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TOPICAL REVIEW

Arteriolar oxygen reactivity: where is the sensor and what is the mechanism of action?

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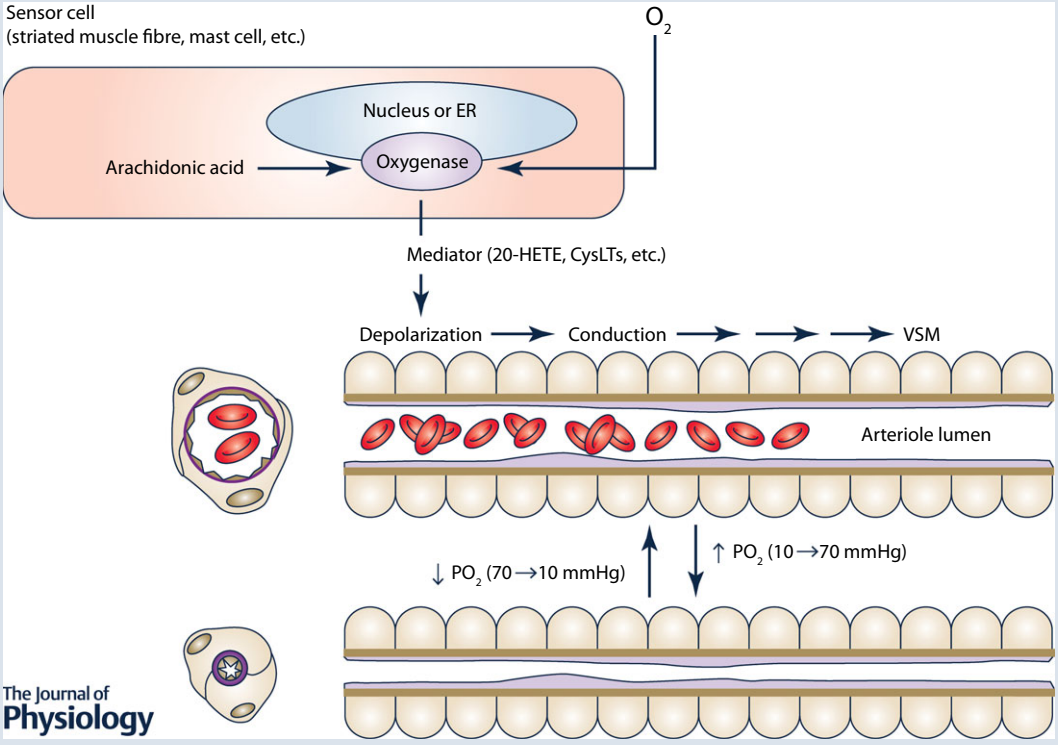

Abstract Arterioles in the peripheral microcirculation are exquisitely sensitive to changes in P_{O_2} in their environment: increases in P_{O_2} cause vasoconstriction while decreases in P_{O_2} result in vasodilatation. However, the cell type that senses O_2 (the O_2 sensor) and the signalling pathway that couples changes in P_{O_2} to changes in arteriolar tone (the mechanism of action) remain unclear. Many (but not all) *ex vivo* studies of isolated cannulated resistance arteries and large, first-order arterioles support the hypothesis that these vessels are intrinsically sensitive to P_{O_2} with the smooth muscle, endothelial cells, or red blood cells serving as the O_2 sensor. However,

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in situ studies testing these hypotheses in downstream arterioles have failed to find evidence of intrinsic O_2 sensitivity, and instead have supported the idea that extravascular cells sense O_2. Similarly, *ex vivo* studies of isolated, cannulated resistance arteries and large first-order arterioles support the hypotheses that O_2-dependent inhibition of production of vasodilator cyclooxygenase products or O_2-dependent destruction of nitric oxide mediates O_2 reactivity of these upstream vessels. In contrast, most *in vivo* studies of downstream arterioles have disproved these hypotheses and instead have provided evidence supporting the idea that O_2-dependent production of vasoconstrictors mediates arteriolar O_2 reactivity, with significant regional heterogeneity in the specific vasoconstrictor involved. Oxygen-induced vasoconstriction may serve as a protective mechanism to reduce the oxidative burden to which a tissue is exposed, a process that is superimposed on top of the local mechanisms which regulate tissue blood flow to meet a tissue's metabolic demand.

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Abstract figure legend The site and mechanism of action of O_2 in the peripheral microcirculation. Schematic diagram showing an extravascular sensor cell and a longitudinal section through an arteriole under low and high P_{O_2} conditions as indicated. Oxygen-sensitive enzymes in the sensor cells (generically labelled as Oxygenase), respond to elevation in the P_{O_2} in their environment by increasing the production of a vasoconstrictor (labelled Mediator) that acts on vascular smooth muscle cells (VSM) to produce membrane depolarization, opening of L-type Ca^{2+} channels and vasoconstriction. The depolarization will be conducted electrotonically along the wall of the arteriole via gap junctions to produce conducted vasoconstriction to sites distant from the initial action of the mediator. The opposite occurs when the P_{O_2} falls from a high to a lower level. ER, endoplasmic reticulum; CysLTs, cysteinyl leukotrienes. See text for more information.

Abbreviations BK_{Ca} channels, large-conductance, Ca^{2+}-activated K^+ channels; Ca_L channels, L-type Ca^{2+} channels; Cl_{Ca} channels, Ca^{2+}-activated Cl^- channels; CYP450, cytochrome P450; CysLTs, cysteinyl leukotrienes; CysLTRs, CysLT receptors; DDMS, *N*-methylsulfonyl-12,12-dibromododec-11-enamide; DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; ETYA, eicosatetraynoic acid; FLAP, 5-lipoxygenase-activating protein; 20-HETE, 20-hydroxyeicosatetraenoic acid; 5-LO, 5-lipoxygenase; LTA_4, leukotriene A_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; 17-ODYA, 17-octadecynoic acid; P_{O_2}, partial pressure of oxygen; PSS, physiological salt solution.

Introduction

Oxygen has been implicated in the local control of blood flow for more than a century (Sparks, 1980; Renkin, 1984; Kontos & Wei, 1985; Golub & Pittman, 2013). In tissues such as skeletal muscle, heart, brain, kidneys and the gut, blood flow (O_2 supply) is directly proportional to tissue oxygen consumption (O_2 demand) (Sparks, 1980; Renkin, 1984; Kontos & Wei, 1985; Golub & Pittman, 2013). Oxygen as one link between O_2 demand and O_2 supply provides a compact control system that would help ensure that O_2 supply meets the tissues' demand for O_2. For O_2 to participate in the local regulation of blood flow, arterioles in the microcirculation must be able to sense changes in the P_{O_2} of the surrounding tissue, and then respond appropriately to either constrict, if O_2 supply exceeds O_2 demand and tissue P_{O_2} increases, or dilate, if O_2 supply is less than O_2 demand and tissue P_{O_2} decreases.

Consistent with a possible role for O_2 in the local regulation of blood flow, there is consensus that O_2 is vasoactive. Arterioles in the peripheral microcirculation constrict when exposed to increases in P_{O_2} produced either by increases in inspired P_{O_2} (Hutchins *et al.* 1974; Zhu *et al.* 1998; Demchenko *et al.* 2000; Vucetic *et al.* 2004; Sakai *et al.* 2007; Kisilevsky *et al.* 2008; Justesen *et al.* 2010) or increases in the P_{O_2} of solutions flowing over microvascular beds (Duling, 1972; Prewitt & Johnson, 1976; Lombard & Duling, 1977, 1981; Tuma *et al.* 1977; Kontos *et al.* 1978; Lindbom *et al.* 1980; Proctor *et al.* 1981; Sullivan & Johnson, 1981*a*,*b*; Proctor & Duling, 1982*a*,*b*; Jackson & Duling, 1983; Jackson, 1986, 1987, 1988, 1989, 1993; Lombard *et al.* 1986, 1999, 2004; Kaul *et al.* 1995; Welsh *et al.* 1998; Hungerford *et al.* 2000; Kunert *et al.* 2001*a*,*b*, 2009; Frisbee & Lombard, 2002; Drenjancevic-Peric *et al.* 2003, 2004; Wang *et al.* 2009; Ngo *et al.* 2010, 2013). Conversely, decreases in P_{O_2}, produced by lowering inspired P_{O_2}, cause vasodilatation (see Marshall, 2000 for references). What remains unclear are the cellular location of the process that detects changes in P_{O_2} (the O_2 sensor) and the mechanism that

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transduces the changes in P_{O_2} to changes in arteriolar tone (the mechanism of O_2 action). This review will focus on the site and mechanism of action of O_2 on arterioles in peripheral tissues at rest. Changes in arteriolar tone induced by systemic hypoxia or hyperoxia produced by changes in inspired P_{O_2} will not be considered because the mechanisms involved may be different. Systemic hypoxia and hyperoxia are complicated by changes in neural outflow to arterioles (Marshall, 1994; Guyenet, 2000), changes in circulating hormones and vasoactive substances (Mazzeo *et al.* 1991), and changes in circulating cytokines released from macrophages in the lungs (Shah *et al.* 2003). Oxygen reactivity of microvessels in active tissue (contracting striated muscle, the heart, etc.) will also not be considered as there are probably interactions among mechanisms that have not been fully explored, and to maintain the focus on arteriolar O_2 reactivity *per se*. Evidence will be presented that: (1) O_2 acts as a vasoconstrictor on arterioles in the peripheral microcirculation, (2) there is regional heterogeneity in the specific vasoconstrictor pathway that mediates arteriolar O_2 reactivity, and (3) arterioles may be tuned to detect changes in tissue P_{O_2}, whereas upstream feed arteries may be tuned to respond to changes in blood P_{O_2}.

Setting the stage

Microvascular architecture and the study of arteriolar O_2 reactivity. The microcirculation is the business end of the cardiovascular system. It is here that exchange of O_2, CO_2, substrates, hormones, waste products, etc. occurs. This exchange function depends on a continuous, regulated flow of blood through the microvasculature. The microcirculation originates when a feed artery (100–200 μm internal diameter) that is external to the tissue branches into a first-order arteriole (50–100 μm). Feed arteries provide up to half of the precapillary vascular resistance to blood flow in tissues such as skeletal muscle and importantly contribute to the regulation of total blood flow to the tissue (Segal & Duling, 1986). Arterioles are embedded in the parenchyma that they perfuse and have 1–2 layers of smooth muscle in the media of their walls. Arterioles contribute the remaining 50% of the precapillary vascular resistance to blood flow and determine the distribution of blood flow within the tissue. However, the primary effectors of local blood flow control to and within a tissue are the arterioles. Upstream feed arteries are external to the tissue, and are recruited to the process of blood flow regulation by indirect mechanisms such as conducted vasomotion (Segal & Duling, 1986) or flow-dependent responses (Davis *et al.* 2011) that are initiated by changes in tone of the downstream arterioles. First-order arterioles usually have ~two layers of smooth muscle, while smaller arterioles are invested with only a single layer of smooth muscle. In striated muscle, for example, second- to fifth-order arterioles divergently branch from the first-order arterioles, ending in terminal arterioles that supply 10–20 capillaries each. Post-capillary venules collect blood flow from two or more capillaries. These then drain into higher order venules, which converge on veins draining a tissue or organ. While capillaries are a major site of exchange of materials and heat between tissues and blood because of their large surface area, O_2 and CO_2 also readily diffuse across the wall of arterioles and venules contributing to the exchange of these respiratory gases in the microcirculation (Renkin, 1984).

Methods and approaches used to study arteriolar O_2 reactivity. Two general approaches have been used to explore microvascular O_2 reactivity: (1) pressure myography (Duling *et al.* 1981; Schjorring *et al.* 2015) and other techniques for the *ex vivo* study of isolated vessels, and (2) intravital microscopy of arterioles, *in situ*, in exteriorized, autoperfused tissues (Baez, 1973; Duling, 1973). Pressure myography involves the surgical removal of a vessel from a tissue. The vessels are then cannulated onto micropipettes and pressurized to a physiological pressure. The vessels are superfused with a physiological salt solution (PSS; to allow treatment with drugs, altering the P_{O_2}, and temperature control), and imaged with a microscope to measure internal diameter. Intravital microscopy usually involves the exteriorization of a tissue from an anaesthetized animal retaining the tissue's blood and nerve supply. The tissue is superfused with PSS (to control the P_{O_2} and temperature), and the vessels imaged using a microscope. Both approaches allow the P_{O_2} in the environment around vessels to be measured, controlled and changed while the diameters of the vessels are measured, the main requirements to study vascular O_2 reactivity.

