Cytochrome *P*-450 ω-hydroxylase senses O_2 in hamster muscle, but not cheek pouch epithelium, microcirculation

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Lombard, Julian H., Mary Pat Kunert, Richard J. Roman, John R. Falck, David R. Harder, and William F. Jackson. Cytochrome *P*-450 ω-hydroxylase senses O_2 in hamster muscle, but not cheek pouch epithelium, microcirculation. *Am. J. Physiol.* 276 (*Heart Circ. Physiol.* 45): H503–H508, 1999.—The goal of this study was to investigate the role of cytochrome *P*-450 ω-hydroxylase in mediating O_2-induced constriction of arterioles in the microcirculation of the hamster. Male Golden hamsters were anesthetized with pentobarbital sodium, and the cremaster muscle or cheek pouch was prepared for observation by intravital microscopy. Arteriolar diameters were measured during elevations of superfusate P_{O_2} from ~5 to 150 mmHg. Arteriolar responses to elevated P_{O_2} were determined in the cremaster muscle, in the retractor muscle where it inserts on the cheek pouch, and in the epithelial portion of the cheek pouch. Elevation of superfusion solution P_{O_2} caused a vigorous constriction of arterioles in the cremaster and retractor muscles and in the epithelial portion of the cheek pouch. Superfusion with 10 μM 17-octadecynoic acid, a suicide substrate inhibitor of cytochrome *P*-450 ω-hydroxylase, and intravenous infusion of *N*-methylsulfonyl-12,12-dibromododec-11-enamide, a mechanistically different and highly selective inhibitor of cytochrome *P*-450 ω-hydroxylase, caused a significant reduction in the magnitude of O_2-induced constriction of arterioles in the cremaster and retractor muscles. However, arteriolar constriction in response to elevated P_{O_2} was unaffected by 17-octadecynoic acid or *N*-methylsulfonyl-12,12-dibromododec-11-enamide in the epithelial portion of the cheek pouch. These data confirm that there are regional differences in the mechanism of action of O_2 on the microcirculation and indicate that cytochrome *P*-450 ω-hydroxylase senses O_2 in the microcirculation of hamster skeletal muscle, but not in the cheek pouch epithelium.

arterioles; oxygen; 20-hydroxyeicosatetraenoic acid; 17-octadecynoic acid; vasoconstriction

ARTERIOLES IN THE peripheral microcirculation constrict in response to elevations in P_{O_2} (6, 17–23). However, the mechanism by which O_2 produces this effect is controversial. In vitro studies suggest that changes in the release of prostaglandins from the vascular endothelium may mediate the dilation of isolated resistance arteries in response to reduced tissue P_{O_2} (3, 4, 12, 13, 29) and the constriction of isolated first-order arterioles of the cremaster muscle to elevated P_{O_2} (30). However, other mechanisms appear to mediate arteriolar constriction in response to elevated P_{O_2} in situ (18, 36). Similarly, evidence has been presented for (36) and against (22) nitric oxide as a mediator of O_2-mediated changes in vascular tone.

In the hamster cheek pouch, there is considerable evidence that the 5-lipoxygenase senses changes in O_2 and that leukotrienes mediate the constriction of arterioles in response to increased O_2 availability (20, 21, 23). However, a different mechanism appears to mediate O_2-induced constriction of arterioles in the cremaster muscle of the same species (23). Recent studies (17) have suggested that formation of 20-hydroxyeicosatetraenoic acid (20-HETE) by cytochrome *P*-450 4A ω-hydroxylase may be involved in O_2-induced constriction of arterioles in the rat cremaster muscle.

In the present study we tested the hypothesis that cytochrome *P*-450 ω-hydroxylase senses changes in P_{O_2} and that a product of this pathway mediates O_2-induced constriction of hamster skeletal muscle arterioles, but not arterioles in the epithelium of the hamster cheek pouch. To this end, we compared the effects of 17-octadecynoic acid (17-ODYA), a suicide substrate inhibitor of ω-hydroxylases (17, 40), and *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), a water-soluble and highly specific inhibitor of cytochrome *P*-450 ω-hydroxylase that inhibits the formation of 20-HETE without affecting other arachidonic acid metabolites (1, 38), on the changes in the diameter of arterioles in the hamster cremaster muscle, retractor muscle, and cheek pouch epithelium in response to elevations in superfusion solution P_{O_2}. The results of these experiments indicate that cytochrome *P*-450 ω-hydroxylase senses O_2 in the hamster skeletal muscle microcirculation, but not in the epithelial portion of the cheek pouch.

