Venular-arteriolar communication in the regulation of blood flow

ROBERT L. HESTER AND LEAH W. HAMMER
Department of Physiology and Biophysics, University of Mississippi
Medical Center, Jackson, Mississippi 39216-4505

Hester, Robert L., and Leah W. Hammer. Venular-arteriolar communication in the regulation of blood flow. Am J Physiol Regulatory Integrative Comp Physiol 282: R1280–R1285, 2002; 10.1152/ajpregu.00744.2001.—Muscle blood flow is regulated to meet the metabolic needs of the tissue. With the vasculature arranged as a successive branching of arterioles and the larger, >50 μm, arterioles providing the major site of resistance, an increasing metabolic demand requires the vasodilation of the small arterioles first then the vasodilation of the more proximal, larger arterioles. The mechanism(s) for the coordination of this ascending vasodilation are not clear and may involve a conducted vasodilation and/or a flow-dependent response. The close arteriolar-venular pairing provides an additional mechanism by which the arteriolar diameter can be increased due to the diffusion of vasoactive substances from the venous blood. Evidence is presented that the venular endothelium releases a relaxing factor, a metabolite of arachidonic acid, that will vasodilate the adjacent arteriole. The stimulus for this release is not known, but it is hypothesized that hypoxia-induced ATP release from red blood cells may be responsible for the stimulation of arachidonic release from the venular endothelial cells. Thus the venous circulation is in an optimal position to monitor the overall metabolic state of the tissue and thus provide a feedback regulation of arteriolar diameter.

vasodilation; venular endothelium; relaxing factor

Tissue blood flow is regulated to meet the metabolic needs of each tissue and can change quite dramatically. Increases in tissue metabolism cause increases in blood flow (functional hyperemia), whereas excess oxygen delivery causes a decrease in blood flow. Blood flow to skeletal or cardiac muscle increases proportionally with increases in metabolic rate to ensure adequate delivery of nutrients and removal of waste products. Although the precise mechanisms governing this close relationship between metabolic state of the tissue and blood flow remain to be elucidated, the anatomical arrangement of arteries and veins may provide a unique mechanism allowing the exchange of information or “cross-talk” between the pre- and postcapillary vessels.

The vasculature is arranged as a series of blood vessels ranging in size from several millimeters to several micrometers, with the largest contribution of total resistance to blood flow coming from the large “feed” arterioles. Thus, to achieve optimal increases in blood flow during periods of increased muscle metabolism, large decreases in resistance of these arterioles must occur. As these upstream or feed vessels are not necessarily in contact with the metabolically active tissue, mechanisms other than or in addition to direct stimulation by tissue metabolites must be important in functional hyperemia. Mechanisms that have been proposed to regulate the diameter of upstream vessels include 1) conducted vasodilation (14), 2) flow-dependent vasodilation (29), and 3) myogenic vasodilation (36). Numerous studies have provided evidence both for and against each of these mechanisms, and there is little doubt that they are all important in control of vascular tone. However, the precise role of these mechanisms in functional hyperemia in directly sensing
requirements for oxygen delivery to the tissue or waste product removal is unclear.

In this review we will present evidence for venular-arteriolar communication in the regulation of cardiovascular function associated with altered muscle metabolism. We propose that in many tissue beds the venous circulation is in an optimal position to monitor the overall metabolic state of the tissue and thus modulate arterial tone. The majority of studies venular-arteriolar communication have addressed the control of blood flow and this will be the major focus of this review. However, additional studies showing a role for venular-arteriolar communication in pathological states will also be discussed.

ANATOMICAL EVIDENCE FOR VENULAR-ARTERIOLAR COMMUNICATION

The vasculature of all tissues is arranged as a series of branching blood vessels. To achieve a maximal increase in tissue blood flow, an increase in diameter of each vessel branch order supplying the tissue is required (12). This is particularly important in the large arterioles, which contribute the major portion of the overall resistance of the vascular bed. Zweifach et al. (51) showed that, in the rat spinotrapezius, ~60% of the pressure drop occurs before the transverse arterioles (diameter 8–30 μm). Similar results have been observed in other tissues (7, 35, 48), although the distribution of resistances in larger, conscious animals has not been determined.