The study of isolated vessels, *ex vivo*, offers the advantage of precise control of experimental conditions: the composition of the bathing solutions, perfusion solutions, pressure, etc. can be precisely controlled. In addition, the cell type responsible for O_2-mediated responses can be identified (Busse *et al.* 1983; Fredricks *et al.* 1994*a*). However, this approach is suitable mainly for the study of feed arteries and first-order arterioles due to the technical difficulties associated with the dissection and cannulation of vessels smaller than 50 μm. A major assumption of the *ex vivo* study of feed arteries is that the reactivity of these larger upstream vessels accurately models the reactivity of the smaller downstream arterioles, an assumption that has not been adequately tested. In addition, loss of input from hormones, extravascular cells and other vessels in the microcirculation may alter the function of isolated vessels. Trauma due to dissection of the vessels can also be problematic.

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The primary advantage of the intravital microscopy approach is the ability to study arterioles of any size in their native environment. However, most studies have focused on smaller arterioles (third- to fifth-order arterioles $< 40\ \mu$m). As will be pointed out repeatedly in sections below, this difference between *ex vivo* and *in situ* studies (the study of arteries *vs.* arterioles) may contribute to the lack of consensus on the site and mechanism of action of O_2 in the microcirculation. The main disadvantage of the *in situ* approach is the lack of control of the environment. The arterioles are embedded in a connected microvascular network that allows conduction of signals along the vessel wall. They are perfused with systemic blood and surrounded by parenchymal cells, mast cells, nerves, etc. Therefore, the signals to which a given vessel is exposed are not always apparent. As will be outlined below, there are strategies to circumvent some of these issues, but these approaches are technically challenging. Because of the requirement to control tissue P_{O_2} and to observe vessel diameter, intravital microscopy is limited to the study of thin tissues such as the hamster cheek pouch or hamster, mouse, or rat cremaster muscles, although surface vessels can be studied in thicker tissues. The control of P_{O_2} in superfused exteriorized tissues can also be problematic. Care must be taken to limit the depth of the superfusion solution to 0.5 mm or less, and to use relatively high superfusate flow rates (5–10 ml min^{-1} for cheek pouches or cremaster muscles) to obviate diffusional boundary layers and lack of the ability to accurately control P_{O_2} at the surface of the preparations. As with the *ex vivo* study of isolated vessels, surgical trauma and the resultant inflammation can also alter microvascular reactivity in intravital preparations.

What is the relevant P_{O_2} range over which arterioles are O_2 sensitive?. Intravital microscopy studies have defined the P_{O_2} range over which arterioles display O_2 reactivity as illustrated in Fig. 1*A*. Arterioles respond to changes in P_{O_2} over a range from ∼10 to >70 mmHg in the tissue (Klitzman *et al.* 1982) and ∼30 to 150 mmHg at the surface of arterioles (Gorczynski & Duling, 1978; Jackson & Duling, 1983; Jackson, 1987) (Fig. 1*A*). In Fig. 1*A*, several P_{O_2} scales are provided, because the cell type in which changes in P_{O_2} are sensed, that will be termed the sensor, has yet to be firmly established. Thus, it is not clear what P_{O_2} (tissue P_{O_2} or arteriolar P_{O_2}) is particularly relevant to the vasomotor effect of O_2 on the arterioles. However, these values (tissue P_{O_2} and arteriolar P_{O_2}) should provide the lower and upper bounds, respectively, for defining the O_2 sensitivity of the system.

Several points should be made regarding the relationship between O_2 and arteriolar diameter (tone) in superfused microvascular preparations as depicted in Fig. 1. First, arterioles in intravital preparations display substantial myogenic tone (diameter at rest = 50–80% of the maximum diameter obtained with a vasodilator) with low P_{O_2} in the superfusion solution (the solutions shown with $P_{O_2} = 12$ mmHg were equilibrated with gases containing 0% O_2 and 5% CO_2). Arterioles in microvascular preparations such as hamster (Jackson, 1986), mouse (Hungerford *et al.* 2000; Figueroa *et al.* 2003) or rat cremaster muscle (Jackson, 1986), hamster cheek pouch (Jackson & Duling, 1983; Jackson, 1987), hamster retractor muscle (Lombard *et al.* 1999) rat spinotrapezius muscle (Marvar *et al.* 2007) or mouse gluteus maximus muscle (Sinkler & Segal, 2014) all retain substantial tone; they are not maximally dilated at the low P_{O_2} values experienced by the preparations (note the right *y*-axis scale in Fig. 1*A*). Second, at rest, arterioles and the tissue surrounding them experience relatively 'low' P_{O_2} values due to diffusional loss of O_2 from precapillary vessels and consumption of O_2 by the tissue as well as diffusion of O_2 from the arterioles into the superfusion solution (for low P_{O_2} equilibrated solutions) (Duling & Berne, 1970). Early studies using recessed-tip O_2 microelectrodes (Whalen *et al.* 1974) or multi-point surface O_2 electrodes (Lund *et al.* 1980*a*,*b*) indicated that there was a broad range of P_{O_2} values measured in the microcirculation of skeletal muscle, from < 1 to 100 mmHg. The P_{O_2} distributions recorded by both methods were dominated by relatively low P_{O_2} values with the means of the P_{O_2} distributions being in the order of 17 mmHg in the anaesthetized cat (Whalen *et al.* 1974), 33 mmHg in the anaesthetized rat (Lund *et al.* 1980*b*) and 15 mmHg in the conscious human (Lund *et al.* 1980*a*). More recent measurements support the findings of these early studies (Smith *et al.* 2002, 2004; Johnson *et al.* 2005). The average minimum resting tissue P_{O_2} (defined as the P_{O_2} midway between the venous end of two capillaries away from arterioles or venules) in a relatively undisturbed striated muscle microvascular bed, *in vivo*, is ∼25 mmHg as measured with phosphorescence probes (Smith *et al.* 2002, 2004; Johnson *et al.* 2005). Thus, a superfused microvascular preparation allows the study of both the response to mild decreases in tissue P_{O_2} (from 25 down to ∼10 mmHg, with the superfusate serving as a sink for O_2) and increases in P_{O_2} (>25 mmHg, with the superfusate serving as a source for O_2). An additional weakness of the superfused intravital microvascular preparations is the lack of ability to study more severe hypoxia (below 10 mmHg). Tissue P_{O_2} values below 10 mmHg can be attained during increases in metabolic activity (e.g. striated muscle contraction) (Lash & Bohlen, 1987; Boegehold & Bohlen, 1988; Smith *et al.* 2002), occlusion of blood flow (Lombard & Duling, 1977) or breathing gases with reduced O_2 content (Shah *et al.* 2003). However, each of these approaches adds additional complexity beyond simple effects of O_2 on the arterioles, and thus, will not be considered in this review.

Finally, it is important to recognize that elevated P_{O_2} causes profound, maintained vasoconstriction of

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arterioles in well-prepared microvascular beds (preparations with substantial resting arteriolar tone at physiological P_{O_2} values as presented above and without leukocyte adherence to arteriolar endothelium or other signs of inflammation) (see Fig. 1*B* in this review, Fig. 2 in Ngo *et al.* 2010 and Fig. 1 in Ngo *et al.* 2013, for examples). This point is particularly important when considering the data obtained from the *ex vivo* study of isolated arteries and arterioles.

Location of the sensor: what cell type senses changes in P_{O_2} relevant to arteriolar O_2 reactivity?. At least four cellular sites have been postulated to sense changes in P_{O_2} and signal vascular smooth muscle cells to contract (elevated P_{O_2}) or relax (reduced P_{O_2}) in response to this change. These include the arteriolar wall (vascular smooth muscle or endothelial cells), red blood cells in the lumen of the vessels, and extravascular cells (parenchymal cells, nerves, mast cells, etc., see Fig. 2).

Arteriolar smooth muscle cells as an O_2 sensor. Studies testing the hypothesis that arteriolar smooth muscle cells are intrinsically sensitive to changes in P_{O_2} within the range of 10–150 mmHg have lead to equivocal results. Early organ bath studies of preparations from conduit arteries (as model systems), *ex vivo*, indicated that smooth muscle cells are intrinsically sensitive to changes in bath P_{O_2} (Carrier *et al.* 1964; Detar & Bohr, 1968; Coburn *et al.* 1979; Chang & Detar, 1980). However, this intrinsic O_2 sensitivity appears to result from the formation of an anoxic core in the tissue due to O_2 consumption by the vascular smooth muscle cells and the multicellular thickness of these conduit artery preparations (Pittman & Duling, 1973). Thus, these studies do not appear to be

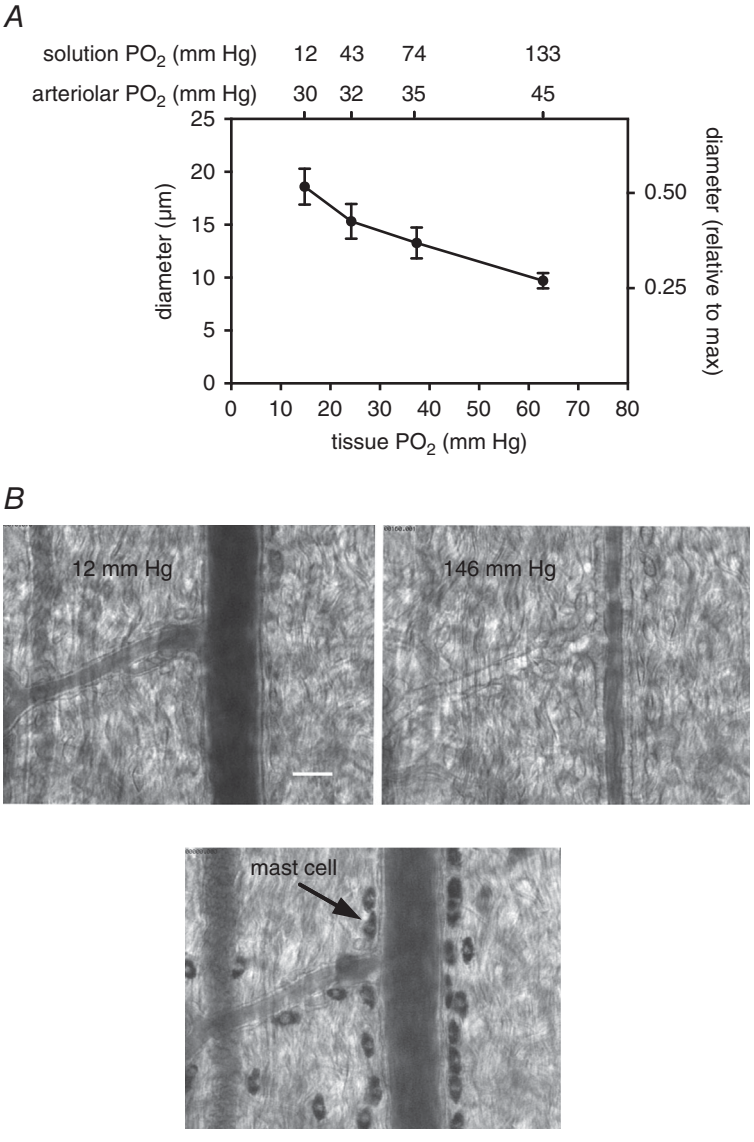

Figure 1. Arteriolar O_2 reactivity in superfused microvascular preparations
A, data from Klitzman *et al.* (1982). Data are mean ± SEM diameters of arterioles in hamster cremaster muscles when exposed to solutions equilibrated with gases containing different P_{O_2} values and 5% CO_2 (solution P_{O_2} values, upper *x*-axis labels). The P_{O_2} values shown were measured with O_2 microelectrodes in the free solution flowing over the preparations. The tissue P_{O_2} values shown on the lower *x*-axis were measured at the midpoint between the venous ends of two capillaries. The arteriolar P_{O_2} values (top *x*-axis labels) were estimated from haemoglobin oxygen saturation measurements in hamster cheek pouch (Jackson & Duling, 1983). *B*, typical O_2-induced vasoconstriction in a hamster cheek pouch preparation exposed to superfusion solutions with approximate P_{O_2} values as indicated using methods as described (Jackson, 1987). *C*, same vessel as in *B* after exposure to ruthenium red (0.001%) to label mast cells (Shepherd & Duling, 1995). Scale bar in *B*, 25 μm.