METHODS

Preparation of tissues for intravital microscopy. Male Golden hamsters were anesthetized with pentobarbital sodium (60 mg/kg ip). The trachea was cannulated with polyethylene tubing to ensure a patent airway, and a femoral vein was cannulated for the administration of small supplemental doses of pentobarbital as necessary to maintain anesthesia. The cremaster muscle or a single-layered cheek pouch was prepared for observation by intravital video microscopy, as described previously (17–23, 26). The animal was placed on the stage of a Leitz microscope, and the preparation was transilluminated and monitored with a closed-circuit televi-

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sion system (17, 26). The diameters of third-order arterioles in the cremaster muscle, the retractor muscle at its insertion onto the proximal surface of the cheek pouch, and the epithelial portion of the cheek pouch were measured with a video micrometer (model IV-550, For-A, Tokyo, Japan). The preparations were superfused at 35°C with physiological salt solution (PSS) equilibrated with a 0% O_2-5% CO_2-95% N_2 gas mixture to ensure that all O_2 delivery was via the microcirculation. Under these conditions, the P_{O_2} of the PSS as it flows over the preparation was 3–5 mmHg. The PSS used in these experiments had the following composition (in mM): 131.9 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, and 20.0 $NaHCO_3$. In each experiment the preparation was allowed to equilibrate for 30–60 min under 0% O_2 superfusion before any measurements were performed.

Experimental protocol. After resting tone in the vessels was verified by demonstration of dilation in response to topical application of 10^{-4} M adenosine solution, the response of third-order arterioles to increased O_2 availability was assessed by measuring the constriction of the vessels in response to increases in superfusion solution O_2 content produced by switching from a gas mixture containing 0% O_2 to one containing 21% O_2 (P_{O_2} 140–150 mmHg). Arteriolar responses to elevated superfusion solution P_{O_2} were assessed before and after treatment of the preparations with 10 μM 17-ODYA and before and after treatment of the animal with DDMS.

17-ODYA (Biomol, Plymouth Meeting, PA) was dissolved in 375 μl of 99% ethanol to prepare a 10 mM stock solution and then diluted with PSS to prepare 10 ml of the 10 μM 17-ODYA solution. To administer the 17-ODYA, the superfusion solution was stopped for 30 min after recovery from the first exposure to 21% O_2 superfusion. The preparation was covered with tissue paper to keep the cremaster muscle or cheek pouch moist and to maintain contact with the inhibitor. PSS containing 17-ODYA was added topically to the preparation with a Pasteur pipette over a 30-min period. In a separate series of experiments the vehicle for 17-ODYA was administered in a similar manner. After the topical application of 17-ODYA or its vehicle was completed, the flow of 0% O_2 superfusion solution from the reservoir was restored, and the preparation was allowed to recover for 30 min before the response to 21% O_2 superfusion was tested again.

In the DDMS experiments the superfusion solution was equilibrated with a 0% O_2-5% CO_2-95% N_2 gas mixture for a 1-h equilibration period. Control measurements of arteriolar diameter were obtained each minute for 5 min. The superfusion solution was then equilibrated with a 21% O_2-5% CO_2-74% N_2 gas mixture, and arteriolar diameter and mean arterial pressure were measured each minute for 10 min. The superfusion solution was then equilibrated with a 0% O_2 mixture. After the control measurements were obtained, DDMS was injected as a bolus (6 mg/kg iv), then at 10 mg/kg over 1 h. After the infusion of DDMS, arteriolar diameter and mean arterial blood pressure were measured each minute for a 5-min control period, and then the superfusion solution was equilibrated with a 21% O_2-5% CO_2-74% N_2 gas mixture. Arteriolar diameter and mean arterial pressure were then measured each minute for 10 min.

Statistics. Values are means ± SE. Comparisons were made using a Student's *t*-test when two groups were compared or ANOVA with a subsequent Newman-Keuls test when multiple comparisons were made. All comparisons were performed at the 95% confidence level.