In most tissues, arteries and veins are closely paired, and this paired arrangement persists throughout many branch orders. Although most studies of the microcirculation acknowledge the existence of pairing of arterioles and venules, only a few have suggested a physiological consequence of such an arrangement. In the human heart for instance, there is a very close relationship between arterioles and venules, with almost complete enveloping of the arterioles by the venules in some instances (25). These authors suggest that diffusible substances carried in the venous blood could have a direct effect on the diameter of the arterioles. This study also demonstrated the existence of a coronary venous system in which the veins are not paired with arteries. The authors speculate that these vessels are alternative drainage routes for the blood, which could prevent a rapid washout of the end products of myocardial metabolism, allowing for prolonged arterial vasodilation. In the hamster cremaster muscle, pairing usually persists down to the arterioles, which are two or three generations upstream from the capillaries (20, 38). Depending on the nomenclature used to classify the arterioles of the microcirculation, these smaller paired vessels give rise to the transverse arterioles (38) or unpaired third- or fourth-order arterioles (20). As noted in the previous paragraph, arterioles upstream from the transverse arterioles provide the most resistance to blood flow within a vascular bed and it is these vessels that are tightly paired with venules. This anatomical arrangement would appear to be ideal for venular control of the resistance arterioles.

EVIDENCE OF CHEMICAL COMMUNICATION BETWEEN ARTERIOLES AND VENULES

Arteriolar diameter can be influenced by many factors, including tissue metabolites, endothelium-derived factors, and changes in flow and shear stress. However, in several laboratories, including our own, the focus of research has been to quantify the role of venular-arteriolar communication in control of blood flow, particularly in the microcirculation. Certainly, as described above, the anatomy of the vasculature allows for such a mechanism to exist.

A role for the venous circulation in the regulation of arteriolar diameter can be traced indirectly back to studies by Stainsby and Otis (46) and Granger et al. (18). These studies examined the effect of increases in oxygen consumption on oxygen extraction and blood flow. When initial venous Po2 was high, consistent with a low oxygen extraction, oxygen delivery to the tissue in response to muscle stimulation was increased by an increase in oxygen extraction, with minimal changes in blood flow. However, when the initial venous Po2 was lower than normal, oxygen delivery in response to the same level of muscle stimulation was increased by an increase in blood flow. These results suggest that as venous Po2 decreased, the control of blood flow shifted from the more terminal blood vessels to the more proximal resistance vessels. This could lead to the hypothesis that the contents of the venous blood may be important in the control of arteriolar diameter.

There is little doubt that substances can move between arteries and veins. Studies have shown significant diffusional shunting of soluble gases, including oxygen from arterial to venous blood (13). Several studies have suggested that there could exist diffusional shunting of CO2 from post- to precapillary vessels, resulting in a relaxation of the precapillary vessels and an increase in blood flow (4, 45). Thus indirect evidence supports a role for the control of blood flow by the diffusion of substances from venous to arterial blood.

As the studies described in the previous paragraph presented evidence for the diffusion of substances from the arterioles to the venules, evidence also exists demonstrating the movement of substances from venules to arterioles. This could occur through either diffusion of tissue-generated metabolites from the venous blood or the generation of relaxing factors from the venular endothelium. Tigno and colleagues (47) provided evidence that substances could diffuse from venules to arterioles and alter capillary blood flow. Injection of norepinephrine into precapillary vessels resulted in a decrease in capillary blood flow. The initiation of the arteriolar constriction occurred upstream from the injection site at a point where the venule draining the precapillary vessel into which norepinephrine was injected crossed or ran parallel to the upstream arteriole. This norepinephrine-induced constriction of the up-
stream arteriole was prevented by occlusion of the draining venule, providing evidence that diffusion of vasoactive substances from venules to upstream arterioles was a potential mechanism for the regulation of blood flow.