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relevant to arteriolar O_2 reactivity except, perhaps, under conditions when the P_{O_2} in the microcirculation falls to very low levels ($P_{O_2} \ll 10$ mmHg).

Studies of the O_2 reactivity of smooth muscle cells of resistance arteries and arterioles have lead to conflicting results. Endothelium-denuded rat gracilis muscle feed arteries studied *ex vivo* by pressure myography display O_2 reactivity in the appropriate P_{O_2} range (10–150 mmHg), but the response is only 15–25% of that observed in vessels with intact endothelium (Frisbee *et al.* 2002). Low P_{O_2} (~12 mmHg) also inhibits vasoconstriction of first-order rat cremaster arterioles constricted by activation of α_2-adrenoreceptors (Tateishi & Faber, 1995*a*,*b*). In addition, noradrenaline-induced contraction of isolated hamster cremaster arteriolar smooth muscle cells is inhibited by exposure to low P_{O_2} (Jackson, 2000*b*; Cohen & Jackson, 2003). In the latter studies, the low O_2-induced inhibition of noradrenaline-induced contraction of hamster cremaster arteriolar smooth muscle cells was not due to the activation of K^+ channels (Jackson, 2000*b*), the inhibition of L-type Ca^{2+} channels (Cohen & Jackson, 2003), nor diminution of noradrenaline-induced Ca^{2+} transients (Cohen & Jackson, 2003). These data suggested that the low P_{O_2} reduced the Ca^{2+} sensitivity of the contractile machinery, consistent with several studies of macrovascular smooth muscle cells (Gebremedhin *et al.* 1994; Aalkjaer & Lombard, 1995; Shimizu *et al.* 2000). On the other hand, patch clamp studies of isolated vascular smooth muscle cells from systemic arteries have demonstrated activation of ATP-sensitive K^+ (K_{ATP}) channels (Dart & Standen, 1995), activation of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (Gebremedhin *et al.* 1994), or inhibition of L-type voltage-gated Ca^{2+} (Ca_L) channels (Franco-Obregon *et al.* 1995; Franco-Obregon & Lopez-Barneo, 1996*a*,*b*) when the cells are exposed to low P_{O_2} (15–20 mmHg), any of which could contribute to O_2 reactivity.

In contrast to the studies described above, removal of the endothelium from first-order rat cremaster arterioles abolished O_2 reactivity in *ex vivo* pressure-myography studies (Messina *et al.* 1992, 1994). These data exclude smooth muscle as the O_2 sensor in these isolated rat cremaster arterioles. Furthermore, several additional *ex vivo* studies of intact, endothelium-replete rat first-order cremaster arterioles have failed to demonstrate effects of changes in P_{O_2} between 10 and 150 mmHg on myogenic tone (Tateishi & Faber, 1995*a*,*b*; Kerkhof *et al.* 1999), again not supporting smooth muscle as the O_2 sensor. In the studies by Tateishi and Faber (Tateishi & Faber, 1995*a*) reduced O_2 tensions ($P_{O_2} < 6$ mmHg) inhibited myogenic tone suggesting intrinsic O_2 reactivity at much lower P_{O_2} values.

Intravital microscopy studies performed in hamster cheek pouches have also failed to support smooth muscle, or other components of the vascular wall, as the O_2 sensor (Duling, 1974; Jackson, 1987). Altering the P_{O_2} of the wall of arterioles through the use of fluid-filled micropipettes (Duling, 1974; Jackson, 1987) (Figs 3 and 4), or by *in situ* perfusion of segments of arterioles in the hamster cheek pouch (Jackson, 1987) (Fig. 5) failed to demonstrate arteriolar O_2 reactivity intrinsic to the arteriolar wall. In contrast to these findings, elevation of the P_{O_2} of the superfusion solution flowing over the entire cheek pouch produced typical O_2-induced vasoconstriction (Duling, 1974; Jackson, 1987) (Figs 3–5). The lack of effect of local changes in P_{O_2} on arteriolar diameter (Figs 3–5) do not support the hypothesis that arteriolar smooth muscle cells are intrinsically sensitive to changes in P_{O_2} between 10 and 150 mmHg.

Thus, smooth muscle *per se*, in some vessels (resistance arteries and first-order arterioles), under some conditions, may indeed sense changes in P_{O_2} in the range of 10–150 mmHg. However, there does not appear to be significant support for smooth muscle cells as the O_2 sensor, particularly based on *in situ* studies of arterioles in the intact microcirculation.

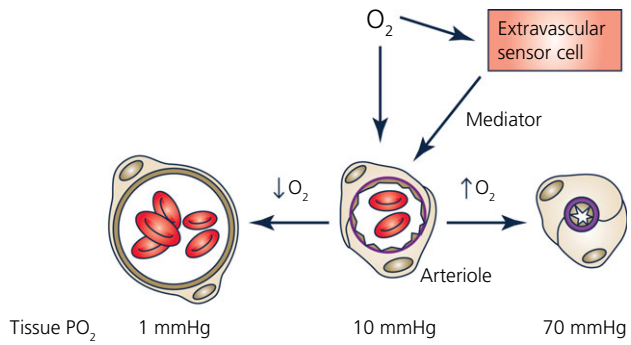

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Figure 2. Schematic diagram of O_2 signalling in the microcirculation
Oxygen in the environment of arterioles can act directly on the arteriolar wall or on cells in the lumen to produce a vasomotor effect (dilatation in the case of reduced P_{O_2}, or constriction in the case of elevated P_{O_2}). Alternatively, changes in P_{O_2} may be sensed by extravascular cells (parenchymal cells, mast cells, nerves, etc.), a mediator produced, which then acts on the vessel wall to produce the appropriate vasomotor effect. The P_{O_2} values shown below the cross section of the arterioles refer to tissue P_{O_2} in a superfused, intravital preparation measured at the midpoint between the venous ends of two capillaries as reference values, only.

Arteriolar endothelial cells as an O_2 sensor. There is considerable evidence from *ex vivo*, pressure-myography studies of isolated vessels that endothelial cells may serve as an O_2 sensor, at least in small arteries and large arterioles. Busse and coworkers first demonstrated endothelium-dependent O_2 reactivity in rat tail arteries,

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canine femoral artery branches and canine epicardial coronary arteries (Busse *et al.* 1983, 1984). These findings were subsequently confirmed in rabbit aortas and rabbit femoral arteries (Pohl & Busse, 1989), hamster carotid arteries (Jackson *et al.* 1987), first-order rat cremaster arterioles (Messina *et al.* 1992, 1994), rat middle cerebral arteries (Fredricks *et al.* 1994*b*), large arterioles from rat diaphragm (Ward, 1999), and rat gracilis muscle feed arteries (Fredricks *et al.* 1994*a*; Frisbee *et al.* 2001*a*,*b*,*c*, 2002).

In contrast, several pressure-myography studies of endothelium-intact first-order rat cremaster arterioles have failed to demonstrate effects of P_{O_2} changes on myogenic tone (Tateishi & Faber, 1995*a*,*b*; Kerkhof *et al.* 1999), as noted above. The apparent difference between the studies showing endothelium-dependent O_2 reactivity (Busse *et al.* 1983, 1984; Messina *et al.* 1992, 1994; Fredricks *et al.* 1994*a*,*b*; Ward, 1999; Frisbee *et al.* 2001*a*,*b*,*c*, 2002) and those not displaying O_2 reactivity (Tateishi & Faber, 1995*a*,*b*; Kerkhof *et al.* 1999) is the presence of luminal flow in the former and not in the latter. The exception to this relationship is a pressure-myography study by Dietrich *et al.* (2000) using endothelium-intact rat cerebral arterioles. These investigators found that PSS or dextran–PSS-perfused arterioles did not display O_2 reactivity in the absence of luminal red blood cells.

Lack of evidence for endothelium-dependent O_2 reactivity also can be gleaned from the hamster cheek pouch studies already cited in the previous section (Duling, 1974; Jackson, 1987). In those studies, either blood (Duling, 1974; Jackson, 1987) or O_2-equilibrated PSS (Jackson, 1987) was flowing through the arterioles. In these studies, despite effectively changing the P_{O_2} of endothelial cells, O_2 reactivity was not observed when only the vessel wall was exposed to changes in P_{O_2}. These data do not support an endothelial O_2 sensor in arterioles in the hamster cheek pouch. They, along with the studies by Dietrich *et al.* (2000), suggest that shear stress is not the key to promoting endothelial cell O_2 sensing.

Thus, there is evidence both for and against an endothelial cell O_2 sensor. However, the extant data supporting this hypothesis stem from pressure-myography studies of isolated arteries and large arterioles, *ex vivo*. No data from intravital preparations

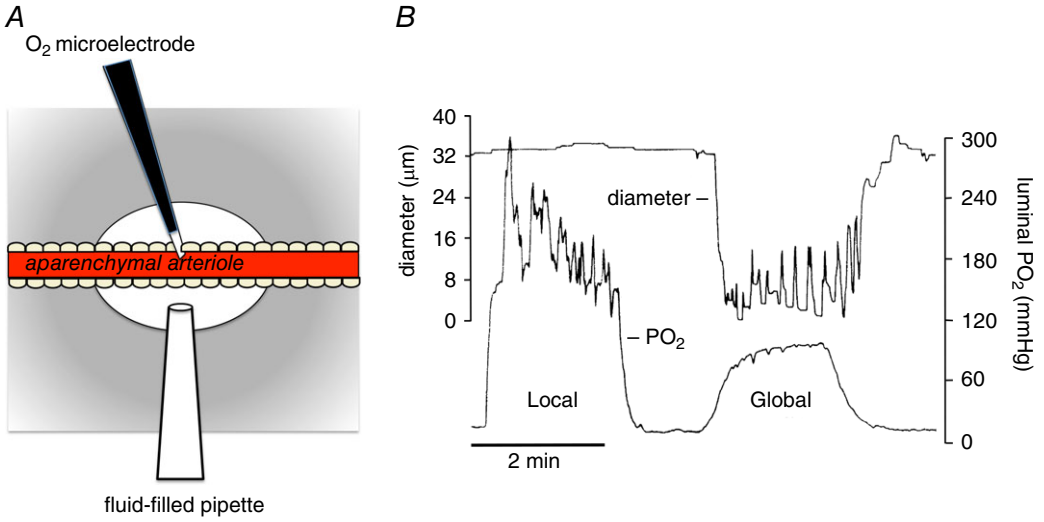

Figure 3. Local increases in P_{O_2} have no effect on arteriolar tone
Data shown are modified from Jackson (1987). *A*, schematic diagram of a segment (1–8 mm) of an arteriole in a hamster cheek pouch from which the parenchyma has been surgically removed (aparenchymal arteriole) to obviate effects of local P_{O_2} changes on parenchymal and other extravascular cells. A Whalen-type recessed tip O_2 microelectrode was inserted through the wall of the vessel into the lumen as shown to monitor luminal P_{O_2}. A temperature-controlled micropipette filled with PSS equilibrated with varied P_{O_2} (fluid-filled micropipette) was positioned opposite the O_2 microelectrode. Pressurization of the fluid-filled micropipette ejected the O_2-equilibrated solution onto the surface of the arteriole to produce a local change in P_{O_2}. The entire cheek pouch preparation was superfused with PSS, the P_{O_2} of which could be varied to produce global changes in P_{O_2} that affected both the aparenchymal arteriole and all other vessels and cell types in the cheek pouch. The diameter of the arteriole was measured by intravital video microscopy. *B*, results from a typical experiment in which either local increases in P_{O_2} were produced using the fluid-filled pipette (Local) or global increases in P_{O_2} were produced by changing the P_{O_2} of the entire superfusate (Global) (replotted data are from Fig. 3 in Jackson, 1987). Local increases in P_{O_2} that effectively changed the P_{O_2} across the wall of the arteriole had no significant effect on arteriolar diameter, whereas a global increase in P_{O_2} produced sustained vasoconstriction. These data suggest that components of the arteriolar wall (endothelial cells, smooth muscle cells or perivascular nerves) are not the sensor cells responsible for arteriolar O_2 reactivity in the hamster cheek pouch. See Jackson (1987) for details.

