Influence of Venular Prostaglandin Release on Arteriolar Diameter During Functional Hyperemia

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Abstract—Indomethacin treatment or removal of the venular endothelium will attenuate functional arteriolar vasodilation in the hamster cremaster muscle. We tested the hypothesis that prostaglandin release from venular endothelial cells was responsible for the functional vasodilation of the paired arteriole. The hamster cremaster muscle was prepared for in vivo microscopy and stimulated for 1 minute (10V, 40 μsec, 1 Hz). Before a second muscle stimulation, the venular endothelium was removed by perfusing the venule with several air bubbles. A third muscle stimulation was performed during prostaglandin inhibition (28 μM/L indomethacin superfusion). Arterioles (n=9, 5.5±5 μm) dilated 25±4% during the initial muscle stimulation. After removal of the endothelium from the paired venules, there was no effect on resting arteriolar diameters (53±4 μm), but the functional arteriolar dilation was attenuated to 15±5% (P<0.05). The additional indomethacin treatment had a significant effect on resting diameter (50±4 μm) but did not alter the magnitude of the functional vasodilation (11±4%, P>0.05). In a second set of experiments, the order of the experimental protocol was reversed. Muscle stimulation resulted in a 23±2% increase in diameter (47±2 to 57±2 μm). Indomethacin treatment significantly attenuated the functional dilation to 8±3% (45±2 to 48±2 μm). Arteriolar diameter was significantly smaller after disruption of the venular endothelium with air bubbles (40±2 μm), but there was no effect on the functional vasodilation, 8±3% increase in diameter (to 43±2 μm). These results suggest that the arteriolar dilatory response to muscle stimulation is mediated, in part, by prostaglandin release from the venular endothelium. (Hypertension. 1998;31[part 2]:213-217.)

Key Words: endothelium • prostaglandins • venule • arteriole • hyperemia

Numerous studies have shown a functional role for the arteriole and the venular endothelium in the control of arteriolar diameter and thus the control of blood flow. On the venular side of the circulation, the endothelium has been shown to regulate arteriolar tone in response to changes in flow and in response to changes in oxygen tension. More recently, studies have focused on the role of the venular endothelium in the regulation of arteriolar diameter. On the venular side of the circulation, it has been shown that vasoactive substances can diffuse from the venule to affect the tone of the adjacent arteriole. Falcone and Bohlen have shown that acetylcholine to a venule will cause dilation of its paired arteriole resulting from the release of a venular endothelium-derived relaxing factor. More recently, Boegehold has shown that acute increases in venular shear rate will cause paired arteriolar dilation as the result of a release of a venular endothelial relaxing factor. We have shown that in the absence of an intact endothelium, the functional hyperemic response of the paired arteriole is significantly attenuated, a result suggesting a role for the venular endothelium in the regulation of arteriolar diameter during exercise.

The identity of the diffusible substance released from the venular endothelium that can affect arteriolar diameter has been speculated about. In the study by Falcone and Bohlen, the diffusible substance was most likely nitric oxide, released from the venule in response to the acetylcholine application. In their study, an inhibitor of nitric oxide synthase blocked the arteriolar dilatory response to venular application of acetylcholine. Further, in Boegehold's work, the arteriolar vasodilation that was observed in response to the acute increase in venular shear rate was blocked by Nω-monomethyl-L-arginine (L-NMMA). In this same study by Boegehold, in response to muscle contraction, venular shear rate was comparably increased, and paired arterioles dilated. The functional hyperemia, however, was not blocked by L-NMMA. Similarly, we have found that Nω-nitro-L-arginine methyl ester treatment does not attenuate functional arteriolar dilation of first order arterioles in the hamster cremaster muscle when arteriolar tone is normal. These findings suggest that some venular endothelial-derived factor other than nitric oxide is responsible for paired arteriolar dilation during functional hyperemia. In addition to nitric oxide, cyclooxygenase metabolites can be released from both the arteriolar and venular endothelium. In the present study, we tested the hypothesis that prostaglandins of venular endothelial origin mediate the functional hyperemic response of adjacent arterioles.

To determine the role of venular endothelium-derived prostaglandin release in the functional vasodilation of paired arterioles, we used the paired venular-arteriolar arrangement in the hamster cremaster muscle. Arteriolar diameter response to

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Figure 1. A, The treatment protocol used for the first experiments. Arteriolar diameter was measured during a control period and after 1 minute of muscle stimulation (1). A second stimulation was performed after disruption of the venular endothelium (2), with a third stimulation in the presence of venular endothelial disruption and indomethacin treatment (3). B, The experimental protocol used for the second set of experiments. Arteriolar diameter was measured during a control period and after 1 minute of muscle stimulation (4). A second stimulation was performed after indomethacin treatment (5) with a third stimulation in the presence of indomethacin treatment and after venular endothelial disruption (6).