Studies from our own laboratory provided further evidence that vasoactive metabolites can diffuse from venules to arterioles. We performed a series of experiments to determine whether microinjections of adenosine into striated muscle venules could alter arteriolar diameter at sites proximal to the injection sites. Injection of adenosine into venules resulted in a concentration-dependent increase in the diameter of the adjacent arteriole (22). After it was established that infusions of adenosine into venules resulted in arteriolar dilation, further studies were designed to determine whether increases in tissue levels of metabolites would result in venular uptake of these substances (23). Fluorescein sodium was injected into hamster cremaster muscle near a large venule. After a period of ~10 s, the dye appeared in the venule downstream from the injection site. There was also a radial appearance of fluorescence along the entire length of the venule, and, at a distance of 75 μm from the center of the venule, the peak intensity of fluorescence in the tissue was ~60% of the peak intensity in the venule. When adenosine was added to the fluorescein sodium, arteriolar dilation was observed in the companion arteriole, immediately after the increase in fluorescence in the venule. These studies suggest that the washout of vasoactive metabolites from a tissue may cause upstream arteriolar dilation, resulting in localized increases in blood flow until the excess metabolites are removed from the tissue.

An alternative way in which venules may communicate with arterioles within close proximity is through vasoactive factors released from the venular endothelium. Many studies have shown that arterial endothelial cells release a variety of vasoactive factors, including nitric oxide, prostaglandins, and cytochrome P-450 products (9, 29). Relatively fewer studies have demonstrated release of vasoactive substances from veins and venules.

The first study to demonstrate that vasoactive substances from the venular endothelium could affect arteriolar diameter was by Falcone and Bohlen (16). These authors showed that iontophoresis of ACh into the tissue immediately beside the outer wall of a venule and on the opposite side to the companion arteriole resulted in dilation of the arteriole. When applied the same distance away but on the arteriolar side, ACh had minimal effect on arteriolar dilation. Inhibition of ACh-mediated arteriolar dilation with methylene blue or dithiothreitol indicated that communication between the venule and the arteriole was through release of endothelium-derived nitric oxide from the venule. This study provided the first evidence that substances released from the venular endothelium could influence arteriolar tone.

**PHYSIOLOGICAL ROLE FOR VENULAR-ARTERIOLAR COMMUNICATION**

Although the studies described to date provide evidence, either directly or indirectly, of communication between venules and arterioles, they do not provide clear evidence of a physiological role. The following describes a series of experiments undertaken in our laboratory to establish a physiological role for venular-arteriolar communication. We proposed that the venous circulation is in an optimal position to monitor the metabolic state of the tissue and thus modulate arterial tone. If our hypothesis is correct, then interruptions to venous flow might be expected to block the communication between upstream venules and arterioles. In a study by Saito et al. (42), a silicone stopcock was placed across the distal portion of the hamster cremaster muscle to localize treatment with the metabolic stimulator 2,4-dinitrophenol (DNP). Application of DNP to the distal portion of the cremaster resulted in an increase in metabolic rate of this portion only. This increase in metabolism was associated with an increase in arteriolar diameter of the upstream arteriole in the section of the cremaster proximal to the Silastic dam (non-DNP treatment). Regardless of whether the venule was occluded before or during treatment of the distal portion with DNP, prevention of venular blood flow significantly attenuated the DNP-mediated dilation of the upstream arteriole. These results suggest that factors released from the metabolically active tissue or changes in venular blood chemistry as a result of increases in metabolic activity are detected by the larger, proximal venules and, through an unknown mechanism, result in arteriolar dilation.

To determine whether communication between upstream venules and arterioles was due to diffusion of tissue metabolites or factors released from the venular endothelium, cremaster preparations were field stimulated to induce functional hyperemia (44). Disruption of the venular endothelium was achieved by infusing several air bubbles into the venule adjacent to an arteriole. A second muscle stimulation resulted in a significant decrease in the hyperemic response of the arteriole adjacent to the venule with disrupted endothelium, suggesting that the venular endothelium released a vasodilator in response to muscle stimulation. Arterioles adjacent to venules with intact endothelium (venules in which air did not enter) exhibited a normal hyperemic response during the second electrical stimulation. Thus, although there was no experimental manipulation of the arterioles, we were able to significantly attenuate the arteriolar dilation associated with the muscle stimulation by disrupting the venular endothelium. These results suggest that a substance(s) from the venular endothelium is important for dilation of upstream arterioles during muscle stimulation. It is noteworthy that disruption of the venular endothelium was not associated with a change in control diameter of the arteriole, suggesting that factors from the venular endothelium are not important for maintaining arteriolar tone under resting conditions in this prepara-
tion. These experiments suggested that the venular endothelium is responsible for a portion of the hyperemia in response to an increase in metabolic rate.