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support endothelial cells as the sensors mediating arteriolar O_2 reactivity.

As noted in the previous paragraph, all of the *ex vivo* studies of vascular O_2 reactivity have been performed on arteries or large, first-order arterioles (diameters $> 50\ \mu$m). In contrast, most *in situ* intravital microscopy studies of arteriolar O_2 reactivity have focused on small, third- to fifth-order arterioles (diameters $< 40\ \mu$m). Given the known differences in mechanisms regulating myogenic tone between small arteries and downstream arterioles (Westcott & Jackson, 2011; Westcott *et al.* 2012), it may be that there are different mechanisms (and hence, sites of action) coupling changes in P_{O_2} with changes in vascular tone in different parts of the vascular tree. In addition, as noted above, *in situ*, elevated P_{O_2} produces dramatic, long lasting constriction of arterioles (Fig. 1). This degree of O_2 reactivity has not been described in the *ex vivo* study of isolated vessels, perhaps reflecting that mostly feed arteries and first-order arterioles have been studied *ex vivo*. However, these data may also indicate that sites other than the arteriolar wall are more important for O_2 reactivity of arterioles in the intact, living microcirculation.

Red blood cells as an O_2 sensor. Red blood cells also have been proposed as the O_2-sensitive cell type responsible for arteriolar O_2 reactivity. Three lines of evidence support this hypothesis. First, *in vitro*, red blood cells release ATP as P_{O_2} is reduced from 85 mmHg down to 17 mmHg (Ellsworth *et al.* 1995). Second, at concentrations consistent with the amount of ATP released from red blood cells, intraluminal ATP produces conducted dilatation of arterioles in the hamster retractor muscle *in situ*, through a mechanism mediated by NO (McCullough *et al.* 1997). Intraluminal application of ATP into collecting venules also produces dilatation of upstream arterioles suggesting conduction of vasodilatation initiated by ATP from venules to arterioles through capillaries (Collins *et al.* 1998) (see below for more on conducted responses). Third, inclusion of red blood cells in the solution perfusing rat cerebral arterioles in pressure-myography studies instills O_2 reactivity to isolated arterioles that do not respond to changes in P_{O_2} in their absence (Dietrich *et al.* 2000).

In contrast, the studies outlined in Figs 3–5 (Jackson, 1987) suggest that red blood cells may not be the O_2 sensor that mediates arteriolar O_2 reactivity *in situ*. In those studies, local changes in P_{O_2} that effectively altered blood P_{O_2} had no effect on arteriolar tone (Figs 3 and 4). Oxygen reactivity was not observed whether blood was flowing through the lumen of the arterioles (Fig. 3) or not (Fig. 4). In addition, if red blood cells were major O_2 sensors coupling changes in P_{O_2} with changes in vasomotor tone, then perfusion of arterioles *in situ* with PSS should eliminate arteriolar O_2 reactivity. This was not the case in the hamster cheek pouch (Fig. 5) (Jackson, 1987) or in the mouse cremaster muscle (Ngo *et al.* 2010); in both tissues, arteriolar O_2 reactivity was retained when

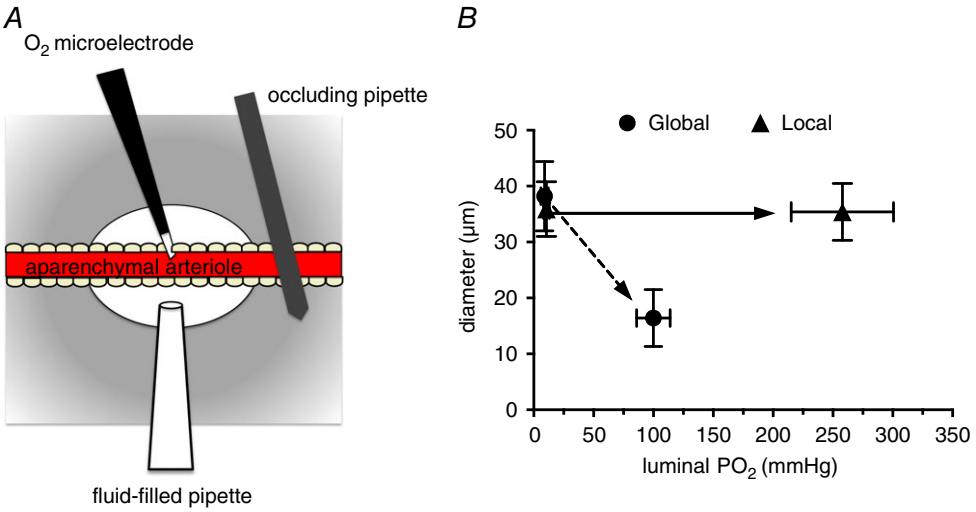

Figure 4. Local increases in P_{O_2} have no effect on arteriolar tone in occluded arterioles
A, a preparation similar to that depicted in Fig. 3, but with the inclusion of an occluding pipette that was pressed down on the arteriole to eliminate blood flow through the aparenchymal segment. *B*, summary data from these experiments (data are means ± SEM, $n = 5$). Local changes in P_{O_2} across the arteriolar wall produced by the fluid-filled pipette (Local) were ineffective in producing arteriolar constriction. However, raising the P_{O_2} of the superfusate over the entire preparation (Global) produced consistent, sustained arteriolar constriction. These data, along with those shown in Fig. 3, suggest that components of the arteriolar wall (endothelial cells, smooth muscle cells or perivascular nerves) are not the sensor cells responsible for arteriolar O_2 reactivity in the hamster cheek pouch. Data are replotted from Fig. 4*B* in Jackson (1987); see this reference for more details.

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the tissues were perfused with physiological salt solution and no red blood cells were present. Thus, *in situ*, red blood cells may not be a major site that senses changes in P_{O_2} values greater than 10–15 mmHg.

Extravascular cells as an O_2 sensor. To test the hypothesis that extravascular cells (parenchymal cells, etc.) are the location of the O_2 sensor involved in arteriolar O_2 reactivity in the hamster cheek pouch, Jackson and Duling surgically removed the parenchyma along long segments of blood-perfused second-order arterioles (Jackson & Duling, 1983) (Figs 3, 4 and 6). Surprisingly, this manoeuvre had no significant effect on O_2 reactivity: the aparenchymal arteriolar segments continued to respond to changes in superfusate P_{O_2} as the solution flowed over the entire preparation (Jackson & Duling, 1983). Furthermore, sealing the aparenchymal arteriolar segments under glass (to prevent superfusate access) and occluding the arterioles (to obviate convection of O_2 in the blood to the segments) did not eliminate O_2 reactivity (Fig. 6). To complicate matters, we found that some isolated, cannulated cheek pouch arterioles retained O_2 reactivity. However, the responses of these isolated arterioles were ephemeral. Taken as a whole, the data from this study suggested that: (1) changes in P_{O_2} are probably sensed at extravascular sites that are non-uniformly distributed along the arterioles (to account for the lack of O_2 reactivity in some isolated vessels), and (2) O_2-dependent vasoconstriction, once initiated, can be conducted along the arteriolar wall (to account for the maintained O_2 reactivity of aparenchymal segments sealed under glass: Fig. 6) (see below for more on the topic of conducted O_2 responses). Subsequent studies, outlined above (Fig. 4), in which segments of cheek pouch arterioles were perfused *in situ* with solutions with varied P_{O_2} supported and extended these conclusions (Jackson, 1987).

Mast cells: a possible O_2 sensor in the hamster cheek pouch. Arterioles in the hamster cheek are intimately associated with mast cells as shown in Fig. 1*C*. These cells cluster, non-uniformly, near the arterioles and they produce cysteinyl leukotrienes (CysLTs) (Storch *et al.* 2015), vasoconstrictors that mediate arteriolar O_2 reactivity in the hamster cheek pouch (see below for more on this topic). Mast cells as the location of the O_2 sensor may also explain why some isolated cannulated cheek pouch arterioles retained O_2 reactivity in a previous study (Jackson & Duling, 1983). Dissection of these arterioles may remove or damage some/all of these perivascular cells accounting for the loss and the labile nature of O_2 reactivity in the *ex vivo* study of cheek pouch arterioles.

Conduction of O_2-induced vasoconstriction: a complicating factor. As noted above, we (Jackson & Duling, 1983) found that even when arterioles in the

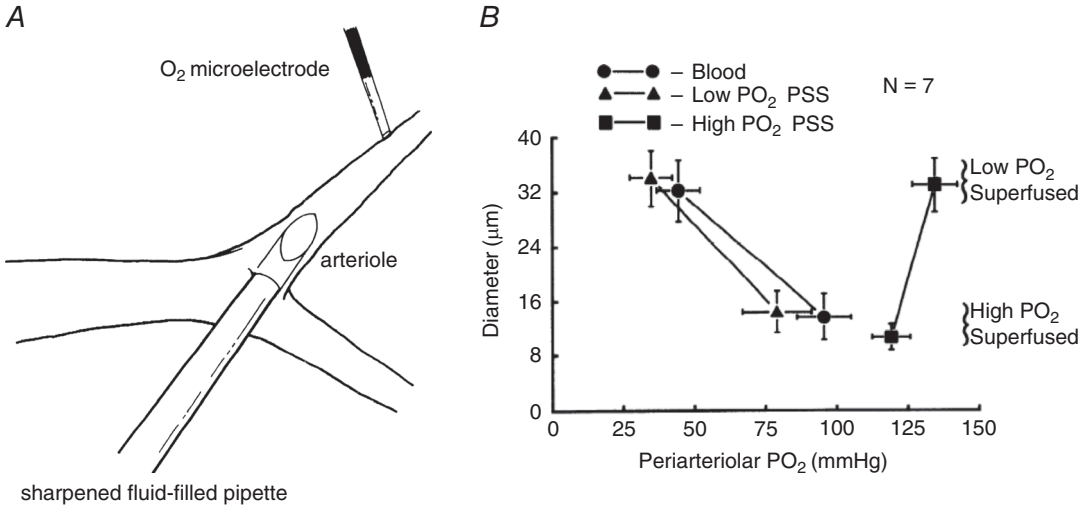

Figure 5. Perfusion of arterioles *in situ* with solutions equilibrated with high P_{O_2} has no effect on arteriolar tone
A and *B* are reproduced from Jackson (1987). *A*, schematic diagram of a hamster cheek pouch arteriole in which a sharpened fluid-filled pipette has been inserted through the wall of an arteriole allowing perfusion of the arteriole with PSS equilibrated with varied P_{O_2}. The entire cheek pouch preparation was superfused with PSS to allow global changes in P_{O_2}. *B*, summary data (means ± SEM). Perfusion of the arterioles with solutions equilibrated with high or low P_{O_2} had no significant effect on arteriolar diameter. Only when the global P_{O_2} was elevated via the superfusate did the arterioles constrict (compare low P_{O_2} superfusate points with high P_{O_2} superfusate points). These data suggest that components of the arteriolar wall (endothelial cells, smooth muscle cells or perivascular nerves) are not the sensor cells responsible for arteriolar O_2 reactivity in the hamster cheek pouch. See Jackson (1987) for details.

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hamster cheek pouch were sealed under glass (to prevent superfusion solution access) and occluded (to obviate convection of O_2 into the segment by blood flow) the vessels retained O_2 reactivity (Fig. 6). We hypothesized that O_2-induced vasoconstriction could be conducted along the arterioles over considerable distance (at least several millimetres). Mouse cremaster muscle arterioles also display conducted O_2-induced vasoconstriction (Ngo *et al.* 2010; Riemann *et al.* 2011), suggesting that this phenomenon is not restricted to the hamster cheek pouch.