Muscle contraction was recorded before and after removal of the venular endothelium by using air bubble perfusion (to determine the origin of the prostaglandin release) in combination with indomethacin treatment (to determine the role of prostaglandin release).

Methods

Animal Preparation
The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out according to the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and according to the guidelines of the Animal Welfare Act. Male golden hamsters (120 to 190 g, n=23, Charles River) were anesthetized with 60 mg/kg ip sodium pentobarbital (Butler Company). A catheter was placed in the left jugular vein for a continual infusion of sodium pentobarbital in 0.9% saline solution (5 mg/mL at 0.01 mL/min). Deep esophageal temperature was maintained at 37 to 38°C by convective heating. The trachea was intubated, and the hamster spontaneously breathed 30% oxygen, with a balance of nitrogen to mimic blood gases typical of conscious animals. The cremaster muscle of the hamster was prepared for in vivo microscopy as described previously. The cremaster muscle was spread over a clear Lucite pedestal, and the edges were secured with insect pins. During both the dissection and experimental periods, the cremaster muscle was superfused with a warm physiological salt solution, pH 7.35 at 34°C, of the following concentrations (in mmol/L): 131.9 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, and 20 NaHCO₃. The superfusion solution was equilibrated with gas containing 95% O₂, 5% CO₂ and 95% N₂.

Experimental Measurements
The microcirculation of the cremaster muscle was observed with a Nikon MM-22 microscope fitted with a Leitz 25X long working distance objective (0.38 N.A.). The microscopic image was televised with a Dage closed-circuit television camera and displayed on a Sony monitor. The final magnification of the display was ×1000. Vessel diameter was measured by using a Colorado Video 321 Analyzer modified to function as a video micrometer. By using this device, two movable lines were positioned on the inside walls of the vessel and a DC voltage proportional to the line separation was recorded by using a computerized data collecting system. The resolution of this system is ±1 μm.
Two silver-silver chloride electrodes were placed across the pedicle of the cremaster muscle and were connected to a Grass S44 stimulator. A square-wave pulse (10 V amplitude, 40 μsec duration, 1 Hz frequency) was used to elicit muscle contraction. A 30- to 40-minute recovery period was allowed after the surgery for the vessels to reach a steady-state baseline tone. A first- or second-order arteriole paired with a venule was selected for study, and in all experiments, the diameter of the arteriole was measured immediately after the cessation of a 1-minute period of muscle stimulation.

Reproducibility of Arteriolar Responses to Muscle Stimulation

All of the described experiments involved three stimulations of the hamster cremaster muscle after various perturbations. To verify that three stimulations would not result in significant changes in either resting diameter or vasodilatory responses, we performed a control set of experiments in which we measured arteriolar diameter before and after 1 minute of stimulation of the cremaster muscle three times with each stimulation period separated by 20 minutes. At the end of the final stimulation period, maximal diameter was determined by addition of sodium nitroprusside to the superfusion solution to yield an effective concentration of 100 μmol/L.

Venular Prostaglandin Release and Functional Dilation

We have previously shown that perfusion of venules with air will result in disruption of the venular endothelium. The same procedure was used in the current experiments. Briefly, a micropipette was inserted into the distal portion of the first-order venules, and air was perfused by applying pressure to the back of the micropipette by using a micropipette manipulator (Narishige). Micropipettes were pulled with a vertical pipette puller (Kopf Instrument model 700D) and sharpened to a tip diameter of 8 to 15 μm to facilitate entry of the tip into the venules. A micromanipulator (Narishige) was secured to the stage and was used for positioning the micropipette.

Figure 3. Vasodilation expressed as percent change from the resting diameter. The changes in arteriolar diameter in response to muscle stimulation after the venular air bubble infusion and the subsequent indomethacin treatment were significantly different from the vasodilation after the first stimulation (P < .05). The second and third stimulations were not significantly different from each other.

Figure 4. The vasodilation expressed as percent change from the resting diameter. The changes in arteriolar diameter in response to muscle stimulation after the indomethacin treatment and the subsequent air bubble infusion were significantly different from the vasodilation after the first stimulation (P < .05). The second and third functional vasodilations were not significantly different from each other.
the venular endothelium. At the end of the experiments, arterolar diameter in response to 0.1 mmol/L of adenosine (Sigma Chemical Company) was determined. The animals were then euthanized by an overdose of sodium pentobarbital.

**Analytical and Statistical Methods**

Arterolar diameter was collected at 1 Hz and stored to disk for later analysis using a Gateway 486/33 personal computer equipped with a Metabyte DASH-8 12 bit A/D converter. Statistical significance was determined by using one-way repeated measures of ANOVA with Scheffe's test for multiple comparisons on both the absolute diameters to determine the effect of treatment on resting diameter and on percent changes in arterolar diameter in response to muscle stimulation (GB-Stat, Dynamic Microsystems). All data presented are mean ± SE. A value of \( P < 0.05 \) was considered significant.

