Propagation of Vasodilation in Resistance Vessels of the Hamster: Development and Review of a Working Hypothesis

Steven S. Segal and Brian R. Duling

In many tissues, a substantial fraction of total vascular resistance resides in the feed arteries that give rise to the microcirculation. We have explored the thesis that control of tissue blood flow is integrated over several levels of the vascular network, including feed arteries and microvessels. In response to muscular contraction, feed arteries (resting diameter 100–125 μm) of hamster cremaster and gracilis muscles dilated by 20–25%. Acetylcholine applied to distal microvessels of the cremaster induced a dilation that ascended into feed arteries not having direct contact with acetylcholine. In the hamster cheek pouch, isomorphotropic application of acetylcholine onto an arteriole (diameter 20–30 μm) triggered a vasodilation that propagated along the arteriole. Propagation was not dependent on blood flow, indicating that the dilator response was conducted along the vessel wall. We found that preventing diameter changes in an arteriole segment along the apparent conducting pathway did not block propagated vasodilation, indicating that propagation was not mediated by a myogenic mechanism requiring changes in smooth muscle length. We investigated whether the conduction of a vasodilatory stimulus may be mediated by either a neural plexus intrinsic to microvessels or cell-cell coupling between the cells composing the arteriole. Tetrodotoxin (10−6 M) did not block propagated vasodilation, indicating that propagation was not mediated by a neural pathway. Hypertonic sucrose solution applied to an arteriole segment along the apparent conducting pathway attenuated propagation significantly, which is consistent with its reported effect to decouple gap junctions between cells. Thus, propagated vasodilation in arterioles may be mediated by direct cell-cell conduction. (Circulation Research 1987;61(suppl II):II-20–II-25)

Classic views maintain that the resistance to blood flow and the control of tissue perfusion resides in the arteriolar segments of the vascular bed (i.e., vessel diameter <60 μm). However, intravascular pressure measurements in many tissues, including the heart and brain, indicate that a major fraction of total vascular resistance resides in the larger arteriolar vessels that give rise to the microcirculation.1,2,3 It can be deduced that if the resistance of feed arteries remained fixed at 50% of total resistance, then maximal dilation of microvessels could result in no more than a twofold increase in tissue blood flow (Figure 1). The fact that many tissues manifest more than a twofold increase in blood flow indicates that coordination of feed artery dilation with that of arterioles may be a general component of functional hyperemia.

The present study is one of a series4,5 exploring various aspects of the thesis that the control of tissue perfusion involves an interplay among resistance segments at several levels of the vascular network. Our initial experiments tested and supported the hypothesis that the feed arteries supplying striated muscle participate in, and are essential to, the hyperemic response to muscular contraction.1 Subsequent experiments have been aimed at understanding the mechanism(s) for coordinating vasodilation in the resistance network as mediated through propagation of a vasomotor response. In the microcirculation, we have shown that propagated vasodilation occurs in the absence of flow, indicating that a vasodilatory stimulus can be conducted along the vessel wall.4 Our subsequent efforts have focused on defining the mechanism of conduction.

Materials and Methods

Feed Arteries

The preparation of feed arteries of the hamster cremaster and gracilis muscle for observation in situ has been described in detail.3 Briefly, hamsters were anesthetized with sodium pentobarbital (70 mg/kg i.p.), the trachea was intubated to ensure a patent airway, and either a jugular or femoral vein was cannulated for maintenance of anesthesia and continuous replacement of respiratory fluid loss. For the cremaster muscle, after preparing the microcirculation for observation in the standard manner, the incision was continued into the abdomen, viscera were retracted, and the feed arteries exposed. For the gracilis muscle, the skin over the tissue was opened, and the feed arteries, which enter the undersurface of the muscle, were exposed by gently retracting the anterior border of the muscle. Cremaster and gracilis muscle preparations were superfused continuously with bicarbonate-buffered physiologic salt solutions (PSS; pH 7.4) maintained at...
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35° and 37° C, respectively. Feed arteries were observed with reflected light using videomicroscopy.