Table 1. *Arteriolar constriction in response to 21% O_2 superfusion in hamster microcirculation*

Vascular Bed	*n*	Resting Diameter, μm	Constriction, μm
Cremaster muscle	19	19.1 ± 0.85	8.7 ± 0.67
Retractor muscle	11	22.4 ± 1.07	10.9 ± 1.19
Cheek pouch epithelium	10	20.9 ± 1.24	7.3 ± 0.58

Values are means ± SE; *n*, number of vessels.

RESULTS

Response of arterioles to elevated superfusion solution P_{O_2}. The constrictor responses of arterioles of the cremaster muscle, retractor muscle, and cheek pouch epithelium to 21% O_2 superfusion are summarized in Table 1. During the control period, arterioles of all three regions exhibited a significant constriction in response to elevated superfusion solution P_{O_2}.

Effect of 17-ODYA on resting diameter and arteriolar responses to elevated superfusion solution P_{O_2} in the cheek pouch and the cremaster muscle. All arterioles studied in these experiments exhibited resting tone, demonstrated by the occurrence of a robust dilation in response to topical addition of 10^{-4} M adenosine solution. 17-ODYA, the suicide substrate inhibitor of cytochrome *P*-450 4A ω-hydroxylase, had no effect on resting diameter of arterioles in the cremaster muscle, retractor muscle, or epithelial portion of the cheek pouch (Table 2). However, superfusion with 17-ODYA inhibited O_2-induced arteriolar constriction in the cremaster muscle (Fig. 1). In the cheek pouch, 17-ODYA inhibited arteriolar constriction in response to elevated P_{O_2} in the retractor muscle but had no effect on O_2-induced constriction of arterioles in the epithelial portion of the cheek pouch (Fig. 1). As previously reported in the rat cremaster muscle (17), 17-ODYA did not prevent norepinephrine (10^{-7} M)-induced constriction of the arterioles in any of the vascular beds, where the arterioles constricted by 16 ± 2 μm (n = 5) in the epithelial portion of the cheek pouch, 14 ± 1 μm (n = 5) in the retractor muscle, and 15 ± 1 μm in the cremaster muscle (n = 5) after treatment with the inhibitor. O_2-induced constriction of arterioles was unaffected by the vehicle for 17-ODYA in any of the tissues (data not shown).

Table 2. *Effect of 17-ODYA and DDMS on resting diameter of arterioles in hamster microcirculation*

	17-ODYA		DDMS	
Vascular Bed	Pre	Post	Pre	Post
Cremaster muscle	19 ± 1.4 (9)	18 ± 1.9 (9)	23 ± 0.9 (6)	20 ± 0.9 (6)
Retractor muscle	25 ± 1.3 (5)	24 ± 1.2 (5)	21 ± 0.9 (6)	24 ± 1.8 (6)
Cheek pouch epithelium	20 ± 2.2 (7)	22 ± 2.2 (7)	19 ± 0.8 (5)	19 ± 0.8 (5)

Values are means ± SE in μm for number of animals in parentheses. 17-ODYA, 17-octadecynoic acid; DDMS, *N*-methylsulfonyl-12,12-dibromododec-11-enamide.

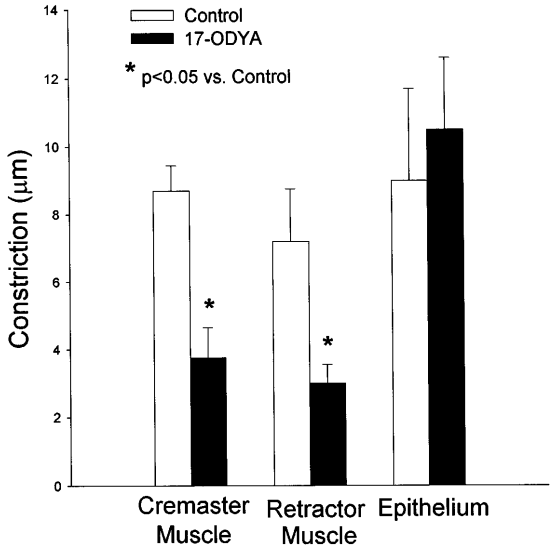

Fig. 1. Effect of 17-octadecynoic acid (17-ODYA) on constriction of arterioles in response to elevation of superfusion solution O_2 concentration from 0 to 21% in hamster cremaster muscle (n = 9), cheek pouch retractor muscle (n = 5), and epithelial portion of hamster cheek pouch (n = 7). Values (means ± SE) are expressed as mean decrease in diameter from control value measured during superfusion in 0% O_2 solution.

