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Role of Endothelium-Derived Prostaglandins in Hypoxia-Elicited Arteriolar Dilation in Rat Skeletal Muscle

Edward J. Messina, Dong Sun, Akos Koller, Michael S. Wolin, and Gabor Kaley

The aims of the present study were to determine the response of rat cremaster muscle first-order arterioles to hypoxia and the role of endothelium-derived prostaglandins in the response. Isolated arterioles were cannulated, pressurized to 65 mm Hg, and studied in a no-flow condition in a bath containing Krebs' bicarbonate solution, pH 7.4, equilibrated with 21% O_2–5% CO_2–74% N_2 (P_{O_2}, 150 mm Hg) or 95% N_2–5% CO_2 (P_{O_2}, 15 mm Hg [hypoxia]). Responses to hypoxia and vasoactive substances were studied before and after removal of the endothelium or blockade of prostaglandin synthesis by the administration of indomethacin (10^{-5} M). Addition to the suffusion solution of arachidonic acid (10^{-7} and 10^{-6} M), prostaglandin E_2 (10^{-9} and 10^{-8} M), acetylcholine (10^{-8} and 10^{-6} M), or sodium nitroprusside (10^{-8} M) evoked significant arteriolar dilation. When the bath P_{O_2} was reduced from 150 to 15 mm Hg, arteriolar diameters increased by 58.8±9.3 μm (61%). Removal of the endothelium completely inhibited responses to hypoxia, acetylcholine, and arachidonic acid, whereas responses to sodium nitroprusside and prostaglandin E_2 remained unaltered. In arterioles with an intact endothelium, indomethacin completely inhibited the responses to hypoxia and arachidonic acid, whereas responses to acetylcholine and sodium nitroprusside were unaltered. These findings support the conclusion that endothelium-derived prostaglandins mediate the arteriolar dilation to hypoxia in rat skeletal muscle arterioles. (*Circulation Research* 1992;71:790–796)

KEY WORDS • endothelium • vascular smooth muscle • hypoxia • oxygen • prostaglandins • cremaster muscle • skeletal muscle arterioles

The contribution of changes in blood or tissue oxygen tension to the local regulation of blood flow has been of interest for over 100 years. Earlier studies have suggested a role for the lack of oxygen in vascular responses such as reactive hyperemia[1] and autoregulation of blood flow.[2] However, changes in oxygen tension in vivo might be acting not only through a direct effect on the blood vessels but also through an indirect effect on tissue metabolism, which can affect changes in blood flow through the generation of local vasoactive metabolites and/or hormones. In vitro studies of isolated blood-perfused skeletal muscle arteries (0.5–1 mm in diameter) indicated a direct effect of decreases in oxygen tension on vascular tone[3] and a greater sensitivity of the smaller (0.5 mm in diameter) compared with the larger arteries to the changes in oxygen tension.[4] Decreases in vascular tone associated with decreases in oxygen tension have also been reported to occur in large vessels such as the aorta,[5] carotid artery,[6] and isolated intestinal[3] and coronary[7,8] arteries. Taken together, these studies indicate that large blood vessels are sensitive to changes in oxygen tension. However, local blood flow regulation is dependent on the responses of small vessels, primarily the arterioles, in the microcirculation.

The direct effects of oxygen and the response of arterioles to changes in oxygen tension are not completely understood. In vivo, arterioles are exposed to oxygen tensions in the range of 0–52 mm Hg.[9,10] Because this is the range of oxygen tensions that induces vascular relaxation of large arteries, it is quite possible that it may also evoke dilation of arterioles. Changes in arteriolar tone to changes in oxygen tension have previously been demonstrated in vivo[11–13]; however, it was not possible in these studies to completely separate the direct effects of oxygen on the arterioles from those on the parenchyma and local metabolism.[11,13]

Mounting evidence indicates that the endothelium is an important local source of powerful vasoconstrictor and vasodilator factors[14] that can affect vascular smooth muscle tone and contribute to local microvascular regulation.[15] Thus, the purpose of the present study was to determine the effects of changes in oxygen tension on isolated arterioles in the absence of parenchymal elements and to test the hypothesis that the arteriolar response (dilation) to decreases in oxygen tension is dependent on the release from the endothelium of vasodilator mediators. Toward this end, we subjected isolated first-order rat cremaster muscle arterioles to changes in oxygen tension before and after either the removal of the endothelium or the administration of indomethacin to inhibit prostaglandin synthesis.