The magnitude of resting tone in feed arteries supplying the cremaster and gracilis muscle was evaluated by recording vasomotor responses to topical application of supramaximal doses of acetylcholine chloride (ACh; Sigma Chemical Co., St. Louis, Mo.). To determine whether feed arteries participated in functional hyperemia, contraction of either muscle was induced by stimulating the respective motor nerve (8 V/30 sec/8 Hz), and vasomotor responses were observed. In cremaster preparations, the effect of supramaximal doses of ACh applied to distal microvessels on the diameter of proximal feed vessels was also examined. The data for control and response diameters of cremaster and gracilis muscles to these experimental interventions have been published; the significance of these observations with respect to flow control is summarized below.

Microvessels

The cheek pouch of anesthetized hamsters was exteriorized for observation with videomicroscopy as described previously. Details of ACh iontophoresis onto cheek pouch arterioles have been reported. Briefly, glass micropipettes were fabricated with internal tip diameters of 2 μm and were filled with 1.0 M ACh dissolved in distilled water. An ACh micropipette was positioned adjacent to an arteriole with a Leitz micromanipulator, and the ACh was ejected using electrical pulses (typically 1.000 nA/200 msec) via an iontophoresis programmer (model 160, World Precision Instruments, New Haven, Conn.). Arteriolar diameter responses were recorded at the pipette tip and at selected distances along the vessel from the ACh pipette (Figure 2). To control for ACh diffusion, identical ACh pulses were delivered in the parenchymal tissue at distances from the arteriole equivalent to those studied for propagation. To control for nonspecific effects of iontophoresis, sodium chloride was prepared and applied to arterioles in a fashion identical to that used for ACh.

In experiments designed to assess the flow dependence of microvessel responses, arterioles were occluded by positioning glass micropipettes (diameter 35 μm) on the arteriole with a second micromanipulator. Paired observations of diameter responses were made at identical vessel locations during control (free flow) and during occlusion.

To study the mechanism of propagation in arterioles, substances thought to influence events associated with the conduction of signals either along nerves or between excitable cells were added to the superfusion solution bathing the preparation (global application) or were microapplied to an arteriole using a second micropipette. Tetrodotoxin (TTX, Calbiochem-Behring, La Jolla, Calif.) and atropine sulfate (ICN Pharmaceuticals, Plainview, N.Y.) were dissolved in distilled water at a concentration of $1 \times 10^{-4}$ M and kept refrigerated; for experimental use, aliquots were diluted to $1 \times 10^{-6}$ M by addition to the PSS superfusing the tissue.

For microapplication experiments, sucrose (Sigma) was added to fresh PSS (final concentration of solutes 600 mOsm), and papaverine hydrochloride was used.
undiluted from injection vials (30 mg/ml; Lilly, Indianapolis, Ind.). The desired solution was backfilled into a glass micropipette (tip i.d. 3–4 μm), which was then positioned with the tip lying adjacent to an arteriole at 0.5–0.6 mm upstream from the ACh pipette, and the sucrose or papaverine solution was ejected by applying pressure to the butt end of the pipette (Picospitzer II, General Valve Corp., Fairview, N.J.). To determine the effect of these substances on propagated vasodilation, diameter responses were observed at 1.1–1.5 mm upstream from the ACh pipette both prior to and during microapplication of the test solution to the intermediate segment of the arteriole.

Comparisons of propagated diameter responses obtained during control conditions and during experimental intervention were performed using paired t tests and were considered significant if p<0.05. Data are reported as mean ± SEM.

Results

Feed Arteries

We have inferred that dilation of feed arteries is required if muscle blood flow is to increase substantially above rest (Figure 1). Our experiments show that feed arteries from both the cremaster and gracilis muscles (typical resting diameter 100–125 μm) displayed maximal vasodilation in response to topical ACh that was consistent with fourfold to fivefold increases in feed artery conductance (Figure 1). Feed arteries from both muscles exhibited 20–25% increases in diameter in response to muscle contraction.1 When ACh was restricted to the distal portion of the cremaster preparation, the direct, local vasodilation spread upstream and induced feed artery dilations that were similar to those occurring in response to muscle contraction.1

Arterioles

The vasodilation induced by ACh decayed in amplitude as it spread along the arteriole (Figure 2). The propagation of arteriolar dilation was bidirectional and occurred more rapidly than could be accounted for by diffusion.4 Iontophoresis of sodium chloride adjacent to vessels was without effect on vessel diameter, as were ACh stimuli when delivered into the tissue parenchyma at sites removed from the vessel.

