery. After their removal, the IMA and RA segments were immediately placed in ice-cold (4°C) plasmalyte solution and transported to the laboratory for tension studies.

Preparation of IMA and RA for tension studies:

IMA and RA segments (3-4 mm in length) were studied without removal of the endothelium in individually thermostated (37°C) 10-ml baths (Metro Scientific) on wire hooks.
attached to force displacement transducers (model FT-03, Grass) for measurements of changes in isometric force on a polygraph (model 7, Grass), employing adaptations of previously published methods (Mohazzab et al., 1996). After a 2 h incubation at an optimal passive tension in Krebs bicarbonate buffer (pH 7.4) containing (in mM) 118 NaCl, 4.7 KCl, 1.5 CaCl₂, 25 NaHCO₃, 1.1 MgSO₄, 1.2 KH₂PO₄, and 5.6 glucose and gassed with 21% O₂-5% CO₂-74% N₂, the vessels were depolarized with Krebs bicarbonate containing KCl in place of NaCl. The arteries were then re-equilibrated with Krebs solution for 20-30 min before the experiments were conducted.

To study the effects of hypoxia-reoxygenation the arteries were contracted with 30 mM KCl. In a subset of experiments, the arteries were pretreated with drugs (10 µM indomethacin; 1 µM SQ-29548; 300 nM BQ 123) once a steady-state level of force was observed, 15-20 min prior to exposing the arteries to a hypoxia-reoxygenation cycle. This cycle consisted of exposure to 30-min of hypoxia (95% N₂-5% CO₂, pO₂=30-40 mmHg), followed by reoxygenation with 21% O₂-5% CO₂-74% N₂ for 60 min. IMA and RA rings were also contracted by phenylephrine (10 µM) or by PDBu (10 µM), a PKC activator, to examine the role of these systems in the relaxation elicited by hypoxia.

**Estimation of 6-keto PGF₂α and TxB₂ released in the tissue by IMA and RA:**

Stable products of the vasodilator PGI₂, 6-keto PGF₂α, and the vasoconstrictor TxA₂, TxB₂, were measured in samples collected periodically from the tissue baths containing IMA
or RA by an EIA method using kits purchased from Cayman Chemical Company, Ann Arbor, MI. Samples were collected 20 min after addition of 30 mM KCl under normoxic conditions, 10 and 30 min after induction of hypoxia, and 10 min after reoxygenation.

**Detection of COX-1, COX-2, and TxA₂ synthase enzyme localization by immunohistochemistry:**

IMA and RA were frozen in liquid nitrogen and the frozen sections of 6-7 μm thickness were mounted on glass slides, air-dried for 30 min and fixed in acetone for 15 min at room temperature. Each tissue section (up to 5 of each tissue) was washed twice with PBS containing 0.1% Triton-X 100. After washing in PBS pre-incubation was carried out with 20% goat serum in PBS, followed by over night incubation at 4°C with polyclonal antibodies against COX-1, COX-2, and TxA₂ synthase (Purchased from Cayman Chemical Co) diluted in PBS containing 5% BSA and 0.1% Tween-20. After three washing in PBS sections were incubated with secondary goat IgG anti-rabbit conjugated with alkaline phosphatase for 2 hrs. Negative control in which tissue sections were not incubated primary antibodies was performed all the three enzymes examined in this study. Staining was done by a protocol provided by Vector Lab, Burlingame, CA, USA. Counter staining with Hematoxylin solution and mounting in Aquamount completed the procedure.

**Statistical analysis:**

Data are expressed mean±SEM. Relaxation of the arteries to hypoxia is determined as %
changes in force from the pre-contracted steady-state level. The contractile force during reoxygenation is expressed as % changes in force of the steady-state level prior to 30-min hypoxia. Statistical significance between the results was analyzed by employing ANOVA and post-hoc Fisher’s LSD (protected t-test) or Student’s t-test.
Results:

Effects of hypoxia and reoxygenation on force generation in IMA and RA precontracted with KCl (30 mM)

