Arteriovenous Pairing: a Determinant of Capillary Exchange

Norman R. Harris
Department of Bioengineering, Pennsylvania State University, University Park, Pennsylvania 16802

Venuloarteriolar signaling helps mediate microvascular function and dysfunction. Mediators produced at venular sites of inflammation appear to constrict arterioles and increase capillary permeability. In contrast, venules beneficially dilate arterioles to enhance capillary flow according to metabolic demand. These mechanisms are altered with cardiovascular risk factors, contributing to microvascular complications.

Capillary exchange has captured the attention of physiologists throughout the past century. In the early 1900s, Ernest Starling and Eugene Landis pioneered work describing fluid filtration from plasma into tissue. Transvascular filtration provides a continual turnover of the interstitial milieu, a convective flow for large molecules, and a transport mechanism for cells and solute in the lymphatic system. Through the years, investigators of capillary exchange have engaged issues such as mediator regulation of hydraulic conductivity and solute permeability, transvascular pathways of fluid and solute transport, and, recently, the influence of the endothelial glycocalyx on exchange. Despite extensive research, aspects of these issues remain controversial and under continued study. Additional research is justified due to the importance of understanding capillary exchange, both as a vital physiological mechanism and also with respect to pathological consequences of excessive transport.

Capillary perfusion is often studied as a related but also somewhat separate issue from transvascular exchange. One well-known connection between the two is that capillary recruitment is known to influence the surface area for transport. Additionally, other mechanisms appear to concurrently determine both perfusion and filtration. For example, arteriolar dilation not only decreases precapillary resistance that limits capillary flow but also increases the capillary hydrostatic pressure driving filtration. Secondly, enhanced shear forces due to increased flow may alter endothelial permeability through a mechanism that involves nitric oxide (NO). Thirdly, several mediators that affect arteriolar tone, such as histamine, NO, and others, also mediate endothelial permeability. An important mechanism that controls both permeability and perfusion is the topic of this review: arteriovenous communication.

Increased capillary permeability during inflammation

Microvascular permeability is known to increase during an inflammatory response, thereby increasing the rate of fluid and solute transport. Increased venular permeability enhances solute flux, which indirectly increases fluid filtration by osmosis across the endothelial barrier. However, due to the small Starling gradient for fluid transport from venules, most filtration occurs upstream, where hydrostatic pressures are greater. In contrast, even during an inflammatory response, relatively little macromolecular exchange occurs from capillaries and arterioles compared with the higher-permeability venules.

Venular alterations in solute transport may have both leukocyte-dependent and leukocyte-independent components. The leukocyte-dependent component of enhanced permeability is facilitated by leukocyte-endothelial cell interactions. Venules with endothelial cells express adhesion molecules that are found less densely on capillary and arteriolar endothelium. Adhesion is responsible for the leukocyte recruitment that accompanies an inflammatory response; these accumulating cells can beneficially help resolve inflammation but also have been implicated in potentially undesirable increases in permeability. Altered venular permeability is not unexpected given the local accumulation of leukocytes that produce a host of reactive and phagocytic mediators. More difficult to explain, however, is why increases in capillary permeability often accompany the changes in venular permeability, given the scarcity of leukocyte adhesion in these smallest vessels.

Leukocyte-dependent increases in capillary filtration

Despite the fact that leukocytes generally infiltrate tissues through postcapillary venules, upstream capillary permeability is also often leukocyte dependent. We have studied this dependency in several models of inflammation in which leukocyte adherence in postcapillary venules is significantly increased, including platelet-activating factor (PAF) exposure (6–8), inhibition of NO synthase (4), and ischemia-reperfusion (I/R) (5, 9), with the last of these in hypercholesterolemic animals. Each of these studies was performed by using intravital microscopic observation of the rat mesentery, a thin, essentially transparent tissue that allows clear images of the flowing microcirculation.