It has since been demonstrated, both in isolated venules and in situ, that venular endothelial cells can release nitric oxide in response to increased flow rate. Using rat spinotrapezius muscle, Boegehold (6) demonstrated that occlusion of one branch of an arcade venule bifurcation increased flow and wall shear rate in the venule with a subsequent (10–30 s later) increase in diameter of the adjacent arteriole. Similarly, muscle contraction evoked by electrical stimulation resulted in increased wall shear rate of the arcade venules and caused arteriolar dilation. However, inhibition of nitric oxide synthesis with N^G-nitro-L-arginine was only successful at inhibiting the occlusion-induced arteriolar dilation and not the contraction-induced arteriolar dilation. Although this study suggests that nitric oxide released from venular endothelium can affect arteriolar tone, it does not support a role for nitric oxide in regulating arteriolar diameter during increases in muscle metabolic rate. Indeed, the literature to date fails to provide convincing evidence of such a role for venular nitric oxide in functional hyperemia (26, 43).

Metabolites of arachidonic acid are another group of endothelium-derived vasoactive factors that may participate in venular-arteriolar communication. Three enzyme systems, cyclooxygenase, cytochrome P-450, and lipoxygenase, can metabolize arachidonic acid. The cyclooxygenase and cytochrome P-450 systems in arteriolar endothelium are known to be activated by physiological stimuli such as hypoxia (8, 33, 37), hypercapnia (32), and increases in blood flow (28). Release of arachidonic acid metabolites from venous/venular endothelial cells was also previously described (11, 27).

In an attempt to determine the mechanisms of functional hyperemia, several studies from our laboratory provided indirect evidence for the venular release of arachidonic acid metabolites in response to muscle stimulation. In these studies, functional hyperemia in the hamster cremaster muscle was inhibited by global administration of the cyclooxygenase inhibitor indomethacin (19, 34) or by the phospholipase A2 inhibitor, quinacrine (39), which blocks the liberation of arachidonic acid from the cell membrane. As these inhibitors were applied to the whole cremaster muscle, it is possible that the arachidonic acid metabolites were coming from locations other than the venular endothelium. However, two pieces of evidence from these studies would suggest that at least the cyclooxygenase component of the functional response was dependent on an intact venular endothelium. First, in the study by McKay et al. (34), disruption of the venular endothelium inhibited the stimulation-induced dilation of the arterioles by ~50%. If cyclooxygenase from locations within the tissue other than the venular endothelium were playing a role in the functional hyperemic response, then global administration of indomethacin should further reduce the response to stimulation. No significant differences were observed between the responses to stimulation after the air bubbles and after the addition of indomethacin to the preparation. The second piece of evidence supporting a role for cyclooxygenase of venular origin mediating the functional dilation of arterioles in this preparation comes from the study of Hammer et al. (20). In this study, we demonstrated that the functional dilation of arterioles that were paired with a venule was blocked by indomethacin, whereas indomethacin treatment had no effect on those arterioles without a paired venule. These results support the hypothesis that during increased muscle metabolism, a prostanoid, such as prostacyclin, is released from the venular endothelium, which diffuses to and dilates the adjacent arteriole.

All of the studies discussed thus far demonstrate that it is possible for venules to communicate with arterioles and that this is probably achieved via diffusion of endothelium-derived vasoactive agents. However, to be physiologically relevant, there must be a mechanism by which the venules can “sense” the need for the arterioles to dilate. That is, there must be a stimulus originating in the venules, which initiates the sequence of events leading to arteriolar dilation. Dramatic decreases in venous/venular oxygen levels along with increases in venous/venular carbon dioxide and hydrogen ion levels have been observed during periods of increased muscle metabolism (18, 24, 30, 31). Thus we hypothesize that the venular endothelium senses a change in venous blood chemistry.