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Materials and Methods

Six-week-old male Wistar rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). The left cremaster muscle was exposed, cleared of adhering fascia, and separated from the scrotal sac. A ventral incision was made to expose and remove the testis and epididymis. The cremaster muscle was excised by a transverse section across its base, as close to the abdominal wall as possible, and placed into a refrigerated dissecting dish containing a cold (0–4°C) MOPS-buffered (pH 7.4) physiological salt solution (PSS) containing (mM) NaCl 145, KCl 5, $CaCl_2$ 2.0, $MgSO_4$ 1, NaH_2PO_4 1, glucose 5.0, pyruvate 2, EDTA 0.02, and MOPS 3.0. The muscle was splayed open as a flat sheet of tissue and pinned to the bottom of the silicone-lined base of the dissecting dish.

First-order arterioles were separated from the adhering skeletal muscle by careful dissection with microscissors. A 1.0–2.0-mm length of isolated arteriole was transferred to a special myograph chamber (Living Systems Instrumentation Inc., Burlington, Vt.) containing a Krebs' bicarbonate-buffered PSS at room temperature and two glass microcannulas (inflow and outflow). The proximal end of the arteriole was mounted to the inflow cannula, and the pressure was increased to 20 mm Hg with a pressure–servo syringe reservoir system (Living Systems), as previously described.[16] The arteriole was perfused for several minutes to clear the lumen of clotted blood. The PSS used to perfuse the arteriole, as well as to suffuse the vessel in the myograph chamber, was a Krebs' bicarbonate-buffered solution equilibrated with 21% O_2–5% CO_2–74% N_2, with a pH of 7.4, containing (mM) NaCl 110, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1, KH_2PO_4 1, glucose 10, $NaHCO_3$ 24, and EDTA 0.02. After the arteriole was cleared, it was attached to the outflow cannula, and perfusion was continued for several minutes to flush the arteriole and cannula. The outflow cannula was then closed, and pressure slowly increased over 20 minutes to 65 mm Hg. This pressure was used for the study of all vessels because it had been ascertained in preliminary experiments that responses to test doses of vasodilator and vasoconstrictor agents produced the maximum response at this level of pressure. Furthermore, this pressure is within the range reported for the arteriole in the intact cremaster muscle of anesthetized rats.[17] Pressure in the arteriole was measured and maintained constant, and arteriolar diameters were measured and recorded with an automatic measuring device, a video dimensional analyzer[16] (Living Systems).

The suffusion system for bathing the arteriole in the myograph had a reservoir volume of 100 ml, and the flow through the myograph chamber, in which the vessel was placed, was 40 ml/min. Temperature of the Krebs' bicarbonate buffered PSS in the myograph chamber was maintained at 33°C. All drugs were added to the reservoir, and final concentrations are reported.

Arterioles were allowed to equilibrate for 30 minutes. During this time, they spontaneously developed tone and achieved resting diameters similar to those observed in vivo.[17,18] Vessels that did not develop tone spontaneously were not used for further study. The internal diameters measured after the equilibration period, when the myogenic tone had stabilized, averaged 85.8±3.2 μm (n=25) and are referred to as control diameters under these experimental conditions. Internal arteriolar diameters, arteriolar pressure, temperature, and the pH of the suffusion fluid were continuously measured and recorded with a Graphtec Multicorder MC6625.

After the equilibration period and when the control diameter had stabilized, arterioles were tested for their capacity to dilate to acetylcholine (ACh, 10^{-6} M), sodium nitroprusside (SNP, 10^{-8} M), prostaglandin E_2 (PGE_2, 10^{-9} M), and arachidonic acid (AA, 10^{-7} M). Vessels incapable of responding to these agents were deemed unsuitable for further study. Arterioles were then subjected to hypoxia by changing the gas from 21% O_2–5% CO_2–74% N_2 to a mixture containing 95% N_2–5% CO_2. This procedure lowered the oxygen tension in the bathing solution from 150 to 15 mm Hg. After the vascular response had reached its maximum, the gas mixture was switched back to 21% oxygen. The arterioles were then subjected to removal of the endothelium by reducing the intraluminal pressure to 20 mm Hg, opening the stopcock on the outflow cannula, and perfusing the arterioles with 2 ml air. After this procedure, the arterioles were perfused for 10–20 minutes at 20–40 mm Hg to permit the flushing of the separated endothelial layer from within the vessel lumen out of the cannula system. The outflow stopcock was then closed, and the pressure was once again increased to 65 mm Hg. The effects of drugs and the responses to hypoxia on vascular diameters were retested. Thus, each arteriole served as its own control. However, all drugs were not studied in every vessel.

At the conclusion of the experiment, arterioles were removed from the cannula and immersed in 10% formalin, stained with hematoxylin and eosin, and prepared for light microscopic examination to verify removal of the endothelial cell layer. Physiological assessment of the removal of the endothelium was determined during the course of the experiment by the absence of a dilator response to ACh (10^{-6} M) and AA (10^{-6} M). In a separate group of arterioles, responses to hypoxia and the vasodilators were studied before and after the administration of indomethacin (IND, 10^{-5} M).