In one of these studies (4), the mesentery was exposed to the NO synthase inhibitor Nω-nitro-l-arginine methyl ester (l-NAME), and capillary fluid filtration rate (Jv) normalized to surface area (S) was measured with a modified Landis technique. Jv/S increased with l-NAME, despite arteriolar constriction that decreased the hydrostatic pressure driving the filtration, suggesting significantly increased hydraulic conductivity. More-
over, the leukocyte dependency of this response was striking: in untreated animals, $J_{v/S}$ increased by ~70%. However, in animals treated either with an anti-adhesion molecule preventing leukocyte CD11/CD18 from binding endothelial cell ICAM-1 or with antineutrophil serum, $J_{v/S}$ was not only attenuated but decreased significantly compared with baseline values (by ~50%). This partially resolves some controversy regarding the role that NO has on vascular permeability: in this study, NO appears to limit increases in leukocyte-dependent permeability but promotes permeability in the absence of leukocyte adhesion.

Similar results were found in a model of I/R (5, 9). Capillary $J_{v/S}$ increased moderately following I/R in normal rats, with the increase enhanced in hypercholesterolemic rats, in which leukocyte adhesion is more prominent. The increase in $J_{v/S}$ with I/R, as with l-NAME, occurred in spite of decreases in hydrostatic pressure feeding the capillaries, a result of arteriolar constriction. The increase in $J_{v/S}$, therefore, was likely due to an increase in hydraulic conductivity and was found to be leukocyte dependent, with attenuated increases in $J_{v/S}$ occurring in animals treated with either antineutrophil serum or with a P-selectin antibody that inhibited leukocyte rolling on venular endothelium.

**Leukocyte-dependent arteriolar constriction**

A question from our I/R and l-NAME studies remained, however. How is the increase in capillary $J_{v/S}$ dependent on leukocyte adhesion that occurs elsewhere, in the downstream venules? A clue to this question is found in the regulation of arteriolar constriction during I/R. Zamboni et al. (12, 13) observed that some, but not all, arterioles constricted in a model of skeletal I/R. The ones that constricted were physically close (paired) to postcapillary venules, where leukocyte infiltration occurs. Moreover, a CD11/CD18 antibody that inhibited venular leukocyte infiltration also inhibited constriction in these venule-paired arterioles. Another observation from their work was that where a venule and arteriole intersect arteriolar constriction would occur downstream of the intersection in the arteriole, suggesting an overall mechanism whereby venule- and/or leukocyte-derived mediators diffuse to nearby arterioles and become blood borne as they influence arteriolar diameter. We often observe the same phenomenon in inflamed rat mesenteric tissue, as shown in Fig. 1.

**Arteriovenular-dependent capillary filtration**

The finding that venular, leukocyte-derived mediators can initiate an arteriolar response during inflammation opens the possibility that the same signal (which may be a blood-borne mediator) or a different one could reach the capillaries branching from the venule-paired arteriole. We first investigated this possibility in a model of PAF exposure of the rat mesentery (7). We had previously found that capillary $J_{v/S}$ increases following PAF exposure and that the increase could be partially inhibited by either antineutrophil serum or with several treatments aimed at inhibiting leukocyte-endothelial cell adhesion (6, 8). We had also begun to test some initial hypotheses about the mechanism connecting venular leukocyte adhesion and capillary $J_{v/S}$, to no avail. For example, we had hypothesized that venular leukocyte adhesion would increase postcapillary resistance and thereby increase filtration pressure. However, micropressure measurements of feeding arteriolar pressure indicated that this mechanism could only explain a minor fraction of the increase in $J_{v/S}$. We hypothesized two other possibilities: that transient leukocyte-endothelial cell interactions within capillaries increased endothelial permeability or that downstream leukocyte adhesion would initiate an upstream response propagated by gap junctions. However, we did not find evidence for either possibility.