Relaxation to hypoxia was observed in endothelium-intact IMA (n=9) and RA (n=5) precontracted with 30 mM KCl (Fig. 1A); however, the relaxation of RA was greater in magnitude than the IMA. At the end of the 30-min hypoxic period, RA relaxed by 106.3±11.8% and IMA by 69.9±6.9% of the initial steady-state levels of force under 21% oxygen. In addition, 50% relaxation of RA (7.78±0.94 min) occurred significantly faster as compared to IMA (15.01±1.12 min). Reoxygenation caused a rapid increase in force from the low levels seen under hypoxia, and the contraction of IMA was relatively greater in magnitude (p<0.05) than the response seen in RA (Fig. 1B). Right IMA (n=3) also relaxed to 68.1±3.2% to hypoxia and rapidly contracted during re-oxygenation. Similar responses were also seen (Fig. 1C & D) in endothelium-denuded IMA (n=5) and RA (n=4). Although removal of endothelium did not affect hypoxic relaxation responses of IMA and RA, re-oxygenation contraction was significantly suppressed (endothelium-intact: 75±3.4% and endothelium-denuded: 40.9±22.9 % @ 10 minutes and at 60 minutes endothelium-intact was 115±8.5 % and endothelium-denuded was 64.0±19.0 %) in IMA.

Effects of inhibitors of prostaglandin biosynthesis of responses of IMA and RA to hypoxia and reoxygenation
To investigate if prostaglandins mediated the differences in the response of IMA and RA to changes in pO2, we examined the effects of the cyclooxygenase inhibitor, indomethacin and, the TxA2-PGH2 receptor blocker, SQ-29548 on force generation during the hypoxia-reoxygenation cycle. As illustrated in Fig. 2A, indomethacin (10 µM; n=10) and SQ-29548 (1 µM; n=6) increased (p<0.01) the relaxation of IMA to hypoxia. Indomethacin (n=5) did not have any significant effect on the relaxation of RA elicited by hypoxia (Fig. 2B). Moreover, the contraction of IMA elicited by reoxygenation was attenuated (p<0.05) by indomethacin and considerably by SQ-29548 (Fig. 2C). Indomethacin did not affect the contraction of RA (Fig. 2D).

Effects of hypoxia-reoxygenation on the biosynthesis of PGI2 and TxA2 by IMA and RA

The effects of hypoxia-reoxygenation on the synthesis of PGI2 in IMA and RA are shown in Fig. 3A. The levels of PGI2 that accumulated during the 20-min incubation under normoxia appeared to be increased in IMA (n=7), but not in RA, during hypoxia. But, the apparent changes were not statistically significant. Upon reoxygenation PGI2 levels were increased significantly in IMA and RA (n=6). In Fig. 3B, the effects of hypoxia-reoxygenation on TxA2 are reported. The synthesis of TxA2 was elevated in IMA (n=7), but not in the RA (n=6), during hypoxia. To study the source of prostaglandins and TxA2 in normal, hypoxia-reoxygenation untreated IMA and RA, we conducted immunohistochemical studies and the results are illustrated in Fig. 4. Red color staining indicates that COX-1, COX-2, and TxA2 synthase is
localized in the endothelial and smooth muscle cells of IMA and RA (Fig. 4). However, the
color intensity of TxA$_2$ synthase staining is weak in RA and indistinguishable from the
negative control.

**Effect endothelin receptor blocker on the response of IMA and RA to hypoxia and reoxygenation**

To study the effects of endothelin on the hypoxic responses of IMA (n=5) and RA (n=4),
ET-A receptors were blocked with BQ-123 (300 nM). Although BQ-123 did not affect the
hypoxic relaxation (Fig. 5A), it attenuated the force generation during the early phase of
reoxygenation in IMA (Fig. 5C). In the RA, BQ-123 had no effect on the relaxation to hypoxia
(Fig. 5B) or contraction to reoxygenation (Fig. 5D).

**Effects of K$^+$ channel blockade, and inhibition of calcium-reuptake on hypoxia-elicited relaxation in IMA and RA**

To examine, if hyperpolarization of VSM by the opening of K$^+$-channels by hypoxia
could contribute the observed relaxation through the closing of voltage-gated Ca$^{2+}$ channels,
IMA (n=7) and RA (n=5) were exposed to hypoxia in the presence of TEA (10 mM), a
non-specific K$^+$ channel inhibitor. TEA did not significantly prevent relaxation to hypoxia in
IMA and RA (Fig. 6 A & B). In addition, the K$^+$ channel blocker also did not have any effect on
reoxygenation force generation in IMA (Control: 75.25±3.36 % and TEA: 64±12.7 % @ 10
minutes) and RA (Control: 55.7±13.6 % and TEA: 59±12.1 % @ 10 minutes). An inhibitor of
sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase pumps, which mediate calcium-reuptake by the SR, CPA (200 µM) was examined for the role of this system in the relaxation to hypoxia. As shown in Fig. 7, this probe markedly attenuated relaxation to hypoxia, implicating a role for an acceleration of calcium-reuptake in this response.