All salts and chemicals were of analytical grade and were obtained from J.T. Baker Chemical Co., Phillipsburg, N.J. ACh, phenylephrine hydrochloride, and SNP were purchased from Sigma Chemical Co., St. Louis, Mo. AA was obtained from Nuchek Prep Inc., Elysian, Minn. PGE_2 was from The Upjohn Co., Kalamazoo, Mich., and IND was from Merck and Co., Rahway, N.J. All concentrations are in molar amounts and refer to the base. Drugs were prepared as previously described.[19]

The data are presented as mean±SEM. The number of vessels studied from separate animals in each protocol is represented by n. Significant differences were determined by Student's t test at $p<0.05$.

Results

The responses of endothelium-dependent dilators AA and ACh and endothelium-independent dilators SNP and PGE_2 are shown before and after removal of the endothelium in a typical experiment depicted in Figure 1. Removal of the endothelium (right tracing)

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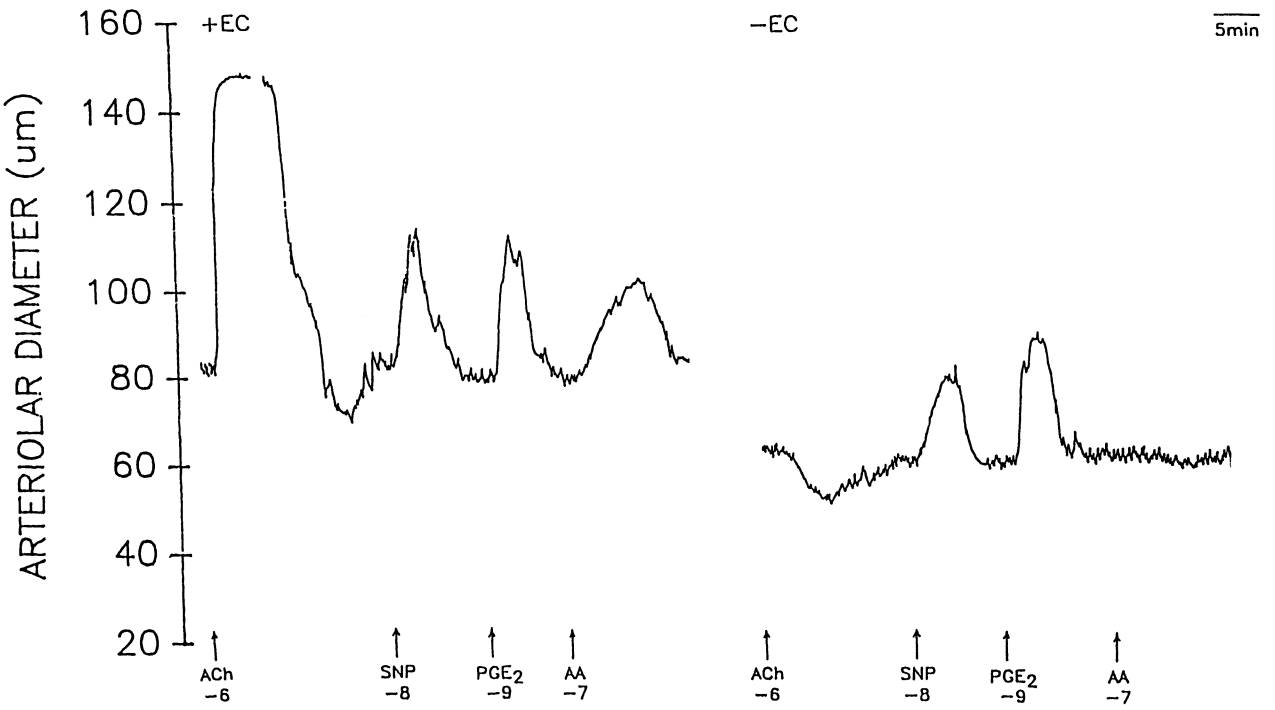

FIGURE 1. *An actual experiment depicting the effects of removal of the endothelium on arteriolar responses to endothelium-dependent dilators acetylcholine (ACh) and arachidonic acid (AA) and endothelium-independent dilators sodium nitroprusside (SNP) and prostaglandin E_2 (PGE_2). +EC, presence of endothelium; −EC, absence of endothelium. Numbers beneath the dilators indicate molar concentrations in powers of 10. Upstrokes in the tracings indicate an increase in diameter (dilation), and downstrokes indicate a decrease in diameter (vasoconstriction) or a return to control diameter. Removal of the endothelium, shown in the right side of the figure, converts the dilation to ACh to a constriction and completely eliminates the response to AA; the responses to SNP and PGE_2 are retained. Removal of the endothelium also decreases the control diameter.*

caused a complete loss in the arteriolar dilator responses to both AA and ACh, whereas responses to PGE_2 and SNP were unaltered. In fact, the dilator response to ACh (10^{-6} M) was completely lost, and a vasoconstrictor response was observed after the removal of the endothelium. Figure 2 summarizes the results of these studies ($n=8$) and shows that responses to ACh and AA were totally eliminated, whereas those to SNP and PGE_2 remained unaltered.