Effect of DDMS on resting diameter and arteriolar responses to elevated superfusion solution P_{O_2} in the cheek pouch and cremaster muscle. As with 17-ODYA, DDMS had no effect on the resting diameter of arterioles in the cremaster muscle, retractor muscle, or epithelial portion of the cheek pouch (Table 2). However, consistent with our findings using 17-ODYA, treatment of the animal with DDMS inhibited O_2-induced constriction of arterioles in the cremaster and in cheek pouch retractor muscles but had no effect on arteriolar constriction in response to elevated superfusion solution P_{O_2} in the epithelial portion of the cheek pouch (Fig. 2).

DISCUSSION

Metabolic autoregulatory mechanisms play a major role in regulating tissue blood flow in the peripheral circulation, and arterioles are highly sensitive to changes in O_2 availability (2, 6, 17–23, 25). Most studies of vascular responses to changes in O_2 availability have investigated the mechanisms that mediate the relaxation of blood vessels in response to reduced P_{O_2} (3, 4, 9, 10, 12, 13, 28, 29, 32–35). Some investigators have hypothesized that arteriolar dilation and increased blood flow in response to reduced P_{O_2} are mediated by increases in the levels of vasodilator metabolites, e.g., adenosine, H^+, K^+, and prostaglandins, produced in the parenchymal tissues (2, 16, 25). Other investigators have proposed that the blood vessels are intrinsically sensitive to reduced P_{O_2}, independent of parenchymal cell metabolites. In the latter case, vascular relaxation in response to reduced P_{O_2} may be due to the release of vasodilator substances, e.g., cyclooxygenase metabolites (3, 12, 13, 29, 31, 37) or nitric oxide (15) from the endothelial cells, or to an intrinsic sensitivity of the vascular smooth muscle cells to changes in O_2 availability (5, 14, 27).

In contrast to the vasodilation that occurs in response to reduced P_{O_2}, increasing tissue O_2 delivery elicits a vasoconstrictor response in most vascular beds (6, 17–23, 25). Arteriolar constriction in response to elevated P_{O_2} may also be sensed at the level of the parenchymal cells or in the wall of the vessel. However, the mechanisms that mediate O_2-induced constriction of arterioles may be different from those that mediate arteriolar dilation in response to reduced P_{O_2}. Major questions that remain unanswered are the identity and location of the "sensor" for arteriolar constriction in response to increased P_{O_2} in the microcirculation and the mechanism that couples changes in P_{O_2} to changes in arteriolar diameter.

One widely held version of the metabolic theory of autoregulation holds that O_2-induced constriction of arterioles is mediated through a reduction in the tonic level of vasodilator metabolites in the tissue (16, 25). An alternative hypothesis to explain vasoconstriction in response to elevated P_{O_2} is that the blood vessels or the parenchymal cells produce a vasoconstrictor metabolite in response to increased O_2 availability. An essential criterion for such a metabolite to be the mediator of O_2-induced constriction of arterioles is that its levels must change over a range of P_{O_2} values that are consistent with the changes in P_{O_2} that occur in the tissue during physiological conditions. Until recently, few enzymes had been identified that generate vasoconstrictor substances and have a Michaelis-Menten constant for O_2 that lies within the normal physiological range of blood and tissue P_{O_2}. As a result, support for the constrictor metabolite hypothesis of O_2-induced autoregulatory vasoconstriction has been limited by