Decreases in venous P_O2 and increases in venous P_CO2 or H^+ may have a direct effect on the release of endothelial-derived factors in venules. However, evidence from several studies suggests that an additional signaling pathway is involved. Bergfeld and Forrester (5) demonstrated that human red blood cells release ATP in response to hypoxia and hypercapnia. These results were confirmed by Ellsworth et al. (15) who exposed red blood cells from hamsters to low P_O2 or low pH environments. A more recent study showed that cerebral arterioles isolated from rats dilated in response to low extraluminal oxygen only in the presence of red blood cells (10). This vasodilatory response was accompanied by an increase in ATP in the vessel effluent, suggesting that the red blood cells released ATP when exposed to a low oxygen environment.

It is well established that ATP can bind to the purine P2Y receptor on endothelial cells where it can induce the production of nitric oxide and/or prostacyclin via a guanylate cyclase-dependent mechanism. The vascular actions of ATP have been extensively researched and are reviewed elsewhere (40). We performed a series of studies to test whether increases in venular levels of ATP would result in an endothelium-dependent dilation of adjacent arterioles through a mechanism involving cyclooxygenase metabolites (19). ATP was injected into hamster cremasteric venules, with a subsequent dilation of the adjacent arteriole. This response was blocked by either disruption of the venular endothelium with air bubbles or by global administration of indomethacin. These studies suggest that in-
creases in venular ATP concentrations will result in the release of prostanoids from the venular endothelium, which can then diffuse to the adjacent arteriole to initiate dilation. Whether there is an actual increase in venular ATP levels during increases in metabolic rate remains to be elucidated. Nonetheless, these studies suggest that the red blood cell may be essential for communication between venules and arterioles during periods of increased muscle metabolism.

VENULAR-ARTERIOLAR DIFFUSION DURING PATHOLOGICAL CONDITIONS

There is evidence that the venular endothelium can affect arteriolar diameter during pathological conditions. Although both the arterial and venous endothelial cells are affected by ischemia, there is an enhanced leukocyte adhesion, oxidant production, and increased permeability at the level of the venous circulation (17). Studies by Zamboni et al. (49, 50) showed that ischemia of the gracilis muscle results in a selective vasoconstriction of arterioles adjacent to venules, hypothesized to be due to thromboxane released from platelets adhering to the venular endothelium. Leukocyte adhesion in venules has been shown to increase capillary permeability (3, 21). Thus, in addition to affecting arteriolar blood flow during normal conditions, venular responses can affect arteriolar vascular function during pathological conditions.

Perspectives

A consequence of a paired arteriolar and venular arrangement is that the concentration of a metabolite in the blood draining a tissue may be lower than the actual tissue concentration. Any diffusional movement of vasoactive substances between venules and arterioles will result in a countercurrent shunting. This hypothesis presumes that the vascular wall is permeable to vasoactive metabolite released by the tissue. Computer models predict that there can be a countercurrent diffusion of carbon dioxide out of venules and into arterioles (2, 41). As a result of this countercurrent shunting, CO₂ may not be washed from the tissue until there is a decrease in metabolism. This shunting mechanism may make venous plasma concentrations of any metabolite a low estimator of tissue levels and thus tend to bias any experimental results against a particular metabolite.

The potential diffusion of vasoactive metabolites from venules to arterioles may be important for the chronic control of blood flow. Adair et al. (1) showed that chronic electrical stimulation of the rat extensor digitorum longus resulted in a 50% increase of both arteriolar and venous diameters. There was also a doubling in the overall length of the paired vessels. We could hypothesize that long-term differences between flow and metabolism would result in new growth of blood vessels through the release of a growth factor from the venous side of the circulation.

SUMMARY

It is clear that the venous circulation plays more than a passive role in the regulation of cardiovascular function. Diffusion of vasoactive products, produced either by metabolically active tissue or from the venous blood (i.e., thromboxane from platelets) and the subsequent diffusion of these products to the adjacent arterioles, is one way in which the venous circulation can affect blood flow. However, the production of endothelium-derived relaxing factors by the venular endothelium and the subsequent diffusion of these products to modulate arteriolar diameter provide a mechanism by which the diameter of the more proximal arterioles can be coupled to the metabolic state of the tissue. Whether the venular endothelium senses the state of the venular blood either directly or through the possible release of ATP from red blood cells is still undetermined.

This work was supported by grants from the National Institutes of Health (HL-51971, HL-63958) and the American Heart Association (National Grant-in-Aid and Southeast Affiliate Postdoctoral Fellowship).

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