To examine the flow dependence of propagated vasodilation, responses to ACh were observed before and after vessel occlusion. Typically, dilation occurred during vessel occlusion; this was offset by raising the PO2 of the superfusate solution to constrict the vessel back to its resting diameter (Figure 3). Thus, responses to ACh iontophoresis during both control (free flow) and occluded conditions were initiated from the same vessel diameter. The magnitude of propagated vasodilation in the absence of flow was not different from that observed during flow.4

The possibility of a role for neural traffic in mediating the propagation of vasodilation was examined in three cheek pouch preparations. In these experiments, propagated vasodilation was observed both before and during global exposure to TTX. The amplitude of the propagated response observed at distances greater than 1 mm upstream from the site of ACh iontophoresis was found typically to be a 20–25% increase in diameter (resting diameter 17 ± 2 μm) and was unaffected by exposure to TTX. In contrast, addition of atropine to the superfusate abolished all responses of arterioles to ACh.

Microapplication of either papaverine or sucrose solutions to a vessel at a site lying between the ACh pipette and the observation site for propagated vasodilation resulted in maximal dilation of a vessel segment.
approximately 250 μm long. With papaverine, this dilation had no effect on propagation; in contrast, propagated vasodilation was attenuated significantly (p<0.05) in the presence of hypertonic sucrose solution (Table 1).

Table 1. Effects of Microapplication of Papaverine and Hypertonic Sucrose Solutions on Propagated Vasodilation in Hamster Cheek Pouch Arterioles.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>Peak</td>
</tr>
<tr>
<td>Papaverine</td>
<td>24±2</td>
<td>29±2</td>
</tr>
<tr>
<td>Hypertonic sucrose</td>
<td>22±2</td>
<td>28±2</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Solutions applied with micropipettes as described in "Materials and Methods." Diameter values recorded at locations where propagated vasodilation was observed (i.e., 1.1–1.5 mm upstream from ACh pipette). Resting diameters recorded prior to and peak diameters recorded during response to ACh stimulus. During microapplication treatment, maximal diameters of intermediate vessel segments (located at 0.5–0.6 mm upstream from ACh pipette) to which papaverine (n=5) or hypertonic sucrose (n=6) solutions were micropplied were 51±2 and 52±2 μm, respectively.

*Significant difference in magnitude of propagated vasodilation between control and treatment, p<0.05.

Discussion

In many tissues, the microvessels are supplied with blood via conduit arteries that are one hundred to several hundred microns in diameter, a class of vessels that we have designated feed arteries.3 Pressure measurements indicate that these vessels represent as much as 50–60% of total vascular resistance in several tissues.4 Our observations indicate that feed arteries are under active control at rest and that diameter changes in these vessels are sufficient to contribute to the integration of muscle blood flow with metabolic demand. Our observations indicate that feed arteries are to the question of whether a local dilation initiated at the level of microvessels could be transmitted to the feed arteries upstream. The observation that feed vessel dilation could be triggered with topical application of ACh to distal microvessels3 indicated that some form of communication exists between microvessels and feed arteries. A flow change per se may underlie the dilation of feed arteries since, in large arterial vessels (e.g., the femoral and coronary arteries), vasodilation can be induced by an increase in flow.5,6 This response appears to be mediated by endothelial cells5 via the release of a vasodilator substance that may be induced by increased wall shear forces associated with hyperemia. Arteriolar vasodilation distal to feed arteries would result in an increase in flow through upstream vessels via the increased total network conductance and could thereby elicit flow-dependent vasodilation. Through such a mechanism, a reduction in resistance initiated at the microvascular level could, in effect, be communicated upstream. As will be shown below, however, flow-dependent vasodilation is only one means by which a vasodilatory response may ascend the vascular tree and thereby coordinate the conductance of feed arteries with that of arterioles.