Conduction of O_2-induced arteriolar responses implies that responses can be initiated away from the site of observation and, importantly, that responses initiated at several sites may summate, as has been observed for conducted vasomotion in response to other vasoactive substances (Segal *et al.* 1989). Based on conducted vasomotor responses induced by other vasoactive substances (Delashaw & Duling, 1991; Xia & Duling, 1995), the conduction of O_2-induced vasomotor responses implies that changes in vascular smooth muscle membrane potential are probably involved in the mechanism of action of O_2 on arterioles. As will be discussed below, this prediction was verified in the hamster cheek pouch where O_2-induced vasoconstriction was preceded by depolarization of the smooth muscle cells (Welsh *et al.* 1998).

Conduction of O_2-induced changes in vascular tone along arterioles complicates identification of the location of the cell type that actually senses changes in P_{O_2} because the sensor cells can be remote from the site of observation. Changes in P_{O_2}, could, for example, be sensed in parenchyma around terminal arterioles or even capillaries or venules downstream from arterioles, a response initiated, and this response conducted upstream to produce the appropriate change in arteriolar tone as suggested by Jackson (1987) and later by Collins *et al.* (1998) and Ellsworth *et al.* (2016). Oxygen sensing in the vicinity of terminal arterioles, capillaries or post-capillary venules might explain why, *in situ*, changes in P_{O_2} that are restricted to segments of second- to third-order arterioles, do not produce a change in arteriolar tone, whereas global changes in P_{O_2} consistently produce an upstream arteriolar response (Duling, 1974; Jackson, 1987). However, in the hamster cheek pouch, conduction of vasoconstrictor-induced depolarization occurs along the smooth muscle layer (Welsh & Segal, 1998). This constraint suggests that, in the cheek pouch, the O_2-sensing cells are probably adjacent to arteriolar smooth muscle cells and not in downstream capillaries or venules (see below for more on this topic). The pathway for conduction in skeletal muscle arterioles has not been as firmly established.

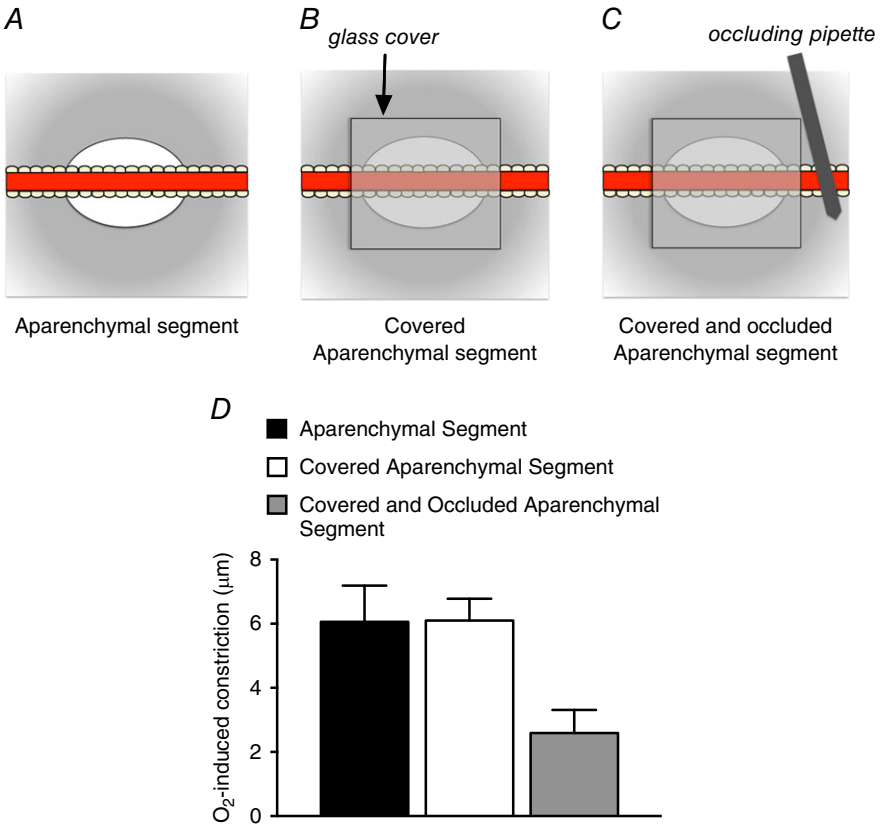

Figure 6. Covering aparenchymal segments with glass and occluding them to eliminate blood flow does not eliminate arteriolar O_2 reactivity
A, schematic diagram of an aparenchymal segment in which the parenchyma has been removed from a long segment of hamster cheek pouch arteriole as described by Jackson & Duling (1983). Elevation of the P_{O_2} of the solution flowing over the preparation from 12 to 150 mmHg resulted in arteriolar constriction as depicted in *D*. As shown in *B*, subsequent covering of the aparenchymal segment with a piece of glass coverslip (sealed in place with silicone grease), to eliminate contact of the arteriole with the superfusate, had no effect on O_2-induced constriction as shown in *D*. To eliminate blood flow through the covered aparenchymal segments, an occluding pipette was used as shown in *C*. Despite the lack of access to the superfusate and flowing blood, these covered and occluded aparenchymal segments retained significant O_2 reactivity as shown in *D*. These data suggest that the constriction induced by elevated P_{O_2} can be conducted along the arteriolar wall. See Jackson & Duling (1983) for details.

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The hypothesis that changes in P_{O_2} can be sensed in capillaries was tested in rat extensor digitorum longus muscles using a microfluidic device approach (Ghonaim *et al.* 2011). The P_{O_2} of regions overlying capillary beds ranging from 100 μm circles (affecting 1 capillary and surrounding skeletal muscle fibres) to 200 μm × 1000 μm rectangles (affecting ∼3 capillaries and surrounding muscle fibres) were changed (Ghonaim, 2013), with capillary red blood cell haemoglobin O_2 saturation (%Sat) and red blood cell flux measured. The authors found that while changes in P_{O_2} applied through 100 or 200 μm circles (affecting only 1–2 capillaries) produced appropriate changes in red blood cell %Sat, they were without effect on red blood cells flux. In contrast, changes in P_{O_2} applied to a 200 μm × 1000 μm area (affecting ∼3 capillaries and associated muscle fibres) produced changes in red blood cell flux through the affected capillaries. These results suggest that there may be summation of responses to altered P_{O_2} that are required to initiate a conducted response sufficient to affect upstream arterioles that control red blood cell flux through the capillaries. Although the authors interpreted these findings as evidence for capillaries as the site where changes in P_{O_2} are sensed (in the context of red blood cells as the O_2 sensors), effects of changes in P_{O_2} on striated muscle fibres, which are also putative O_2 sensors (see below), were not excluded. Striated muscle fibres are much longer than capillaries, and they will contact arterioles in the network. Thus, it is also possible that the striated muscle fibres sensed changes in P_{O_2}, a response initiated and transmitted to upstream arterioles to modulate arteriolar smooth muscle tone and hence capillary perfusion.

Striated muscle fibres as an O_2 sensor. Frisbee and Lombard compared the O_2 reactivity of first-order arterioles in rat cremaster muscles, *in situ*, with similar arterioles studied by pressure myography, *ex vivo* (Frisbee & Lombard, 2002). They found that while these large arterioles retained O_2 reactivity when studied *ex vivo*, the maximal O_2-induced diameter responses of the isolated vessels were only 50% of the responses observed *in situ*. These data support a role for striated muscle fibres as an O_2 sensor in rat cremaster muscle. As will be outlined below, the location of cytochrome $P450_{4A}$ ($CYP450_{4A}$) ω-hydroxylase, a key enzyme involved in arteriolar O_2 reactivity in striated muscle fibres (Kunert *et al.* 2001*a*), also lends support for these cells as O_2 sensors in striated muscle.

Summary of the location of the sensor. *Ex vivo* studies of isolated feed arteries and first-order arterioles support the hypothesis that these vessels are intrinsically sensitive to changes in P_{O_2} within the appropriate range (10–150 mmHg), with smooth muscle, endothelium, or red blood cells as the location of the sensor (see references above). In contrast, intravital microscopy studies (Duling, 1974; Jackson & Duling, 1983; Jackson, 1987), all in the hamster cheek pouch, and all directed at smaller, downstream arterioles, have failed to support the hypothesis that these smaller vessels are intrinsically sensitive to O_2 *in situ*. Furthermore, local changes in P_{O_2} that effectively changed the P_{O_2} of the blood flowing through the arterioles in the cheek pouch were also without effect on arteriolar tone (Jackson, 1987). Microvascular beds perfused with PSS retain O_2 reactivity (Jackson, 1987; Ngo *et al.* 2010). These data do not support the hypothesis that red blood cells are a major sensor mediating arteriolar O_2 reactivity in the intact microcirculation of the hamster cheek pouch or mouse cremaster muscle. Instead, the data from intravital preparations suggest that changes in P_{O_2} are sensed at some extravascular site. These conflicting conclusions may indicate that vascular O_2 sensing varies along the vascular tree with upstream feed arteries possessing intrinsic mechanisms to respond to changes in blood P_{O_2}, and downstream arterioles being tuned to detect local changes in tissue P_{O_2}. However, tissue-dependent heterogeneity also cannot be excluded as studies to evaluate the intrinsic O_2 sensitivity of small arterioles (<30 μm), *in situ*, have not been evaluated in tissues other than the hamster cheek pouch. Conduction of O_2-induced changes in arteriolar tone also complicates identification of the microvascular O_2 sensor that has not been adequately explored in preparations other than the hamster cheek pouch.

Mechanism of action

Several signalling pathways have been proposed to explain arteriolar O_2 reactivity. These can be separated into two groups: (1) mechanisms in which low O_2 results in increased production or decreased destruction of a vasodilator substance, and (2) mechanisms by which increased O_2 results in production of a vasoconstrictor. As will be evident in the sections below, there appears to be significant regional heterogeneity that has complicated the study of the mechanisms responsible for arteriolar O_2 reactivity. As discussed above, conduction of O_2-initiated responses also adds to the complexity of defining the mechanism of action of O_2 on arterioles in the microcirculation.

Oxygen-dependent inhibition of production of prostanoids. A number of *ex vivo* studies of isolated vessels have supported a role for prostaglandins in mediating dilatation of resistance arteries and large arterioles to a reduction in P_{O_2} from 150 mmHg to 20–50 mmHg (Busse *et al.* 1983, 1984; Fredricks *et al.* 1994*a*,*b*; Frisbee *et al.* 2001*b*,*c*, 2002) or constriction of these vessels to increases in P_{O_2} from 20 to 150–600 mmHg (Messina *et al.* 1994; Frisbee *et al.* 2001*a*).

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However, the relevance of these *ex vivo* studies to *in situ* arteriolar O_2 reactivity remains unclear. Effective inhibition of cyclooxygenase has no effect on arteriolar O_2 reactivity in intravital preparations of the hamster cheek pouch, hamster and rat cremaster muscles (Jackson, 1986), rat spinotrapezius muscle (Pries *et al.* 1995) and mouse cremaster muscle (Ngo *et al.* 2013). A weakness in these intravital studies is that only large changes in P_{O_2} (from ~12 to 150 mmHg) were examined. Thus, subtle effects of cyclooxygenase inhibition on the P_{O_2}–response relation could have been missed. Nonetheless, the intravital studies do not support a sole, major role for prostaglandins in mediating arteriolar O_2 reactivity in the intact microcirculation. However, the intravital studies do not exclude a role for prostaglandins in larger feed arteries outside of the tissue proper.

The mechanism by which elevated P_{O_2} inhibits, and lowered P_{O_2} enhances, vasodilator prostanoid formation also is not known. The enzymes catalysing oxygenation of arachidonic acid, the cyclooxygenases (COXs), are half-maximally activated at a P_{O_2} of about 3 mmHg (Lands *et al.* 1978; Mukherjee *et al.* 2007), with maximal activity at P_{O_2} values greater than 18 mmHg (Lands *et al.* 1978; Mukherjee *et al.* 2007). Thus, formation of prostanoids should be P_{O_2} independent over much of the P_{O_2} range to which arterioles are O_2 sensitive (Fig. 1). However, the COXs can undergo auto-oxidation, which inhibits enzyme activity (Tsai & Kulmacz, 2010). Oxygen-dependent oxidation and inhibition of COXs might explain O_2-dependent inhibition of production of vasodilator prostanoids, but this has not been established.