**Results**

**Reproducibility of Arteriolar Responses to Muscle Stimulation**

For these experiments, the hamster cremaster muscle was stimulated three times with a 20-minute recovery period between the stimulations. One minute of muscle stimulation resulted in an increase in the arterolar diameter from 44 ± 3 \( \mu \)m to 57 ± 6 \( \mu \)m \((n=5, \text{Fig } 2)\). Arterolar diameter returned to the control values within 5 minutes after cessation of the muscle stimulation. After a 20-minute recovery period, arterolar diameter averaged 47 ± 7 \( \mu \)m, and with muscle stimulation, arterolar diameter increased to 60 ± 5 \( \mu \)m. After a second 20-minute recovery period, arterolar diameter averaged 48 ± 4 \( \mu \)m. A third muscle stimulation resulted in an increase in arterolar diameter to 61 ± 5 \( \mu \)m. There was no difference in the resting diameters before the three stimulations, nor was there a difference in the magnitude of the functional vasodilation. Maximal diameter in response to sodium nitroprusside averaged 67 ± 5 \( \mu \)m.

**Venular Endothelium, Prostaglandin Release, and Functional Dilation**

For the first set of experiments, arterolar diameter averaged 55 ± 5 \( \mu \)m, and 1 minute of muscle stimulation resulted in a 25 ± 4% vasodilation to a diameter of 68 ± 5 \( \mu \)m (Fig 3, n = 9). After disruption of the venular endothelium with air bubbles, there was no change in arterolar diameter (53 ± 4 \( \mu \)m, \( P > 0.05 \)), but the functional vasodilation was significantly attenuated to 15 ± 5% (60 ± 5 \( \mu \)m) in these animals. Addition of indomethacin (28 \( \mu \)mol/L) to the superfusion solution resulted in a slight but significant decrease in the resting arterolar diameter (50 ± 4 \( \mu \)m, \( P < 0.05 \)). Muscle stimulation in the presence of indomethacin resulted in an 11 ± 4% increase in diameter (55 ± 4 \( \mu \)m). This functional dilation was not statistically different from the vasodilation after the endothelial disruption. Arterolar diameter in response to 0.1 mmol/L of adenosine averaged 71 ± 5 \( \mu \)m.

In the next set of experiments, the protocol was similar to the previous set of experiments except that the indomethacin and air bubble treatments were reversed (Fig 4, n = 9). During the control period, arterolar diameter averaged 47 ± 3 \( \mu \)m, and muscle stimulation increased diameter 23 ± 2% (57 ± 2 \( \mu \)m). Indomethacin treatment (28 \( \mu \)mol/L) resulted in no significant change in resting arterolar diameter (45 ± 2 \( \mu \)m) but significantly attenuated the vasodilatory response to muscle stimulation, an 8 ± 3% increase in diameter (48 ± 2 \( \mu \)m, \( P < 0.05 \)). In these animals, subsequent disruption of the venular endothelium during indomethacin treatment resulted in an arterolar diameter of 40 ± 2 \( \mu \)m, significantly less than the initial resting diameter (\( P < 0.05 \)). After venular endothelial disruption in the presence of indomethacin, arterolar diameter in response to muscle stimulation increased by 8 ± 3% (43 ± 2 \( \mu \)m). This response was not statistically different from the vasodilation that was observed after the indomethacin treatment. Arterolar diameter in response to 0.1 mmol/L adenosine averaged 64 ± 3 \( \mu \)m.

**Discussion**

Reports from our laboratory and others have established that the venular blood or the venular endothelium can influence the diameter of adjacent arterioles. This venular-arteriolar communication represents a logical mechanism by which the needs of the metabolically active tissue can be communicated upstream to the resistance vessels.

Studies to examine mechanisms of venular-arteriolar communication have focused on the potential diffusion of substances from the venules to the paired arterioles to cause vasodilation. Hense showed that perfusion of venules with adenosine caused the paired arteriole to vasodilate resulting from the diffusion of adenosine from the venules to the arterioles. More recently, a number of studies have shown that various venular stimuli resulted in arteriolar dilation. In these studies, the focus was on the role of nitric oxide as the diffusible substance released from the venular endothelium that affected arteriolar diameter. Under certain conditions, venular nitric oxide release is indeed responsible for the paired arteriolar vasodilation. In a study by Falcone and Bohlen, acetylcholine application to the venule resulted in dilation of the paired arteriole. This dilation response was blocked by methylene blue, which is known to block nitric oxide-mediated vasodilation. Further, Boegehold showed that acute increases in venular shear rate resulted in arteriolar vasodilation that was blocked by L-NMMA, a result suggesting that nitric oxide released by the endothelium in response to increased shear caused the arteriolar dilation. In contrast, in the same study by Boegehold, paired arteriolar dilation in response to muscle contraction was not prevented by L-NMMA despite comparable increases in venular shear rate. Similarly, studies from our laboratory have suggested that nitric oxide release is not responsible for the functional vasodilation associated with muscle stimulation. Thus, while it would appear that nitric oxide can be a mediator in venular-arteriolar communication, some other diffusible substance must be released in response to muscle contraction.