Figure 3 is a recording of a typical experiment depicting the relation between bath oxygen tension and arteriolar diameter. As shown in the top portion of the

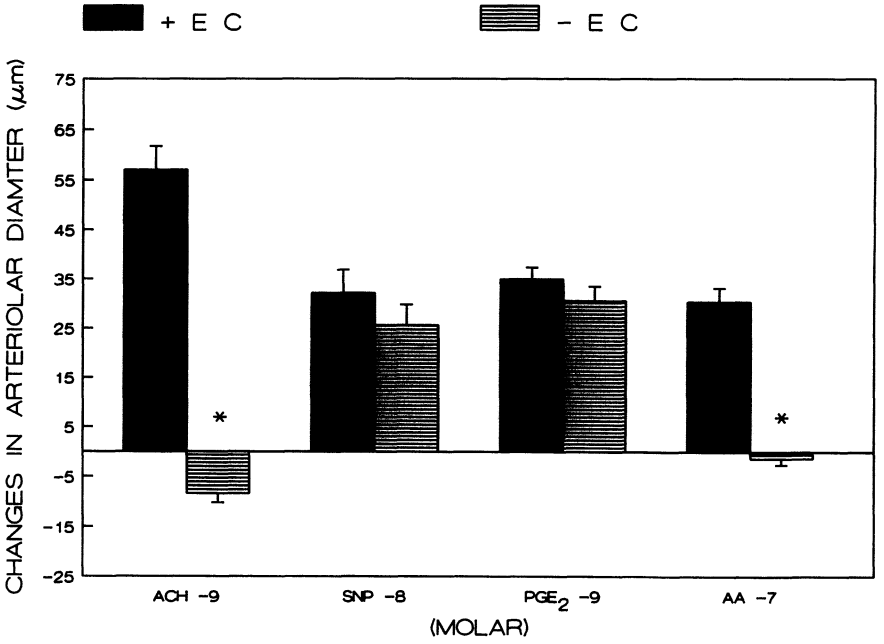

FIGURE 2. *Bar graph of summary data demonstrating the specific loss in arteriolar responses to endothelium-dependent dilators acetylcholine (ACh) and arachidonic acid (AA)* (n=8). *+EC, presence of endothelium; −EC, absence of endothelium; SNP, sodium nitroprusside; PGE_2, prostaglandin E_2. Numbers beside the dilators indicate molar concentrations in powers of 10. Removal of the endothelium (−EC) causes a significant* (*) *loss in the responses to ACh and AA; the responses to SNP and PGE_2, endothelium-independent dilators, are retained.*

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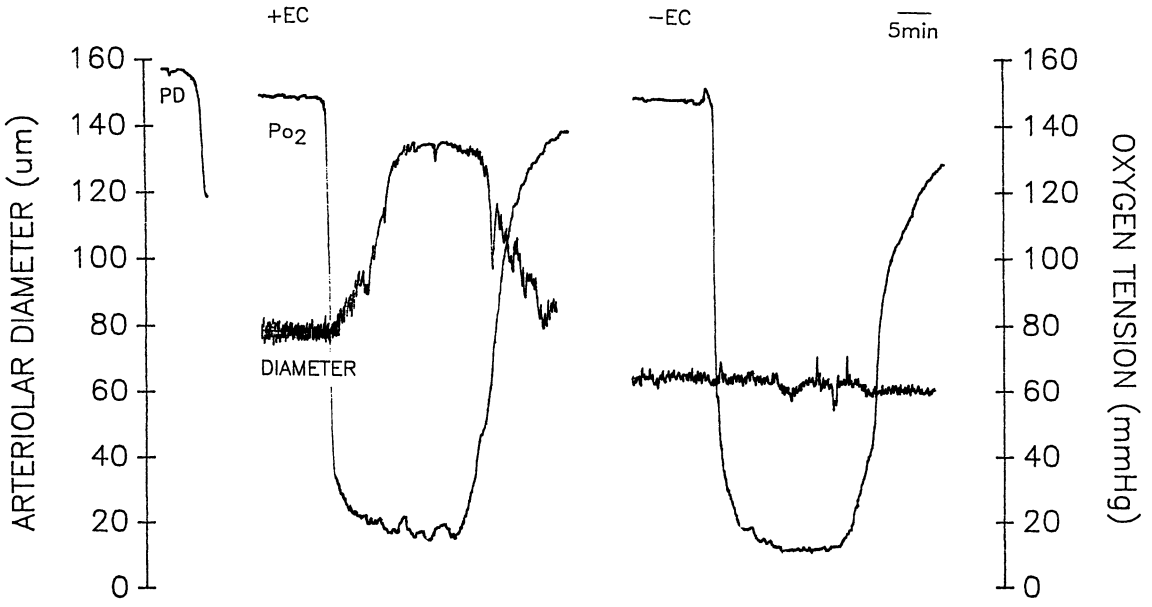