Oxygen-dependent destruction of nitric oxide. Reducing the P_{O_2} of solutions perfusing rabbit aortas and rabbit femoral arteries (Pohl & Busse, 1989) or hamster carotid arteries (Jackson *et al.* 1987), *ex vivo*, from 150 to 20–30 mmHg produces endothelium-dependent vasodilatation mediated by NO. Nitric oxide has also been proposed to mediate arteriolar O_2 reactivity in rat spinotrapezius muscle (Pries *et al.* 1995) and the O_2 reactivity of rat intestinal arterioles (Nase *et al.* 2003) *in situ*. *Ex vivo* studies of rat gracilis muscle feed arteries also support a role for NO, but only at relatively high P_{O_2} values (between 100 and 150 mmHg) (Frisbee *et al.* 2002). Pressure-myography studies of rat first-order cremaster muscle arterioles, *ex vivo*, found that NO mediated O_2 reactivity, but only after application of the $CYP450_{4A}$ ω-hydroxylase inhibitor, 17-octadecynoic acid (17-ODYA) (Kerkhof *et al.* 1999). In the absence of 17-ODYA, these authors found that isolated rat cremaster arterioles did not respond to changes in P_{O_2} suggesting that endogenous production of 20-hydroxyeicosatetraenoic acid (20-HETE) by $CYP450_{4A}$ ω-hydroxylation of arachidonic acid inhibited O_2-dependent signalling by NO.

In contrast to the studies supporting a role for NO in arteriolar O_2 reactivity, intravital studies in the hamster cheek pouch (Jackson, 1991) and in mouse cremaster muscle (Ngo *et al.* 2010, 2013; Riemann *et al.* 2011) found that effective inhibition of NO synthesis had no effect on arteriolar O_2 reactivity. An earlier *ex vivo* study of rat gracilis feed arteries also did not support a role for NO in the reactivity of these vessels to changes in P_{O_2} between 35 and 150 mmHg P_{O_2} (Frisbee *et al.* 2001*a*). These findings may indicate that there is heterogeneity in the mechanism of action of O_2, which is dependent on the region, location in the vascular tree and possibly on the species studied. In addition, another study in the rat spinotrapezius muscle suggests a major role for $CYP450_{4A}$ ω-hydroxylase and 20-HETE in arteriolar O_2 reactivity (see below) (Marvar *et al.* 2007). This observation is difficult to reconcile with the findings of Pries *et al.* cited above, who reported that inhibition of NO synthase abolished arteriolar O_2 reactivity in the same tissue, from the same rat strain (Pries *et al.* 1995). It could be that multiple mechanisms are in play, as suggested by *ex vivo* studies of first-order rat cremaster arterioles noted above (Kerkhof *et al.* 1999). Subtle methodological differences also cannot be excluded. Thus, the contribution of NO to arteriolar O_2 reactivity remains unclear.

Oxygen-dependent production of superoxide anion. If NO mediates arteriolar O_2 reactivity, the relationship between O_2 and NO production cannot be direct, because O_2 is a substrate for NO synthase, with half-maximal activation at about 2 mmHg for the isolated enzyme (Abu-Soud *et al.* 2000), or as high as 38 mmHg in cell-based assays (Whorton *et al.* 1997). Thus, NO production should increase or remain unchanged as O_2 increases. This is opposite to what would be required for direct effects of O_2 on NO production to mediate arteriolar O_2 reactivity.

It has been suggested that O_2-dependent production of superoxide anion ($O_2^{-\bullet}$) and the subsequent destruction of NO may explain arteriolar O_2 reactivity *in situ* (Golub & Pittman, 2013). However, previous direct tests of this hypothesis in striated muscle preparations using exogenous superoxide dismutase (SOD) both failed (Proctor & Duling, 1982*b*; Pries *et al.* 1995). Golub & Pittman (2013) argue that the kinetics of the reaction between $O_2^{-\bullet}$ and NO is so much faster than that between $O_2^{-\bullet}$ and SOD, and that the tissue content of extracellular SOD is so high in striated muscle, the use of exogenous SOD does not adequately test for a role for $O_2^{-\bullet}$. In the rat brain, where there is lower expression of extracellular SOD, hyperbaric O_2-induced constriction of resistance arteries indeed does appear to be mediated by O_2-dependent production of $O_2^{-\bullet}$ and destruction of NO, and can be inhibited by application of exogenous

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SOD (Demchenko *et al.* 2000). Similarly, superfusion with SOD and catalase or the SOD mimetic tempol abolishes O_2-induced constriction of rat sciatic epineural arterioles, supporting a role for $O_2^{-\bullet}$ in these vessels (Sakai *et al.* 2007). Inhibitors of NADPH oxidase or xanthine oxidase also eliminated O_2 reactivity, suggesting that these enzymes may be the O_2 sensors in rat epineural arteriolar O_2 reactivity (Sakai *et al.* 2007). However, unlike the studies in the brain (Demchenko *et al.* 2000), inhibition of NO synthase did not inhibit O_2 reactivity of rat sciatic epineural arterioles suggesting that alterations in NO bioavailability do not explain O_2 reactivity in this tissue. In addition, earlier studies demonstrated that $O_2^{-\bullet}$ is a dilator in cat (Wei *et al.* 1996) and rabbit (Didion & Faraci, 2002) cerebral arterioles, and human coronary arterioles (Sato *et al.* 2003), supporting the idea that the $O_2^{-\bullet}$–NO destruction pathway is not a general mechanism accounting for arteriolar O_2 reactivity. The lack of effect of effective inhibition of NO production on O_2 reactivity in other preparations (Jackson, 1991; Ngo *et al.* 2010, 2013; Riemann *et al.* 2011) also argues that this is not a general mechanism explaining arteriolar O_2 reactivity in all tissues and every species.

All of the mechanisms discussed above involve O_2-dependent modulation of a vasodilator: as P_{O_2} increases, the dilator signal would decrease and as P_{O_2} decreases, the dilator signal would increase. Recall that in the normal resting microcirculation of skeletal muscle, for example, the P_{O_2} in the environment of arterioles is low. Thus, if arteriolar O_2 reactivity involves modulation of a vasodilator, then when arterioles are isolated and cannulated for *ex vivo* study and exposed to ambient O_2 conditions (21% O_2), these arterioles should display substantially greater tone than they displayed *in situ* under low P_{O_2} (~10–15 mmHg) conditions. This simply has not been observed: the degree of myogenic tone developed by cannulated, pressurized arterioles *ex vivo* exposed to room air (21% O_2, P_{O_2} ~150 mmHg) is similar to the tone observed when these same vessels are studied *in situ* by intravital microscopy under low P_{O_2} conditions (P_{O_2} in the order of 10–15 mmHg) (for example, compare Jackson & Blair, 1998 with Burns *et al.* 2004). This observation alone suggests that arteriolar O_2 reactivity *in situ* does not involve a vasodilator and also that it is unlikely that O_2 is sensed directly by cells that comprise the wall of the arterioles.

Oxygen as a vasoconstrictor. In contrast to the mechanisms discussed thus far involving the O_2-dependent modulation of vasodilators, there is a substantial body of literature that O_2-dependent production of vasoconstrictors mediates arteriolar O_2 reactivity in the peripheral microcirculation (see below for references). These vasoconstrictor mechanisms largely have been ignored when considering the role played by O_2 in the local regulation of blood flow (Casey & Joyner, 2011; Marshall & Ray, 2012; Golub & Pittman, 2013; Reglin & Pries, 2014).

Oxygen-dependent production of leukotrienes. The 5-lipoxygenase and CysLTs appear to mediate arteriolar O_2 reactivity in the epithelial portion of the hamster cheek pouch (Jackson, 1988, 1989, 1993) (Fig. 7). General lipoxygenase inhibitors (Jackson, 1988), selective 5-lipoxygenase inhibitors (Jackson, 1989, 1993), a selective inhibitor of the 5-lipoxygenase-activating protein (FLAP) (Jackson, 1993), as well as antagonists of CysLT receptors (Jackson, 1989, 1993) all selectively and effectively inhibit O_2-induced arteriolar constriction in the cheek pouch. Importantly, these same inhibitors have no effect on the O_2 reactivity of arterioles in hamster cremaster muscles, supporting their vascular bed selectivity (Jackson, 1993). These latter findings also strongly support the hypothesis that there are regional differences in the mechanisms coupling changes in P_{O_2} with changes in arteriolar tone.

Leukotrienes are synthesized from arachidonic acid released from membrane phospholipid stores by a multistep process (Peters-Golden, 1998; Storch *et al.* 2015). In activated cells, FLAP presents arachidonic acid to the 5-lipoxygenase that catalyses the first step in this reaction sequence to form an unstable epoxide, leukotriene A_4 (LTA_4). The epoxide LTA_4 is then conjugated with glutathione to form the CysLT leukotriene C_4 (LTC_4), which is converted to leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) (also CysLTs) by consecutive cleavage of peptides from the added glutathione. The K_m for O_2 of the 5-lipoxygenase purified from porcine leukocytes is about 9 mmHg P_{O_2} (Ibe & Raj, 1992). Given the nuclear membrane localization of the 5-lipoxygenase (Peters-Golden & Brock, 2001; Woods *et al.* 1995) and the steep intracellular gradients in O_2 that arise from O_2 consumption by mitochondria, oxidases and oxygenases within cells (Jones, 1981), leukotriene production by intact cells will be O_2 dependent well within the physiological range (10–150 mmHg) required for a microvascular O_2 sensor (see Fig. 1). Oxygen-dependent synthesis of leukotrienes has been reported in several *in vitro* systems (Paterson, 1986; Ohwada *et al.* 1990; Ibe & Raj, 1992; Martin *et al.* 1992) providing support for the hypothesis that this pathway is involved in O_2 sensing, at least in the hamster cheek pouch.

Oxygen-dependent production of 20-HETE. In contrast to the data from the epithelial portion of the cheek pouch, arteriolar O_2 reactivity in striated muscle appears to be mediated by 20-HETE produced by $CYP450_{4A}$ ω-hydroxylase (Harder *et al.* 1996; Lombard *et al.* 1999; Hungerford *et al.* 2000; Kunert *et al.* 2001*a*,*b*, 2009; Drenjancevic-Peric *et al.* 2003, 2004; Marvar *et al.* 2007; Wang *et al.* 2009; Ngo *et al.* 2013) (Fig. 8). This

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pathway also has been implicated in O_2-induced cerebral vasoconstriction in fetal sheep (Ohata *et al.* 2010) and contributes to O_2-induced constriction of retinal arterioles (Zhu *et al.* 1998) produced by increases in inspired P_{O_2}. Importantly, inhibitors of $CYP450_{4A}$ ω-hydroxylase have no effect on the O_2 reactivity of arterioles in the epithelial portion of the hamster cheek pouch (Lombard *et al.* 1999) indicating that the inhibition observed in striated muscle preparations is not simply some non-specific effect. The lack of effect of $CYP450_{4A}$ ω-hydroxylase inhibitors on O_2 reactivity in the epithelial portion of the cheek pouch also further supports the idea that there are regional differences in the mechanism of action of O_2. The K_m for O_2 for formation of 20-HETE by $CYP450_{4A}$ ω-hydroxylase