In previous work in our laboratory, we showed that inhibition of prostaglandin synthesis with indomethacin significantly attenuated functional vasodilation. Further, we have shown a role for an intact venular endothelium in functional vasodilation. Therefore, it is possible that a prostaglandin is released from the venular endothelial cells in response to muscle stimulation. In the present study, we tested the hypothesis that prostaglandins of venular endothelial origin cause paired arteriolar dilation during functional hyperemia. This hypothesis was tested in two ways (Fig 1).

In the first group of experiments, we found that removal of the venular endothelium using air bubble perfusion significantly attenuated the paired arteriolar functional dilation without changing the resting arteriolar diameter. Addition of
superfusate indomethacin appeared to further attenuate functional dilation, however, the difference between the two treatment groups was not statistically significant. These results confirm our earlier finding that the presence of an intact venular endothelium is necessary for full expression of functional vasodilation of paired arterioles. The difference (3 μm) between the functional dilation after the air bubble and after the indomethacin could be the result of a release of prostanoids from the intact venular endothelium present below the level of the air bubble perfusion. In the second group of animals, these results confirmed our earlier observation that functional dilation is mediated in part by prostanoids. Further, as no additional decrease in functional dilation was observed after endothelial denudation, the majority of the prostanoid release appears to be of venular endothelial origin.

In both experiments, there was a significant decrease in arteriolar diameter after both treatments, air bubble perfusion plus indomethacin superfusion or indomethacin superfusion plus air bubble infusion. The reason for the decrease in arteriolar diameter after both treatments is unknown. It is possible that there is a tonic release of some vasodilator other than a prostanoid from the venular endothelium, possibly nitric oxide. Thus, only when prostanoids are blocked and the venular endothelium is removed is there complete loss of vasodilator. Work is continuing in our laboratory to determine the potential relationship between basal prostanoid release, other vasodilators, and arteriolar diameter.

A decrease in reactivity of the paired arterioles in these experiments is ruled out by the fact that addition of adenosine to the tissue caused significant vasodilation of the paired arterioles in the absence of venular endothelium. Further, our control experiments (Fig 2) indicate that there is no decrease in arteriolar responsiveness over the time course of the study.

As reviewed by Lash, the resistance vessels upstream of metabolically active tissue are important in generating the large increases of muscle blood flow that are seen during exercise. Venular-arteriolar communication represents one logical mechanism by which the needs of metabolically active tissue are communicated upstream to the feed vessels. There are a number of potential stimuli that may be involved in this type of communication. Such stimuli could include changes in venular shear rate, changes in metabolic content of the venular blood, and the direct diffusion of tissue metabolites or other stimuli that cause a release of endothelium-derived relaxing factors. In previous work in our laboratory and in work reported by Boegehold, it is evident that more than one endothelium-derived factor can participate in venular-arteriolar communication. As described previously, Boegehold showed that comparable increases in venular shear rate by two different experimental manipulations result in paired arteriolar dilation, but the dilation was evidently the result of two different vasodilators, one being nitric oxide and the other an unidentified substance. Our findings strongly support a role for venular endothelial prostanoid release in paired arteriolar dilation. However, from the study of Boegehold, it would appear that the stimulus for the functional dilation was not an increase in venular shear rate.

As functional hyperemia represents vasodilation due to an increase in tissue metabolism, a potential stimulus for prostanoid release is a change in the oxygen content in and around the venules. Indeed, Lash and Bohlen have shown that while arteriolar oxygen content is relatively unchanged during functional hyperemia, the pvenular oxygen concentration is decreased throughout the hyperemic episode. This decrease in venular oxygen may represent a stimulus to cause the release of venular endothelial-derived prostanoids. In support of this, it has been shown that endothelial cell prostanoid synthesis is increased in endothelial cells under hypoxic conditions, though the time course is different. Future work in our laboratory will focus on the potential for changes in venular oxygen content as a stimulus for venular prostanoid-dependent arteriolar vasodilation during functional hyperemia.

Clearly, there are numerous mechanisms, direct and indirect, that participate in the coordinated response of the vasculature to exercise. In our current experiments, we have shown an important role for the venular endothelium and venular endothelial-derived prostanoid release in the regulation of arteriolar diameter during muscle contraction. Together, these observations suggest that prostanoids released from the venular endothelium in response to an increased flow and/or a decrease in oxygen tension. However, future studies will be needed to fully test this hypothesis.

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