FIGURE 3. *A recording of an actual experiment showing the effects of removal of the endothelium on arteriolar responses to decreases in oxygen tension. +E, presence of endothelium; −E, absence of endothelium; PD, passive diameter. After increasing luminal pressure to 65 mm Hg, the arteriole increased its tone and achieved a control diameter of 78 μm. The arteriole with an intact endothelium (left tracings) responds to the decrease in bath oxygen tension with an increase in diameter. In contrast, the same arteriole after the endothelium has been removed (right tracings) fails to respond to the decrease in bath oxygen tension to 15 mm Hg.*

left tracings of the figure, the arteriole had a passive diameter of approximately 150 μm before tone spontaneously developed, and the diameter decreased 52% to 78 μm (control diameter). When the gas mixture bubbling the bathing solution was changed from 21% oxygen to 95% nitrogen, the oxygen tension decreased from 150 to 15 mm Hg. After a delay of approximately 1 minute, the arteriole, with an intact endothelium (left tracings), began to dilate and in approximately 10 minutes reached a maximum diameter of approximately 137 μm, a 69% increase. In contrast, after removal of the endothelium (right tracings), the same arteriole failed to dilate in response to the decrease in oxygen tension. Figure 4 summarizes the results of 16 such experiments and shows that removal of the endothelium completely eliminated the hypoxia-induced dilation. Endothelial removal also significantly increased tone, as demonstrated by a 14% reduction in control diameter from 85.8±3.2 to 73.5±3.3 μm (Figure 4). Histological examination of arterioles at the end of the experiments revealed an intact endothelium in control vessels, whereas those subjected to perfusion with air showed complete loss of the endothelium.

Responses to the endothelium-dependent dilators AA and ACh and the endothelium-independent dilators SNP and PGE_2 were also studied in arterioles with

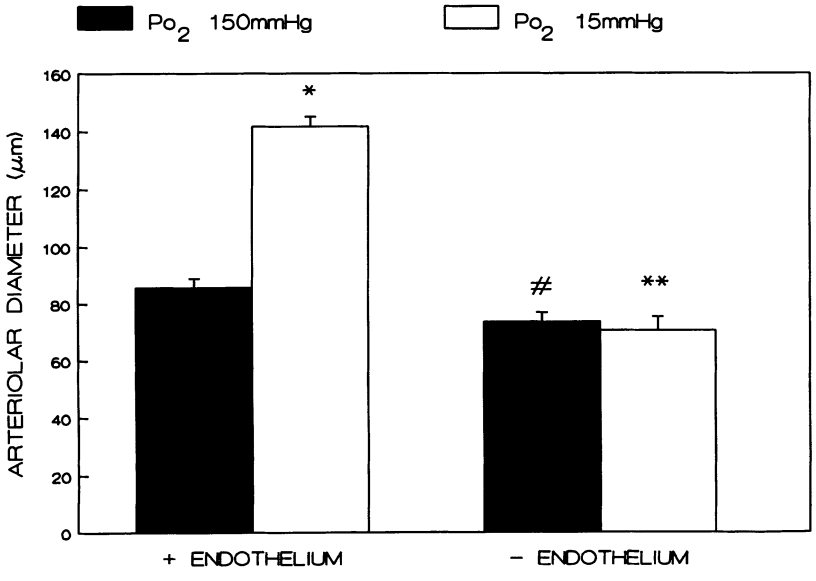

FIGURE 4. *Bar graph of summary data showing the effects of endothelium removal on control diameters and on the arteriolar response to hypoxia (Po_2, 15 mm Hg). +, Presence; −, absence. In arterioles with endothelium, hypoxia produced a significant increase in diameter (*). Removal of the endothelium produced a significant decrease in control diameter during control conditions (#) and elimination of the dilation in response to hypoxia (**) (n=16).*