We then developed a parameter to quantify the extent of arteriovenular pairing, with the hypothesis that a closer arrangement of venules with the arteriolar pathway leading to a selected capillary would facilitate delivery of venule-derived permeability factors. This is what we found, with a correlation between the PAF-induced increase in $J_{v/S}$ and the extent of pairing (7).
We followed this work by a more direct hypothesis and by using a different inflammatory challenge (1). We locally administered N-formyl-methionyl-leucyl-phenylalanine (fMLP) through a micropipette at an arteriovenular pairing site in the rat mesentery to stimulate venular leukocyte accumulation. Micropipette delivery limited the area of exposure to the pairing site, so that the selected branching capillary, an ~400-μm away, was not directly exposed to fMLP. Mediators produced by leukocytes at the pairing site were hypothesized to initiate an arteriolar signal that would be delivered to branching capillaries and increase filtration. This protocol was accompanied by two sets of control experiments to verify that the mechanism depended on venuloarteriolar communication. The role of the venule was determined in the first control set, where fMLP was administered only to an unpaired arteriole, to eliminate the possibility that fMLP simply enters the arteriole and is then delivered to the branching capillary to modify filtration. In the second set, fMLP was administered only to an unpaired venule to eliminate the possibility that fMLP-induced leukocyte adhesion increased capillary I/S by an upstream response independent of an arteriole, that is, by increasing hydrostatic pressure or by initiating an upstream response propagated by gap junction communication. The results of the fMLP study were as follows. When fMLP was delivered to sites of arteriovenular pairing, there was an increase in I/S from capillaries branching from the paired arteriole (Fig. 2), supporting the idea that venular leukocytes mediate capillary permeability. No increase in capillary I/S was observed when fMLP was administered to either unpaired arterioles or unpaired venules, and in fact a decrease in capillary I/S resulted when fMLP was administered to unpaired arterioles, likely due to the resulting arteriolar constriction. The increase in fMLP-induced capillary I/S appeared to be correlated to the number of infiltrating leukocytes at the arteriovenular pairing site (1). Therefore, with both fMLP and PAF exposure, and possibly many other models of inflammation, the mechanism of increased capillary filtration appears to involve communication whereby venular leukocytes initiate a signal received by arterioles and delivered to branching capillaries. However, applying this mechanism to other inflammatory conditions leads to the following scenario: in several models of inflammation (for example, NO synthase inhibition and I/R), venuloarteriolar signaling also results in arteriolar constriction that limits downstream capillary filtration. Therefore, even though capillaries branching from highly paired arteriolar pathways are more likely to have increased permeability during an inflammatory response, the same capillaries are likely to experience the largest decreases in hydrostatic pressure driving filtration. Therefore, to completely understand how arteriovenular pairing affects capillary exchange, it is necessary to understand venular control of arteriolar tone.

### Venular control of capillary perfusion

Most microvascular beds in the body have a common structural arrangement of a feeding arteriole in close, countercurrent pairing with a venule leaving the same capillary bed (Fig. 3). This pairing structure has been noted to provide arteriovenular transport of heat, oxygen, and carbon dioxide and more recently has been noted to help control arteriolar tone. However, before a discussion of diffusional arteriovenular signaling, it should be mentioned that not all venuloarteriolar communication requires close pairing. Collins et al. (2) have determined that venules exposed to adenosine triphosphate send a vasoactive signal to upstream arterioles through the capillaries, most likely by gap junctional communication conducted along the vascular wall. A recent review by Hester and Hammer (10) summarizes much of the work that has investigated venular control of arteriolar tone through the closely paired countercurrent arrangement. Among the initial findings of this mechanism was that venules could dilate closely paired arterioles by a mechanism involving endothelial-derived relaxing factors. Hester's group has also investigated a mechanism whereby the venous circulation might monitor the metabolic state of the tissue and adjust arterial tone accordingly. Studies from their lab indicate that functional hyperemia is facilitated by a venular release of arachidonic acid metabolites (possibly including prostacyclin) that dilate nearby arterioles. Another key finding from their lab, in resting baseline conditions, is that venule-paired third-order arterioles have significantly larger diameters than unpaired arterioles of the same order (3). With this evidence, it could be hypothesized that capillaries branching from highly paired arteriolar pathways normally would have greater flow due to lower precapillary resistance. However, this possibility had not been tested, to our knowledge, before our recent studies (5, 11). Consistent with this hypothesis, we found that baseline capillary red blood cell velocity ($V_{RBC}$) in the rat mesentery is highly correlated with the extent of arteriovenular pairing (5, 11). The same was found to be true for baseline capillary I/S (5): filtration was highest in capillaries branching from arteriolar pathways that were closely paired with postcapillary venules. Figure 4 demonstrates that the mechanism for perfusion appears to be NO dependent. A significant correlation
between $V_{\text{RBC}}$ and arteriovenular pairing was observed during a baseline period before the introduction of L-NAME (11). However, following 20 min of L-NAME exposure, the correlation not only decreased but also became negative. Much of the scatter that is present in the two correlations can be eliminated by calculating the change in velocity ($V_{\text{RBC, L-NAME}} - V_{\text{RBC, baseline}}$) and correlating with arteriovenular pairing. Figure 4 demonstrates that the capillaries affected most by NO synthase inhibition were the ones that branched from highly venule-paired arteriolar pathways: when the extent of pairing was low, L-NAME had a negligible effect on capillary flow.