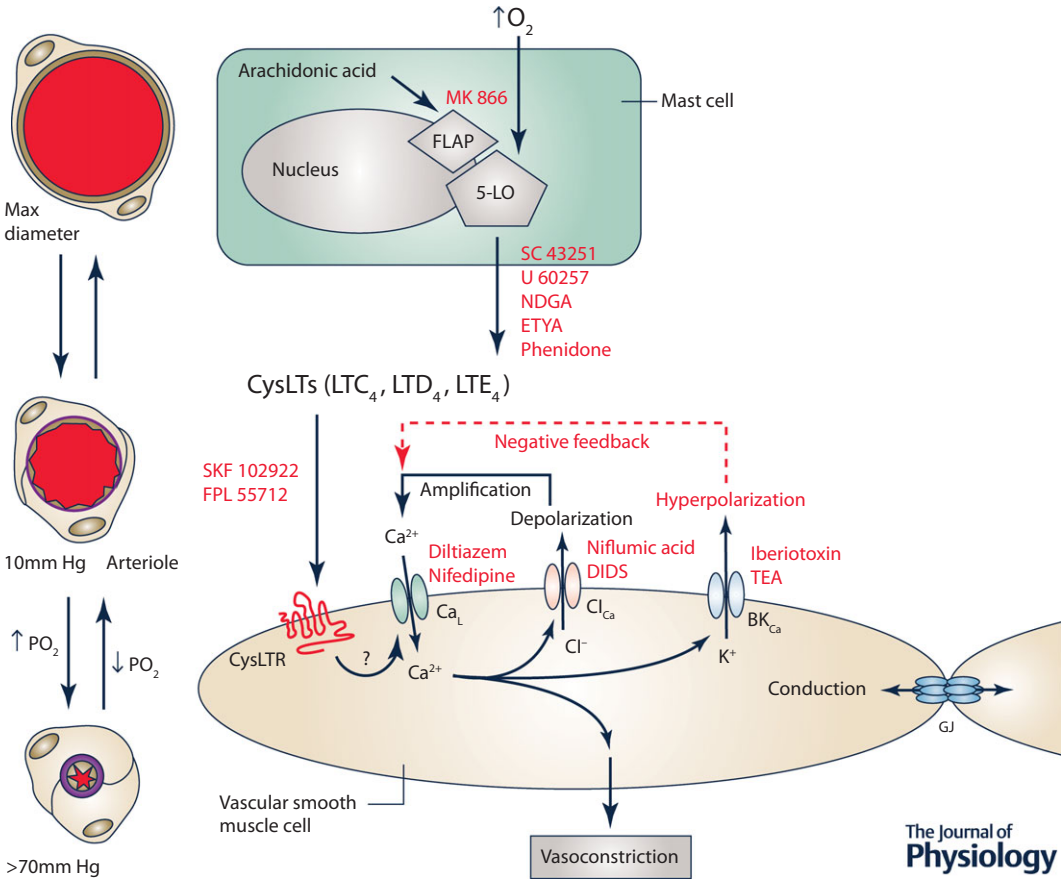

Figure 7. The site and mechanism of action of O_2 in the hamster cheek pouch
Schematic diagram depicting a mast cell (the proposed sensor site in the cheek pouch), a smooth muscle cell replete with receptors for cysteinyl leukotrienes (CysLTRs) and ion channels involved in arteriolar O_2 reactivity in the cheek pouch. Elevated P_{O_2} is sensed by the 5-lipoxygenase (5-LO) in the nuclear membrane of periarteriolar mast cells that decorate arterioles in this tissue (see Fig. 1). This results in conversion of arachidonic acid to cysteinyl leukotrienes (CysLTs) such as LTC_4, LTD_4 and LTE_4 through a process that involves presentation of the arachidonic acid to the 5-LO by the 5-LO-activating protein (FLAP). This process can be inhibited by drugs such as MK 866, that blocks interaction of FLAP with the 5-LO, or SC 43251, U 60257, nordihydroguaiaretic acid (NDGA), eicosatetraynoic acid (ETYA) or phenidone, inhibitors of the 5-LO. The CysLTs then bind to and activate CysLTRs on vascular smooth muscle cells to induce vasoconstriction. CysLTR antagonists such as SKF 102922 or FPL 55712 can inhibit this step in the process. Activation of CysLTRs results in activation of L-type Ca^{2+} channels (Ca_L), Ca^{2+} influx, an increase in intracellular Ca^{2+} and vasoconstriction, which can be antagonized by Ca_L blockers such as diltiazem or nifedipine. The increase in Ca^{2+} activates Ca^{2+}-activated Cl^- channels (Cl_{Ca}). The resulting efflux of Cl^- through these channels causes membrane depolarization, further activating Ca_L and amplifying the initial signal. Blockers of Cl_{Ca} channels such as niflumic acid or DIDs can inhibit this step in the process. The increase in intracellular Ca^{2+} and the membrane depolarization due to activation of Ca_L and Cl_{Ca} activates large conductance, Ca^{2+}-activated K^+ channels (BK_{Ca}). The efflux of K^+ through BK_{Ca} channels blunts the depolarizing effects of activation of Ca_L and Cl_{Ca} providing a degree of negative feedback, and limiting membrane depolarization. This step in the process can be inhibited by iberiotoxin or tetraethylammonium ions (TEA). Oxygen-induced smooth muscle depolarization can be conducted along the vessel wall by gap junctions (GJ) supporting the conduction of O_2-induced vasoconstriction that has been observed experimentally.

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by renal microvessels is in the order of 50 mmHg P_{O_2} (Harder *et al.* 1996), well within the physiological P_{O_2} range experienced by the microcirculation (see Fig. 1).

Studies in rat cremaster muscle have demonstrated expression of $CYP450_{4A}$ ω-hydroxylase in both arteriolar smooth muscle cells and in the surrounding striated muscle (Kunert *et al.* 2001*a*). This observation suggests that O_2 may be sensed either by smooth muscle cells or by striated muscle fibres to mediate arteriolar O_2 reactivity. In first-order arterioles within the cremaster muscle, it appears that striated muscle cells serve as an important source of 20-HETE that is responsible for the bulk of O_2 reactivity in those large arterioles *in situ* (Frisbee & Lombard, 2002). Isolated first-order rat cremaster arterioles studied *ex vivo* were demonstrated to display intrinsic O_2 reactivity that is also mediated, in part, by O_2-dependent production of 20-HETE by the smooth muscle (Frisbee & Lombard, 2002). However, this is not a universal finding as other investigators have failed to demonstrate intrinsic O_2 reactivity in isolated, cannulated first-order arterioles from the rat (Tateishi & Faber, 1995*a*,*b*; Kerkhof *et al.* 1999), as noted above. This is in contrast to the large body of evidence from intravital preparations demonstrating 20-HETE-mediated O_2 reactivity in small arterioles in a variety of striated muscle microvascular preparations (see references above).

Thus, it seems likely that striated muscle fibres and the $CYP450_{4A}$ ω-hydroxylase contained within are the

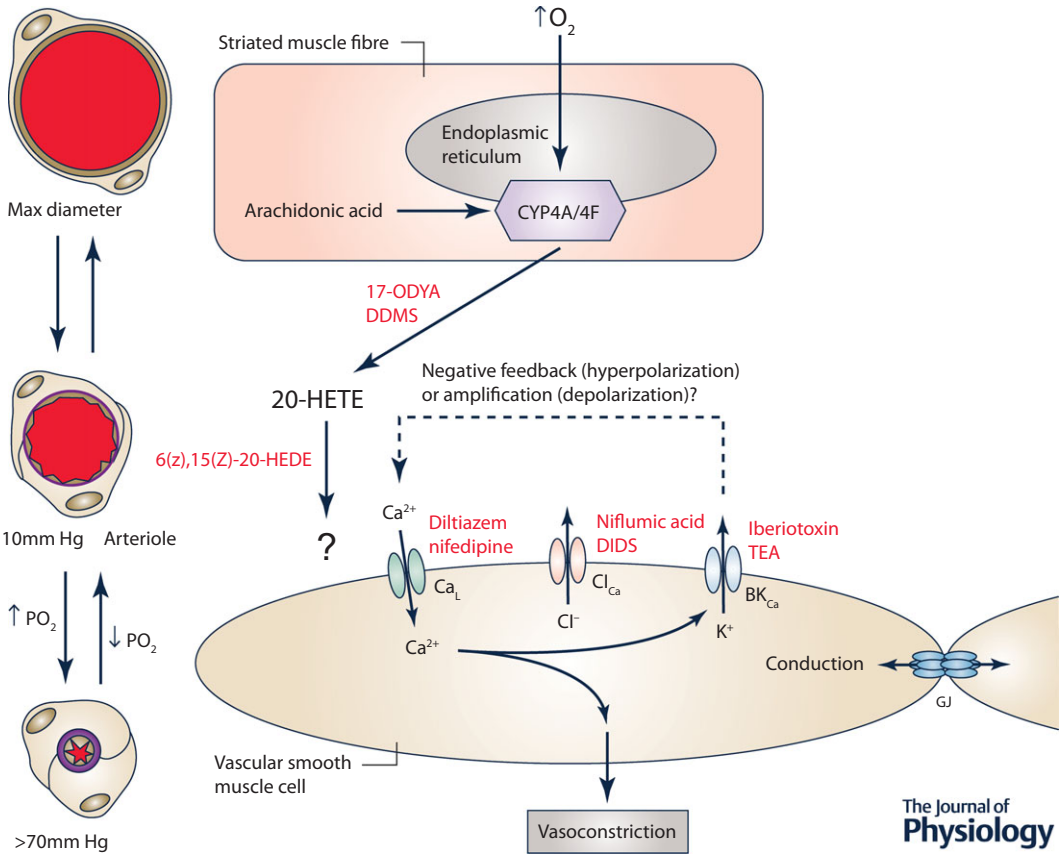

Figure 8. The site and mechanism of action of O_2 in cremaster muscle
Schematic diagram of a striated muscle fibre (the proposed sensor cell in this tissue) and a smooth muscle cell replete with ion channels that may be involved in arteriolar O_2 reactivity in this tissue. Elevated P_{O_2} is sensed by cytochrome $P450_{4A/4F}$ ω-hydroxylase (CYP4A/4F) located in the endoplasmic reticulum of striated muscle fibres, resulting in conversion of arachidonic acid into 20-HETE, a process that can be inhibited by 17-ODYA or DDMS. 20-HETE then acts on smooth muscle cells to induce Ca^{2+} influx through L-type Ca^{2+} channels (Ca_L), an increase in intracellular Ca^{2+} and vasoconstriction. As indicated by the '?' next to the arrow connecting 20-HETE and the smooth muscle cell, the precise receptor for 20-HETE that is responsible for O_2 reactivity is unclear because 20-HETE has been proposed to close large conductance, Ca^{2+}-activated K^+ channels (BK_{Ca}), which would lead to membrane depolarization activating Ca_L. In contrast, other studies suggest that BK_{Ca} serve a negative feedback role as they do in the cheek pouch. As in the cheek pouch it is proposed that O_2-induced depolarization of smooth muscle cells can be conducted along the vessel wall through gap junctions (GJ), consistent with the observed conducted vasoconstriction that has been observed experimentally. 6(*Z*),15(*Z*)-20-HEDE, 20-hydroxy-6*Z*,15*Z*-eicosadienoic acid.

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site where changes in P_{O_2} are primarily sensed, with 20-HETE serving as the mediator linking changes in P_{O_2} with changes in arteriolar tone in the microcirculation of striated muscle. Additional experiments designed to critically test the hypothesis that vascular smooth muscle cell $CYP450_{4A}$ ω-hydroxylase contributes to arteriolar O_2 reactivity *in situ* are needed; perhaps conditional, cell-specific knock-out of this enzyme family in smooth muscle cells might help to resolve this issue. In addition, the location of $CYP450_{4A}$ ω-hydroxylase in striated muscle fibres (and smooth muscle cells) favours O_2 sensing in the muscle fibres rather than capillaries downstream from the arterioles as has been proposed (Ghonaim *et al.* 2011). Additional experiments will be required to positively identify where O_2 is sensed in striated muscle to mediate arteriolar O_2 reactivity.

While there is considerable evidence that $CYP450_{4A}$ ω-hydroxylase serves as the main O_2 sensor mediating arteriolar O_2 reactivity in the microcirculation of striated muscle, this system is not immutable. For example, the $CYP450_{4A}$ ω-hydroxylase antagonist *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) (Kroetz & Xu, 2005) reduces arteriolar O_2 reactivity in cremaster muscles of Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) with established hypertension (Kunert *et al.* 2001*b*). However, DDMS is without significant effect on O_2 reactivity during the development of hypertension in the SHR (Kunert *et al.* 2001*b*). Similarly, DDMS inhibited O_2 reactivity in cremaster arterioles in sham-operated controls and in rats with established Goldblatt hypertension, but was without effect during the early phase of hypertension development in this model (Kunert *et al.* 2009). Spinotrapezius arterioles of Sprague–Dawley rats fed a high salt diet lose $CYP450_{4A}$ ω-hydroxylase-mediated O_2 reactivity, with no change in $CYP450_{4A}$ ω-hydroxylase protein expression (Marvar *et al.* 2007). Conversely, in Dahl salt-sensitive rats, high salt diet increases arteriolar O_2 reactivity and the sensitivity to inhibition by DDMS that is associated with an increased expression of $CYP450_{4A}$ ω-hydroxylase isoforms (Wang *et al.* 2009). These data indicate that the mechanism of action of O_2 on arterioles can change and support the idea that there are multiple mechanisms coupling changes in P_{O_2} with changes in arteriolar diameter. The fact that O_2 reactivity remains but the mechanism of action of O_2 can change suggests that this is an important process that is 'protected' by mechanism redundancy.