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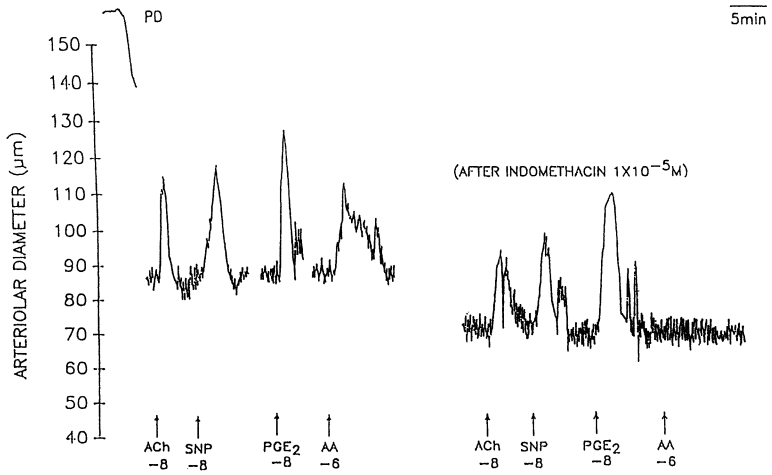

FIGURE 5. *An actual experiment depicting the effects of indomethacin on arteriolar responses to endothelium-dependent dilators acetylcholine (ACh) and arachidonic acid (AA) and endothelium-independent dilators sodium nitroprusside (SNP) and prostaglandin E_2 (PGE_2). PD, passive diameter. Numbers beneath the dilators indicate molar concentrations in powers of 10. Upstrokes in the tracings indicate an increase in diameter (dilation), and downstrokes indicate a decrease in diameter (vasoconstriction) or a return to control diameter. Note the complete inhibition of the arteriolar dilation to AA; the responses to the other agents remain intact.*

an intact endothelium before and after the administration of IND (10^{-5} M), as shown in an actual experiment in Figure 5. IND had no effect on the dilator responses to ACh, SNP, and PGE_2, whereas the response to AA was entirely eliminated. The results of nine experiments are summarized in Figure 6 and demonstrate complete cyclooxygenase blockade by IND, with no significant alterations in dilator responsiveness of arterioles.

The effects of IND on the arteriolar response to hypoxia are demonstrated in the recording of an actual experiment in Figure 7. Before IND (left tracings), hypoxia produced a significant increase in diameter, whereas after IND (right tracings), the response is completely eliminated. Figure 8 summarizes the results from nine experiments depicting the significant increase in arteriolar diameter to hypoxia and the absence of this increase after blockade of prostaglandin synthesis by IND. Although there was a slight reduction in control arteriolar diameter to IND, the change was not significant.

Discussion

The principal findings of this study are that skeletal muscle arterioles dilate in response to hypoxia and that removal of the endothelium or treatment of the arterioles with IND completely eliminates the hypoxia-induced dilation. On the basis of these findings, we have concluded that arteriolar dilation to hypoxia is mediated by endothelium-derived vasodilator prostaglandins and that arteriolar vascular smooth muscle itself is insensitive to changes in oxygen tension in the range of 15–150 mm Hg. To our knowledge, this is the first report of endothelium-dependent responses to hypoxia in isolated skeletal muscle arterioles.

Similar studies have been performed in isolated, perfused segments of the rat tail artery and branches of the canine femoral artery,[20] in which intraluminal and extraluminal P_{O_2} could be controlled and altered independently. A reduction in intraluminal P_{O_2} to 40 mm Hg, without any change in extraluminal oxygen tension (150 mm Hg), evoked an endothelium-dependent increase in diameter (10% in the rat tail artery and 9% in branches of the canine femoral artery). In contrast, reducing extraluminal oxygen tension to 40 mm Hg while maintaining intraluminal tension (150 mm Hg) was without effect.[20] These findings, taken together with ours, indicate that the endothelium may be the oxygen sensor in some blood vessels and that the vascular smooth muscle is insensitive to changes in

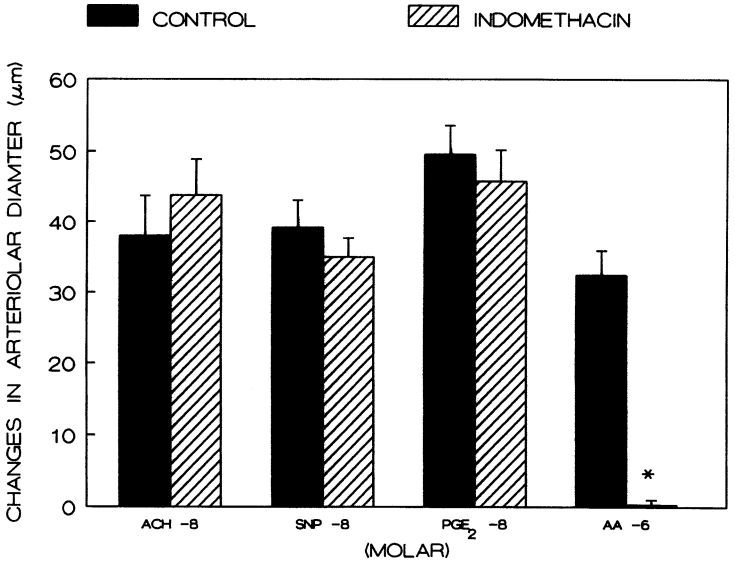

FIGURE 6. *Bar graph of summary data demonstrating the effects of indomethacin (10^{-5} M) on arteriolar dilator responses to acetylcholine (ACh), sodium nitroprusside (SNP), prostaglandin E_2 (PGE_2), and arachidonic acid (AA)* (n=9). *Numbers beside the dilators indicate molar concentrations in powers of 10. Responses to ACh, SNP, and PGE_2 are unaltered; responses to AA are significantly inhibited by indomethacin* (*).