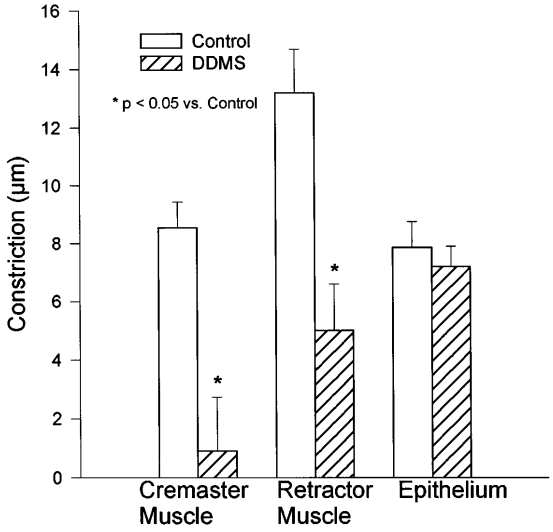

Fig. 2. Effect of *N*-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) on constriction of arterioles in response to elevation of superfusion solution O_2 concentration from 0 to 21% in hamster cremaster muscle (n = 6), cheek pouch retractor muscle (n = 6), and epithelial portion of hamster cheek pouch (n = 5). Values (means ± SE) are expressed as decrease in diameter from control value measured during superfusion with 0% O_2 solution.

the lack of candidate metabolites to mediate the response.

Recent studies (17) have demonstrated that the generation of 20-HETE by cytochrome *P*-450 4A ω-hydroxylase is directly dependent on P_{O_2} in the normal physiological range and that the enzyme that forms 20-HETE is present in rat cremaster muscle homogenates. That study also demonstrated that 20-HETE was made by rat cremaster muscle microsomes and that 17-ODYA inhibits arteriolar constriction in response to increases in superfusion P_{O_2} without affecting resting tone or the vasoconstrictor response to norepinephrine. Taken together, those observations suggested that cytochrome *P*-450 ω-hydroxylase senses changes in P_{O_2} and that 20-HETE is a likely candidate for the vasoconstrictor substance that mediates the local control of blood flow during increased O_2 availability in the rat cremaster muscle.

In the present study we demonstrated that 17-ODYA, a suicide substrate inhibitor of 20-HETE formation (38), also inhibits O_2-induced arteriolar constriction in the hamster cremaster and cheek pouch retractor muscles. The blockade of O_2-induced constriction after exposure to 17-ODYA does not reflect a nonspecific effect of the inhibitor that impairs the ability of arteriolar smooth muscle cells to contract, since we demonstrated in the present experiments and in our earlier study (17) that norepinephrine-induced constriction of arterioles is unaffected by 10 μM 17-ODYA, whereas O_2-induced constriction of the skeletal muscle arterioles was significantly reduced by this inhibitor of cytochrome *P*-450 ω-hydroxylase. More importantly, in the present study, 10 μM 17-ODYA had no effect on O_2-induced constriction of arterioles in the cheek pouch epithelium. Had the effects of the inhibitor been nonspecific, it would likely have affected O_2 reactivity in this tissue as well. The effects of 17-ODYA that we observed also cannot be attributed to the vehicle, since the ethanol vehicle at the same concentration used in the 17-ODYA studies did not affect arteriolar O_2 responses. A role for cytochrome *P*-450 ω-hydroxylase in mediating O_2-induced constriction of arterioles in the hamster skeletal muscle microcirculation in the present study is further supported by our observation that O_2-induced constriction is also blocked by DDMS, a mechanistically different inhibitor of 20-HETE formation that is extremely specific for inhibiting ω-hydroxylation by the cytochrome *P*-450 system (38).

In the hamster microcirculation, elevation of superfusion solution O_2 concentration from 0 to 21% would be expected to increase tissue P_{O_2} from a normal value of ~8–10 mmHg during 0% O_2 superfusion to ~40 mmHg during 21% O_2 superfusion, and P_{O_2} in arterioles of the branching order used in this study would increase from ~28–30 mmHg during 0% O_2 superfusion to 45–50 mmHg during 21% O_2 superfusion (8). This P_{O_2} range is much less than the change in superfusion solution P_{O_2} and would span the physiological range of P_{O_2} values where 20-HETE formation by cytochrome *P*-450 4A ω-hydroxylase is increased by elevated P_{O_2} (17). Thus the present study demonstrates that O_2-induced constriction of arterioles is blocked by two different inhibitors of cytochrome *P*-450 4A ω-hydroxylase in a P_{O_2} range that would be encountered physiologically and that corresponds to the range of P_{O_2} values where 20-HETE formation by this enzyme increases rapidly with elevated P_{O_2} (17).