Effects of hypoxia on force generation in IMA and RA precontracted with PKC activator and \(\alpha\)-adrenergic receptor agonists

IMA and RA precontracted with 10 µM PDBu (n=5), a PKC activator, and \(\alpha\)-adrenergic receptor agonists, 1-10 µM phenylephrine (n=5-7, Fig 6 B) or norepinephrine (n=3-5, data not shown) also relaxed to hypoxic stimulation. Surprisingly, there were no significant differences in relaxation to hypoxia between IMA and RA precontracted with PDBu (Fig. 8 A & B) and \(\alpha\)-adrenergic receptor agonists (Fig. 6B), presumably because interestingly activation of PKC by PDBu significantly inhibited relaxation of RA-induced by hypoxia. Furthermore, PDBu treated IMA (Control: 75.2±3.36 % and PDBu: 53.5±4.5 %) and RA (Control: 55.7±13.6 % and PDBu: 12.1±9.7 %) showed a significantly slower recovery of the contractile force in the early phase (at 10 minutes) of re-oxygenation. However, at the end of re-oxygenation period (@ 60 minutes) contractile force developed in IMA contracted with PDBu was 30-40 % more than that developed in 30 mM KCl treated IMA.
Discussion:

The properties of hypoxia-elicited relaxation of IMA observed in the present study are consistent with this response being mediated by a prostaglandin-independent mechanism, which partly involves an acceleration of calcium-reuptake by the SR. The relaxation to hypoxia was impaired in IMA precontracted with 30 mM KCl (see Fig. 9) through a mechanism involving the production of increased vasoconstrictor prostaglandins, which cause contraction through TxA2 receptors. Interestingly hypoxic relaxation in these conduit grafts was not different when pre-contracted by $\alpha$-adrenergic agonists and the PKC activator PDBu. Reoxygenation causes a rapid contraction, with IMA showing a greater level of force development than RA, with force slowly returning to pre-hypoxic steady-state levels by 60-min of reoxygenation.

Observations in this study indicate that the synthesis of PGI2 and TxA2 are increased by hypoxia-reoxygenation. While it has been reported that IMA show an endothelium-dependent transient relaxation followed by a contraction on exposure to hypoxia associated with increases in these cyclooxygenase-derived metabolites (Pearson et al., 1993), hypoxia-mediated relaxation of IMA or RA was the primary response observed in our study. This is probably because of differences in the experimental design, such as stimulation of the endothelium with calcium was avoided through using a lower, more physiological, level of this ion in our buffer. Increased buffer calcium levels have previously been shown to markedly increase the influence
of endothelial mediators in vascular responses to hypoxia (Mathew et al., 1991). The synthesis of dilator prostaglandins is known to be elevated by hypoxia in isolated coronary arteries (Kalsner, 1977). Paradoxically, the relaxation of IMA, but not of RA, elicited by hypoxia was attenuated by prostaglandins. The relaxation to hypoxia is impaired and contraction during reoxygenation is potentiated through a mechanism that appears to involve stimulation of TxA2-PGH2 receptors by a cyclooxygenase-derived product. This interpretation is based on the alterations of responses in IMA caused by inhibition of prostaglandin synthesis and blocking TxA2-PGH2 receptors. The apparent increased generation of PGI2 during hypoxia in IMA and reoxygenation in RA and IMA were presumably insufficient to modulate pO2-elicited responses under the conditions examined. Although it is thought that the source of prostaglandins, endothelin, NO, and hyperpolarizing factor is endothelium; the response of IMA and RA to hypoxia was not altered by removal of the endothelium. Nevertheless, re-oxygenation contraction was significantly suppressed in endothelium-denuded IMA thereby indicating that endothelin-1 maybe derived from endothelium during re-oxygenation. However, platelets are not a primary source of TxA2, since TxA2 synthase was observed to be present in endothelium and VSM of IMA, suggesting that these cell types appear to be the primary source of TxA2 generation. Thus, IMA, but not RA, produce vasoconstrictor levels of TxA2 under hypoxia.

Studies have suggested that prostaglandins mediate hypoxic relaxation of coronary artery
in various animal species (Kalsner, 1977; Jiang and Collins, 1994). In the present study, prostaglandins did not mediate relaxation of IMA and RA exposed to hypoxia. Instead, the elevated prostaglandins contributed to impairing relaxation to hypoxia and accentuating contraction initiated by reoxygenation of IMA. Additionally, ET-1 synthesized during reoxygenation appeared to have a role in accelerating the post-hypoxic contraction of IMA. It is possible that an interaction between activation of the TxA2-PGH2 receptors and the ET-1 system could be a contributing factor in the response to re-oxygenation, since TxA2 levels were not significantly increased during this period. Thus, the release of these vasoconstrictive factors by CABG may contribute to triggering contractions and spasms of arterial grafts leading to hypo-perfusion of myocardium.