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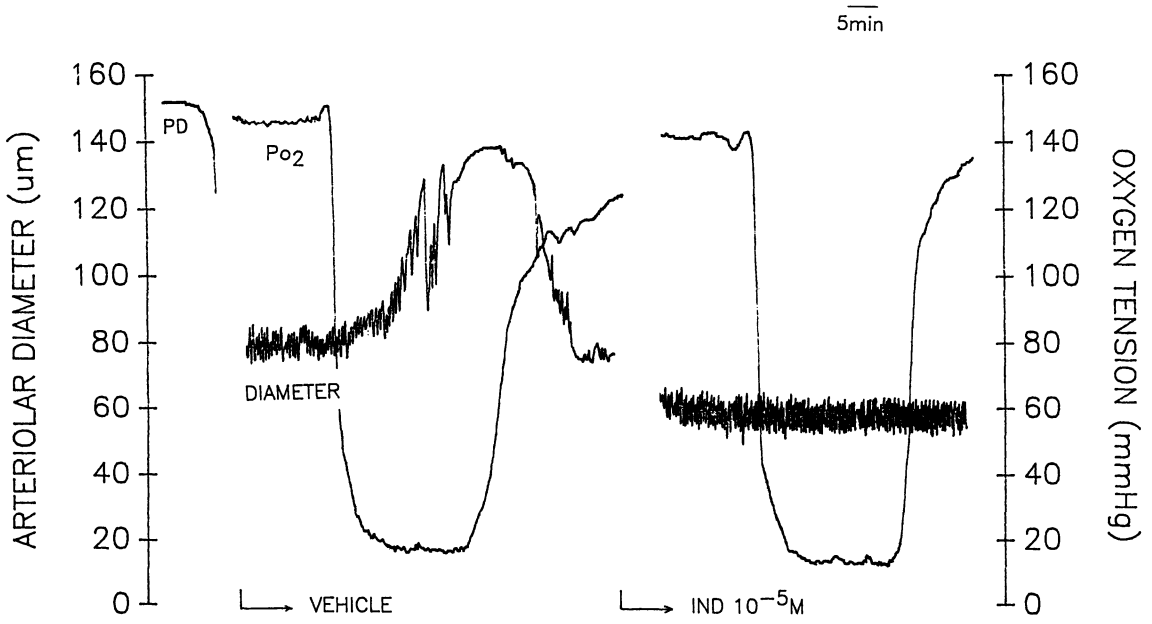

FIGURE 7. *A recording of an actual experiment showing the effects of indomethacin (IND) on arteriolar responses to a decrease in oxygen tension. PD, passive diameter. After increasing luminal pressure to 65 mm Hg, the arteriole increased its tone and achieved a control diameter of 80 μm. Before the administration of IND (left tracings), the arteriole responded to the decrease in bath oxygen tension with an increase in diameter. In contrast, the same arteriole failed to respond to the decrease in bath oxygen tension to 15 mm Hg after IND administration (right tracings).*

oxygen tension. On the other hand, hypoxia-induced dilation was reported to be endothelium independent in rings of canine femoral artery, rabbit thoracic aorta, and lamb ductus arteriosus.[21] What accounts for these differences is unknown at present, but several potential explanations may relate to differences in the function of the blood vessels, in endothelial sensitivity to oxygen of large blood vessels as compared with vessels from the microcirculation, in the capacity of the endothelium to produce prostaglandins, in the quantity and types prostaglandins produced by the endothelium of small and large vessels,[22] and in the differential sensitivity of vascular smooth muscle to dilator prostaglandins. Therefore, it is quite possible that not all vessels demonstrate an endothelium-dependent response to changes in oxygen tension and that vascular smooth muscle may also vary in its response to hypoxia. Furthermore, even within a single arterial tree, such as the canine femoral artery, differences may relate to the size of the branches studied and/or to differences between arterial rings[21] versus perfused segments.[20]