The propagation of vasodilation in arterioles indicates that some form of communication occurs within the microcirculation. Possible stimuli for initiating propagated vasodilation include mechanical, electrical, and chemical events. Our efforts to measure propagation velocity using videomicroscopy failed to resolve the actual velocity; nevertheless, they indicated that the dilatory stimulus moves much more rapidly than can be explained by diffusion of a chemical intermediate along the arteriole (we estimate that propagation velocity is no less than 2 mm/sec4).

We investigated whether the propagation of vasodilation in arterioles was dependent on blood flow and found that elimination of flow in single microvessels with microocclusion did not attenuate the response.4 This finding demonstrated that the spreading dilation...
occuring in response to ACh is conducted along the arteriolar wall. The mechanism for this conducted response in arterioles is, therefore, unlike the flow-dependent coordination between feed artery and microvessel resistance proposed previously.

Using two approaches, we tested whether the propagation of vasodilation in arterioles is dependent on a myogenic mechanism (i.e., that propagation involved connected changes in smooth muscle length or tension along successive vessel segments). In the first approach, a focal, maximal dilation of an arteriole segment was induced by microapplication of papaverine at 0.5 mm upstream from the ACh pipette. We tested whether the ACh response would propagate through the maximally dilated segment and induce dilation 500 μm further upstream (i.e., at distances greater than 1 mm upstream from the ACh pipette). In fact, propagated vasodilation at the upstream site was not affected by papaverine dilation (Table 1).

With the second paradigm, we observed that vasodilation propagated effectively through a vessel segment that was sealed at both ends with microocclusion pipettes and was thus unable to change in volume. Since smooth muscle cell length could not have changed in either the maximally dilated or in the occluded arteriolar segments located along the conducting pathway, we conclude that propagation does not depend on mechanical changes in the arteriolar wall.

**A Working Hypothesis**

We hypothesized that the ACh dilatory stimulus is conducted along the arteriolar wall either via direct electrical continuity between cells or through a neural pathway. Historically, both ideas have received experimental support. Krogh et al. reported that spreading dilation in arterioles of the web of the frog leg was nervously in nature. In contrast, Lutz et al. concluded that a nonnervous conducting mechanism (e.g., a muscle syncytium) mediated the spread of vasodilation. Hilton proposed that the conducting elements responsible for ascending dilation of the femoral artery were the smooth muscle cells of the vessel media. Subsequently, Duling and Berne reported a propagated vasodilation in hamster cheek pouch arterioles that appeared to be mediated by nerve fibers. Thus, these previous studies depicted alternative pathways for the propagation of a dilatory stimulus along the vessel wall: either a neural pathway intrinsic to the vessels or direct electrical communication between cells.

**Innervation**

There is extensive anatomic and physiologic evidence for neurally mediated vasoconstriction and vasodilation in microvessels. Additionally, ganglion cells intrinsic to the arteriolar wall and thought to mediate vasodilation have been reported. If neural elements do mediate propagated vasodilation, blockade of the fast, voltage-sensitive sodium channels should disrupt the response. The failure of TTX to block the propagated response to ACh indicates that propagation does not occur via a neural pathway. However, these conclusions must be viewed with caution since there were no independent measures of the effectiveness of TTX at the level of the arteriole. Preservation of propagated vasodilation following vascular denervation would provide additional evidence against neural mediation of this response.

**Gap Junctions**

Gap junctions are abundant throughout microvessels and provide a direct pathway for electronic conduction between cells. In arterioles of the guinea pig muscosa, smooth muscle cells are coupled electrically. Endothelial cells are also coupled electrically. Furthermore, arteriolar smooth muscle and endothelial cells establish gap junctional contact via the myoendothelial junction. Thus, not only can smooth muscle cells and endothelial cells conduct information between homologous cells, but there also may be direct intercellular communication between the smooth muscle and endothelial cells composing the arteriole.

**Acknowledgment**

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**References**

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