The finding that inhibition of cytochrome *P*-450 ω-hydroxylase blocks O_2-induced constriction in the microcirculation of hamster skeletal muscle is consistent with the results of our previous studies in rat cremaster muscle (17) and strongly suggests that cytochrome *P*-450 ω-hydroxylase is the O_2 sensor in hamster muscles as well. In contrast, the finding that inhibition of cytochrome *P*-450 ω-hydroxylase does not block O_2-induced constriction of arterioles in the cheek pouch epithelium suggests that mechanisms other than 20-HETE formation by the cytochrome *P*-450 pathway can also mediate O_2-induced constriction in the microcirculation. In the case of the cheek pouch epithelium, this mechanism probably involves the formation of leukotrienes by the lipoxygenase pathway, since Jackson (20, 21, 23) reported that leukotriene antagonists and lipoxygenase inhibitors block O_2-induced constriction in the hamster cheek pouch. However, Jackson (23) noted that this mechanism does not appear to mediate O_2-induced constriction of microvessels in the hamster cremaster muscle, since the application of 5-lipoxygenase inhibitors or leukotriene antagonists had no effect on O_2 reactivity in this vascular bed. The latter finding indicated that there may be regional differences in the mechanisms that mediate O_2-induced constriction of arterioles in various vascular beds and suggested that an unknown substance may mediate arteriolar constriction during exposure to elevated P_{O_2} in the hamster cremaster muscle. The current study extends our earlier findings suggesting that 20-HETE may mediate O_2-induced constriction in rat cremaster muscle (17) and indicates that the previously unidentified substance that mediates O_2-induced constriction in the hamster skeletal muscle microcirculation (23) is a cytochrome *P*-450 ω-hydroxylase metabolite of arachidonic acid.

Clarification of the mechanisms of O_2-induced vasoconstriction in individual vascular beds is crucially important, since the mechanisms of O_2-induced changes in vascular tone can differ among vascular beds and even within the same vessel of different species. For example, dilation of the rat middle cerebral artery in response to reduced P_{O_2} is mediated via an endothelium-dependent release of prostacyclin that activates membrane ATP-dependent K^+ channels (13), whereas hypoxic dilation of the cat middle cerebral artery is mediated via a direct effect on Ca^{2+}-activated K^+ channels in the vascular smooth muscle cells themselves (14). Therefore, it is impossible to make any generalizations regarding the mechanism of O_2-induced changes in vascular tone between one vascular bed and another or even in the same vascular bed of different species. In this respect, the identification of distinctly different mechanisms that mediate regional differences in O_2 response within the vascular beds of a

species (the hamster) that has been widely studied for mechanisms of O_2 response in the microcirculation is novel and important.

The observation that superfusion with 17-ODYA and DDMS blocks O_2-induced arteriolar constriction in the cremaster and retractor muscles, but not in the cheek pouch epithelium, suggests that the neural or parenchymal cell environment may be an important determinant of the expression of O_2-dependent signal transduction pathways that mediate vascular O_2 responses. Previous studies have suggested that the mechanisms controlling arteriolar tone differ in the cheek pouch retractor muscle and epithelium. For example, arterioles of the retractor muscle dilate in response to elevated K^+ concentration, whereas those in the epithelial portion of the cheek pouch constrict (7). Sympathetic control of vascular tone also differs in the muscles and cheek pouch epithelium, since the cremaster (11, 24) and retractor (39) muscles are innervated by adrenergic nerve fibers, whereas adrenergic influences do not appear to contribute to the regulation of active tone in the epithelial portion of the cheek pouch (24, 26). However, if the neural or parenchymal cell environment influences the expression of O_2-related signal transduction pathways, the mechanism by which this occurs remains a matter of speculation.

In summary, we have shown that 17-ODYA and DDMS, which are inhibitors of cytochrome *P*-450 ω-hydroxylase, inhibit arteriolar O_2 reactivity in hamster muscles, but not in the cheek pouch epithelium. These results indicate that cytochrome *P*-450 ω-hydroxylase senses changes in P_{O_2} in hamster muscles and suggest that an ω-hydroxylase product of arachidonic acid, such as 20-HETE, may mediate the arteriolar constriction in response to increased O_2 availability in the hamster cremaster and retractor muscles, but not in the epithelial portion of the cheek pouch.

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