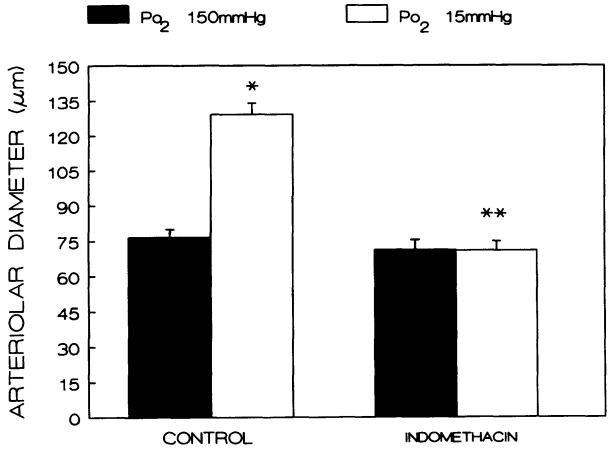

FIGURE 8. *Bar graph of summary data showing the effects of indomethacin on control diameters and the arteriolar response to hypoxia (P_{O_2}, 15 mm Hg). Before the administration of indomethacin, hypoxia produced a significant increase in diameter (*). Indomethacin eliminated the dilation in response to hypoxia (**) (n=9).*

It is generally well recognized that the endothelium of blood vessels can produce a variety of vasoactive factors that can mediate a multiplicity of responses.[14,23] Any of the factors could participate in the mediation of the hypoxia-induced dilation we observed. In the present study, we demonstrated that ACh and AA are endothelium-dependent dilators of isolated first-order rat cremaster arterioles. In previous in vivo studies in the intact rat cremaster muscle microcirculation, we have also shown that third-order arterioles dilate in response to ACh and AA and that the dilations are endothelium-dependent and mediated by endothelium-derived relaxing factor and prostaglandins, respectively.[24–26] Thus, either of these endothelium-derived vasodilator factors could have contributed to the hypoxia-induced dilation. In the present study, removal of the endothelium eliminated the response evoked by hypoxia, indicating that the endothelium is the source of the relaxing factor. Furthermore, indomethacin completely blocked the hypoxia, supporting the conclusion that a product of cyclooxygenase activity (prostaglandins) is the mediator of the dilator response. Consistent with our findings are the results of others who have shown that removal of the endothelium eliminated the hypoxia-induced dilation of perfused arterial segments[20] and that, in several different types of isolated arteries, prostaglandins were re-

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sponsible for hypoxia-elicited relaxation.[8,27,28] Thus, our findings and those of others suggest a role for the endothelium as an oxygen sensor and for prostaglandins as mediators of hypoxia-induced dilation.

To be certain that removal of the endothelium did not alter arteriolar smooth muscle responsiveness nonspecifically, we tested the responses to endothelium-dependent vasodilators (ACh and AA) and endothelium-independent vasodilators (SNP and PGE_2). Removal of the endothelium completely eliminated the responses to the endothelium-dependent vasodilators but did not affect the responses to the endothelium-independent vasodilators.

In this study, removal of the endothelium of isolated first-order arterioles significantly increased their tone, as evidenced by the reduction in control diameter. The decrease in control diameters associated with removal of the endothelium could be explained by the elimination of the production of endothelium-derived relaxing factor or other endothelium-derived vasodilator factors. In this context, in a previous study we observed that N^G-nitro-L-arginine (10^{-3} M), an inhibitor of endothelium-derived relaxing factor/nitric oxide synthesis, significantly reduced control diameters in isolated first-order rat cremaster muscle arterioles.[29] These observations suggest that endothelium-derived relaxing factor has a tonic vasodilator influence; hence, removal of the endothelium might be expected to produce a significant increase in arteriolar tone.

The results of the present study of first-order arterioles indicates that hypoxia stimulates the release of vasodilator prostaglandins. Hypoxia is known to stimulate the release and accumulation of AA,[30] which can lead to the generation of vasodilator prostaglandins that have been reported to mediate the hypoxia-induced relaxation of isolated bovine coronary,[8,28] canine coronary and femoral arteries, and rat tail arteries.[27] In the latter study, radioimmunoassay measurement of prostaglandins in the effluent from these large vessels during hypoxia revealed that the principal prostaglandin released was prostaglandin I_2.[27] The identity of the specific prostaglandins responsible for the mediation of the hypoxia-evoked dilation in microvessels is not known, although previous work from our laboratory indicates that prostaglandin I_2 and PGE_2 are produced in approximately equal amounts by cremasteric arterioles.[22]

In summary, these studies demonstrate that arterioles from skeletal muscle dilate in response to hypoxia and that removal of the endothelium or administration of IND completely eliminates the dilation. We have also shown that arteriolar vascular smooth muscle is insensitive to changes in oxygen tension in the range of 15–150 mm Hg. On the basis of these observations, we conclude that hypoxia stimulates the synthesis of endothelial prostaglandins eliciting dilation of skeletal muscle arterioles in vitro.

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