Signaling pathways in mediating hypoxic relaxation of systemic arteries remain elusive. Some reports have proposed that opening of K⁺ channels, inhibition L-type Ca²⁺ currents, and acceleration of Ca²⁺ uptake by SR may be involved in modulating VSMC responses to changes in oxygen tension (Close et al., 1994; Franco-Obregon et al., 1995; Herrera and Walker, 1998; Shimizu et al., 2000). Opening of K⁺ channels did not mediate hypoxia-elicited relaxation of IMA and RA, because inhibiting K⁺ channels with TEA did not attenuate relaxation. Since hypoxia relaxed IMA and RA contracted with the PKC activator PDBu, this response does not appear to be mediated through changes in the stimulation of PKC. The additional studies on the mechanism of relaxation to hypoxia also generated data indicating that differences in
relaxation of IMA and RA were not observed in arteries contracted with stimuli which activate PKC (PDBu and α-adrenergic receptor agonists) or with KCl contractions in the presence of TEA. While the origins of these observations were not investigated, they could originate from a diminished effect of a further activation of PKC by stimulation of TxA$_2$-PGH$_2$ receptor, or an effect of K$^+$ channels on the generation or action of TxA$_2$. Furthermore, the data in figure 8B suggest that RA relaxation to hypoxia was significantly suppressed by the activation of PKC by PDBu. The attenuation of relaxation to hypoxia by inhibition of calcium-reuptake by the SR Ca$^{2+}$-ATPase pump, suggests that this system is stimulated by hypoxia. While prostaglandins do not appear to mediate the IMA and RA response to hypoxia, additional studies are needed to examine the role of prostaglandins in reducing sequestration of Ca$^{2+}$ by activated SR Ca$^{2+}$-ATPase.

A role of hypoxia-reoxygenation in inducing the release of spasmogens is not well studied. The handling of the arteries used in this study more closely simulates the handling of the IMA and RA as a free graft during surgery, than the pedicle graft. However, differences such as the observed increased expression of enzymes that generate constrictor prostaglandins in the IMA should be independent of the handling of the arteries studied, because they are immediately immersed in ice-cold saline after removal from the pedicle or free graft. IMA, a pedicle grafts, receives blood flow from the sub-clavian artery and does not become hypoxic during CABG surgery, whereas, the RA is routinely used as a free graft and pO$_2$ in this tissue is
not controlled during the CABG procedure. Grafts frequently become hypoxic post-operatively, because hypoxemia commonly occurs in the patients after a CABG surgery due to hypoventilation, atrial or ventricular arrhythmia, respiratory syndromes etc (Christenson et al., 1996; Willems et al., 1997; Tamis-Holland et al., 2000; Weiss et al., 2000). We have demonstrated that a mechanism independent of prostaglandins mediates relaxation of human IMA and RA to hypoxia, whereas vasoconstrictor prostaglandins impaired the relaxation and potentiated the contraction of IMA to hypoxia-reoxygenation. Therefore, hypoxia-reoxygenation maybe a greater stimulus of vasospasm in IMA than RA, through the TxA2-mediated and ET-1 receptor-dependent mechanisms reported in this study. The increased generation of TxA2 by hypoxia-reoxygenation could be a contributing factor to spasm observed peri- and post-operatively which are a leading cause of IMA graft occlusion in the early post-operative stage (Sarabu et al., 1987; Loop and Thomas, 1993). Cyclooxygenase inhibitors, TxA2 receptor blockers, and ET-1 receptor blockers could potentially be beneficial in post operative therapy for preventing the genesis of vasospasm and thrombosis, which promote ischemic events particularly in hypoxic patients as well as all patients undergoing elective CABG operations.

In summary, we have demonstrated that human systemic arteries relax in response to decrease in oxygen tension through mechanisms that involve sarcoplasmic Ca2+ reuptake and do not appear to involve endothelium-derived mediators or the opening of membrane K+
channels. Although the mechanisms involved in increasing uptake of Ca$^{2+}$ by SR by hypoxia in human arteries remains to be identified, our data support the notion that hypoxia elicits relaxation of IMA and RA conduits by accentuating sequestration of intracellular Ca$^{2+}$. IMA conduit are thought to produce more endothelial factors than RA, this study provides evidence that activation of TxA$_2$-PGH$_2$ receptors and elevation of ET-1 maybe contributing factors to impairing relaxation to hypoxia and enhancing contraction to reoxygenation through the generation of TxA$_2$ and endothelin, which could be factors triggering vasospasm, thrombosis, and graft failure early in the post-operative phase.
References:


Figure Legends:

**Figure 1** (A) Relaxation of IMA (n=9) and RA (n=5) on exposure to hypoxia. (B) Contraction of IMA (n=9) and RA (n=5) upon reoxygenation after exposure to hypoxia. (C) Relaxation of endothelium-denuded IMA (n=5) and RA (n=4) on exposure to hypoxia. (D) Contraction of endothelium-denuded IMA and RA upon reoxygenation after exposure hypoxia. Data are reported as the % relaxation of the pre-hypoxic force generated by 30 mM KCl in Panel A & C and as the % of this initial force in Panel B & D; and statistical significances were determined by ANOVA analysis.

**Figure 2** Effects of pretreatment with indomethacin or SQ-29548 on the relaxation of IMA (Panel A, n=7-10) and RA (Panel B, n=5) to hypoxia, and the recovery of this response in IMA (Panel C) and RA (Panel D) upon reoxygenation. The initial level of force generation to KCl (30 mM) was not significantly altered by these probes.

**Figure 3** Effects of hypoxia and reoxygenation on the generation of PGI$_2$ and TxA$_2$ by IMA and RA. The levels of a stable metabolite of PGI$_2$ (6-keto PGF$_{1α}$; Panel A) and a stable metabolite of TxA$_2$ (TxB$_2$; Panel B) produced during normoxia, hypoxia, and reoxygenation of IMA (open bars; n=7) and RA (hatched bars; n=6) are shown. The statistical significances within and between groups was determined by paired Student’s *t*-test.

**Figure 4** Immunohistochemical studies of normal left internal mammary artery (LIMA) and radial artery (RA) showing localization of COX-1, COX-2, and TxA$_2$ synthase in the
endothelial cells (ENDO) and vascular smooth muscle (VSM) as red color staining. Note the
TxA₂ synthase staining in RA is weak and indistinguishable from negative control (NEG CON).
Similar negative controls were seen with the other antibodies (not shown). Magnification is
10x.

**Figure 5** Effects of pretreatment with BQ-123 on the relaxation of IMA (Panel A, n=5) and RA
(Panel B, n=5) to hypoxia, and the recovery of this response in IMA (Panel C) and RA (Panel
D) upon reoxygenation. The initial level of force generation to KCl (30 mM) was not
significantly altered by BQ-123.

**Figure 6** Influence of the K⁺ channel inhibitor 10 mM TEA on relaxation to hypoxia in IMA
(n=5) and RA (n=5) contracted with 30 mM KCl (Panel A), and IMA (n=4) and RA (n=4)
pre-contracted with phenylephrine (10 µM; Panel B).

**Figure 7** Pretreatment with 200 µmol/L cyclopiazonic acid significantly (p<0.05) attenuated
the relaxation of IMA (Panel A; n=5) and RA (Panel B; n=5) to hypoxia. The initial level of
force generation to KCl (30 mM) was not significantly altered by cyclopiazonic acid.

**Figure 8** Comparison of the relaxation to hypoxia of IMA (Panel A) and RA (Panel B)
contracted with PDBu (10 µM; n=5-7). Control is the relaxation of arteries pre-contracted with
30 mM KCl to hypoxia from figure 1.

**Figure 9** Model shows mechanisms which influence the response of IMA and RA to hypoxia.
A: **Hypoxic Relaxation (%)**

- Control (IMA)
- Indo (10 µM)
- SQ-29548 (1 µM)

* * p < 0.05 vs. control

B: **Hypoxic Relaxation (%)**

- Control (RA)
- Indo (10 µM)
- SQ-29548 (1 µM)

C: **Reoxygenation Contraction (%)**

- Control (IMA)
- Indo (10 µM)
- SQ-29548 (1 µM)

* * p < 0.05 vs. Control

D: **Reoxygenation Contraction (%)**

- Control (RA)
- Indo (10 µM)
- SQ-29548 (1 µM)
**A**

6-Keto PGF$_{1\alpha}$ (pg/g. wet wt.)

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**B**

TxB$_2$ (pg/g. wet wt.)

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A

![Graph showing hypoxic relaxation over time for IMA-Control and IMA-PDBu](image)

B

![Graph showing hypoxic relaxation over time for RA-Control and RA-PDBu](image)

* *p<0.05 vs Control

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Hypoxia

Internal Mammary Artery

Indo

↑ PGH₂/TxA₂

SQ-29548

Radial Artery

SERCA

+ CPA

- [Ca²⁺]ᵢ

Relaxation