As demonstrated by Kerkhof *et al.* (1999), there may be substantial interactions among signalling pathways that complicate interpretation of data based on inhibition of single pathways. While such interactions have been explored in isolated vessels *ex vivo* (Kerkhof *et al.* 1999; Frisbee *et al.* 2001*a*,*b*, 2002), there are no similar studies examining the interaction among all of the putative O_2-related signalling pathways in small arterioles *in situ* in the intact microcirculation.

Ionic basis of arteriolar O_2 reactivity. Despite the differences in chemical mediators of O_2 reactivity between the hamster cheek pouch and striated muscle preparations (CysLTs *vs.* 20-HETE, respectively), a common downstream element in the signalling cascade appears to be activation of L-type Ca^{2+} channels. Blockers of these channels eliminate O_2 reactivity in both the cheek pouch and cremaster muscle models (Welsh *et al.* 1998; Jackson, 2012; Ngo *et al.* 2013) (Figs 7 and 8). However, the signalling pathways leading to activation of these Ca^{2+} channels may be different in the two tissues. In the hamster cheek pouch *in situ*, elevated P_{O_2} causes arteriolar smooth muscle cell depolarization (Welsh *et al.* 1998) and a subsequent increase in intracellular Ca^{2+} (Brekke *et al.* 2006). Consistent with a role for CysLTs in arteriolar O_2 reactivity, we have found that LTD_4 depolarizes smooth muscle cells, constricts isolated, cannulated hamster cheek pouch arterioles and induces conducted vasoconstriction of arterioles in cheek pouches *in situ* (Jackson & Segal, 2005). Blockade of L-type Ca^{2+} channels *in situ* obliterates both O_2-induced constriction and depolarization (Welsh *et al.* 1998). There are at least two, non-mutually exclusive mechanisms that could be responsible for the O_2-dependent depolarization in the cheek pouch and that are consistent with the findings that L-type Ca^{2+} channel blockers prevent both constriction and depolarization of the arteriolar muscle cells (Welsh *et al.* 1998). First, it is possible that the inward current of Ca^{2+} through L-type Ca^{2+} channels causes the depolarization. However, this seems unlikely given the high resting K^+ permeability of vascular muscle cells (Jackson *et al.* 1997, 1998, 2000*c*), the density of voltage-gated K^+ (K_V) and BK_{Ca} channels (which will activate as the membrane is depolarized) (Jackson *et al.* 1997, 1998, 2000*c*), and the small currents through L-type Ca^{2+} channels at physiological membrane potentials and Ca^{2+} gradients (Nelson *et al.* 1990; Gollasch *et al.* 1992). More likely, this depolarizing Ca^{2+} signal must be amplified. Calcium-activated Cl^- (Cl_{Ca}) channels could provide a means of amplifying the Ca^{2+} signal and promoting depolarization. These channels are activated by elevation of intracellular Ca^{2+} and, given the electrochemical gradient for Cl^- ions in most vascular muscle cells, result in Cl^- efflux from the cells and hence depolarization (Large & Wang, 1996). Calcium-activated Cl^- channels mediate depolarization associated with vasoconstrictor-induced contraction of vascular muscle in other systems (Large & Wang, 1996). Preliminary studies showed that O_2-induced constriction and membrane depolarization of smooth muscle cells in hamster cheek pouch arterioles could be reversed by the Cl_{Ca} channel blockers niflumic acid and 4,4′-diisothiocyano2,2′-stilbenedisulfonic acid

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(DIDS) (Jackson, 2000*a*), supporting this hypothesis. Additional research will be needed to fill in the gaps in this signalling pathway and to exclude alternative hypotheses.

The details of the signalling pathway by which elevated P_{O_2} causes constriction of arterioles in striated muscle, between 20-HETE and activation of L-type Ca^{2+} channels, remain unclear (Fig. 8). It has been proposed that O_2-induced constriction of arterioles in rat spinotrapezius muscle involves 20-HETE-dependent closure of BK_{Ca} channels, membrane depolarization and subsequent opening of L-type Ca^{2+} channels (Marvar *et al.* 2007). In preparations exposed to low P_{O_2}, they found that the BK_{Ca} channel blocker, iberiotoxin, produced substantial vasoconstriction that was similar in magnitude to that produced by elevation of P_{O_2} (Marvar *et al.* 2007). Iberiotoxin blunted both 20-HETE- and O_2-induced vasoconstriction, while arteriolar constriction induced by noradrenaline remained intact. Studies of isolated rat gracilis arteries *ex vivo* also support this signalling pathway (Frisbee *et al.* 2001*a*). In contrast, it was previously shown in hamster cremaster muscle that arteriolar BK_{Ca} channels are silent at rest under low P_{O_2} conditions and that inhibition of these channels potentiated, rather than inhibited, O_2-induced vasoconstriction (Jackson, 1998) even though O_2 reactivity in this preparation is also mediated by $CYP450_{4A}$ ω-hydroxylase (Lombard *et al.* 1999). These data suggest that in the hamster microcirculation, BK_{Ca} channels participate in the negative feedback regulation of arteriolar tone and actually blunt O_2-induced vasoconstriction (Jackson & Blair, 1998). Blockade of BK_{Ca} channels also potentiates O_2-induced constriction of hamster cheek pouch arterioles (W. F. Jackson, personal observation) (Fig. 7). Thus, in hamster cremaster muscle and in the cheek pouch, BK_{Ca} channels appear to lie downstream from L-type Ca^{2+} channels in the O_2-dependent signalling cascade, rather than upstream as was proposed by Marvar *et al.* (2007). In addition to inhibiting BK_{Ca} channels (Zou *et al.* 1996), 20-HETE augments currents through L-type Ca^{2+} channels (Gebremedhin *et al.* 1998), possibly through activation of protein kinase C (Lange *et al.* 1997) or tyrosine kinases (Sun *et al.* 1999). In porcine coronary resistance arteries, 20-HETE-induced vasoconstriction appears to involve activation of Rho kinase (Randriamboavonjy *et al.* 2003). Thus, alternative signalling pathways are possible that could reconcile these data. Additional research in which both membrane potential and diameter are measured before and after blockade of L-type Ca^{2+} channels, BK_{Ca} channels and/or $CYP450_{4A}$ ω-hydroxylase in striated muscle arterioles, *in situ*, will be required to resolve these apparent differences. It is also not known if 20-HETE can induce conducted vasoconstriction, as does O_2 in striated muscle arterioles. Nonetheless, as stated above, the observation that O_2-induced vasomotor responses can be conducted in mouse cremaster muscle (Ngo *et al.* 2010; Riemann *et al.* 2011) strongly suggests that changes in arteriolar smooth muscle membrane potential are part of the mechanism of action of O_2 in this tissue and presumably other striated muscle beds.

Summary and conclusions

The location of the cell type responsible for arteriolar O_2 reactivity, *in situ*, remains unclear, because there is regional heterogeneity in the site and mechanism of action of O_2 in the peripheral microcirculation. As a target for future investigation, it is assumed that the models shown in Figs 7 and 8 apply to arteriolar O_2 reactivity over the tissue P_{O_2} range of 10–70 mmHg for tissues such as the hamster cheek pouch (Fig. 7), or striated muscle preparations such as the hamster, rat or mouse cremaster muscles (Fig. 8). Based largely (but not exclusively) on studies in the hamster cheek pouch, it is proposed that changes in P_{O_2} are sensed, extravascularly, by O_2-dependent enzymes located in the membrane of the nucleus or endoplasmic reticulum in parenchymal or other cell types (e.g. mast cells in the case of the hamster cheek pouch). This results in the O_2-dependent production of a vasoconstrictor (CysLTs in hamster cheek pouch or 20-HETE in striated muscle) that acts on arteriolar smooth muscle cells in the wall of arterioles. The vasoconstrictors produce membrane depolarization and activation of L-type, voltage-gated Ca^{2+} channels, leading to an increase in intracellular Ca^{2+} and local vasoconstriction at the site of action of the mediator. The membrane depolarization is conducted along the vessel via gap junctions to produce conducted vasoconstriction along the arteriole allowing for summation and integration of O_2-generated signals throughout the microvascular network. In the hamster cheek pouch, vasoconstrictors that depolarize smooth muscle cells produce conducted constriction transmitted along the smooth muscle cell layer (Welsh & Segal, 1998; Bartlett & Segal, 2000). These data suggest that, in this tissue, O_2-induced vasomotor effects are initiated near smooth muscle-containing arterioles, rather than in downstream capillaries or venules, to provide an arteriolar smooth muscle pathway for conduction of the O_2-initiated response. A similar scenario is assumed in striated muscle with the understanding that the tremendous length of striated muscle fibres (relative to the length of arterioles, capillaries and venules) may also contribute to transmission of O_2-induced signals within a microvascular network. However, it is also recognized that additional research is required to exclude alternatives in the microcirculation of striated muscle. It should also be noted that detailed studies of arteriolar O_2 reactivity have only been studied in rodents (hamsters, mice and rats). Whether similar mechanisms are functional in larger mammals (including humans) remains to be established.

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While there is a plethora of *ex vivo* studies suggesting that smooth muscle cells, endothelial cells, red blood cells or some combination contribute to O_2 reactivity, it is not clear that these *ex vivo* findings accurately represent the arteriolar O_2 reactivity that is consistently observed, *in situ*. This may partly be due to the use of relatively large vessels (small arteries and larger arterioles) for the majority of *ex vivo* studies. Given the regional heterogeneity of mechanisms modulating myogenic tone between arteries and arterioles (Westcott & Jackson, 2011; Westcott *et al.* 2012), it seems likely that different mechanisms, and hence different O_2 sensors and mechanisms, may exist for sensing and responding to changes in P_{O_2} in different segments of the vascular tree. Resistance arteries upstream from the microcirculation may be tuned to respond primarily to changes in blood P_{O_2} (systemic hypoxia/hyperoxia), whereas downstream arterioles may be more tuned to respond to changes in local tissue P_{O_2}.

It is also possible that different mechanisms operate over different P_{O_2} ranges, as suggested by the *ex vivo* studies of Frisbee *et al.* (Frisbee *et al.* 2002). Most studies have examined responses of vessels to relatively large changes in P_{O_2} and have not carefully examined the concentration–response relationship between O_2 and vessel tone. Nor have detailed concentration–response relationships been performed for antagonists and inhibitors to define their efficacy and potency on O_2 reactivity. Thus, when a single concentration of an inhibitor is used and only partial inhibition is observed, is this because there are multiple mechanisms involved, or because of a lack of efficacy of the drug on its target? Interactions among signalling pathways (Kerkhof *et al.* 1999) also complicate the interpretation of inhibitor data. Signalling pathway interactions have not been adequately explored in the O_2 reactivity of small arterioles *in situ*. Conduction of O_2-induced vasomotor responses also complicates the study of arteriolar O_2 reactivity, and should be further examined.

It is clear that there are regional differences in the mechanism of action of O_2 on arterioles in the peripheral microcirculation (e.g. hamster cheek pouch epithelium *vs.* hamster striated muscle). However, there is a remarkably consistent result: elevated P_{O_2} in the environment of small arterioles *in situ* results in profound vasoconstriction. Oxygen-induced arteriolar constriction may be a protective mechanism to defend against oxidative stress and excessive production of O_2-derived reactive oxygen species (ROS) as suggested by Golub and Pittman (Golub & Pittman, 2013). Oxygen-induced arteriolar constriction may be part of a negative feedback mechanism to limit tissue exposure to O_2 to help reduce the oxidative burden that is superimposed on the mechanisms that are involved in the regulation of blood flow to meet the metabolic demands of tissues. That different tissues have different mechanisms (e.g. cheek pouch *vs.* striated muscle) to accomplish the same end-result (O_2-induced arteriolar constriction) suggests that this may be an important response to maintain homeostasis.

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Additional information

Competing interests

None declared.

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