These results are consistent with a hypothesis that NO is involved in a mechanism whereby venules dilate nearby arterioles, allowing enhanced capillary flow and exchange. [However, it should be mentioned that arachidonic acid metabolites might be the primary venule-derived mediators during functional hyperemia (10)]. Inasmuch as basal control of arteriolar diameter may require NO in our studies, a question that we have been addressing is whether the mechanism is intact in the presence of cardiovascular risk factors. Conditions such as aging, diabetes, and hypercholesterolemia share a common deficiency in NO-mediated vasodilation. Therefore, venule-dependent capillary function, which appears to have a NO-dependent component, may also be adversely affected. In preliminary experiments in both diabetic and aging rats, we have found this hypothesis to be confirmed. In hypercholesterolemic rats, we have performed more extensive experiments that not only demonstrate the dysfunction in venular control of capillary function but also show that this dysfunction can be reversed.

In one study of hypercholesterolemia (5), we found that both capillary filtration and perfusion in the rat mesentery demonstrated tendencies toward negative correlations with arteriovenular pairing, similar to that observed with L-NAME exposure in normcholesterolemic rats (Fig. 4). We followed this study with an attempt to restore the desired venular communication that normally enhances capillary function (11). In one group of hypercholesterolemic rats, we supplemented drinking water with L-arginine to augment NO production. This treatment demonstrated moderate success, with the relationship between $V_{\text{RBC}}$ and arteriovenular pairing improving from a slightly negative correlation to a significantly positive one. However, the slope of the correlation obtained with L-arginine was still only one-half that observed in normocholesterolemic rats. We next turned to one possible cause of decreased NO in the microvasculature, that is accumula-
leukocytes that produce reactive oxygen species. With an injection of antineutrophil serum, we reduced the number of circulating neutrophils (and also the total number of leukocytes) to one-third the value normally found, then measured and correlated capillary VRBC as a function of arteriovenular pairing (11). This treatment was highly effective in preventing arteriolar constriction, increasing capillary perfusion, and restoring the correlation between perfusion and venular pairing to nearly the same as that observed in normocholesterolemic rats. We are optimistic that similar protocols may be found to be effective with other cardiovascular risk factors.

Summary

In conclusion, capillary filtration and perfusion appear to be dependent on communication between venules and closely paired arterioles. In a rat mesenteric model, this communication is altered with inflammation: venular accumulation of leukocytes increases capillary filtration through an arteriolar signaling pathway while at the same time constricting these arterioles to limit capillary perfusion. Restricted capillary flow in the mesentery also accompanies the inflammation present with cardiovascular risk factors such as hypercholesterolemia; however, treatments aimed at restoring arteriovenular communication in this model are successful in improving capillary perfusion.

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References