Propagation of vasomotor responses coordinates arteriolar resistances

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SEGAL, STEVEN S., DAVID N. DAMON, AND BRIAN R. DULING. Propagation of vasomotor responses coordinates arteriolar resistances. Am. J. Physiol. 256 (Heart Circ. Physiol. 25): H832–H837, 1989.—We tested the hypothesis that a conduction pathway intrinsic to the arteriolar wall possesses the properties necessary to coordinate vasomotor responses in the microcirculation. Acetylcholine (ACh) or norepinephrine (NE) was iontophoresed onto cheek pouch arterioles (15–35 μm diam) of pentobarbital-anesthetized hamsters, and diameter responses were observed using intravital video microscopy. ACh and NE induced vasodilation and vasoconstriction, respectively, that propagated both upstream and downstream from the site of application. Propagated vasomotor responses decayed with distance along the arterioles; this decay was characterized by mechanical length constants of 1.9 and 1.8 mm for ACh and NE, respectively. Vasodilations and vasoconstrictions initiated on daughter vessels of a branch propagated into parent arterioles that were approximately twice the diameter of the daughter vessels. Iontophoretic stimuli applied simultaneously to paired daughter vessels induced propagated responses that summed linearly in the parent vessel. We conclude that the arteriolar network functions as a highly coordinated syncytium and that diverse vasomotor stimuli can be summed and integrated within the peripheral microvasculature.

VASCULAR RESISTANCE NETWORKS are composed of many vessel segments arranged both in series and in parallel. This complex organization offers the potential for independent control of the magnitude of tissue blood flow or the pattern of flow distribution within the tissue. However, it has been noted that some form of communication among the various resistance vessels is required if tissue perfusion is to be optimally matched to the metabolic requirements of active parenchymal cells (7, 18).

The means by which resistance vessels may communicate with one another are not well understood, but it is clear that vasomotor responses need not be confined to their site of origin (5, 7, 10, 16–19). Acetylcholine infused into distal segments of the femoral artery can induce a vasodilation that “ascends” into the proximal portions of the vessel (10, 16). In the microcirculation, iontophoresis of acetylcholine onto an arteriole induces a response that spreads rapidly away from the site of origin (5, 17). Unlike the flow-dependent nature of the ascending dilation in the conduit arteries (16), propagated vasodilation in arterioles occurs independent of changes in blood flow (17).

In the present study, we examine a conduction pathway intrinsic to arterioles. Using in vivo microscopy and microiontophoresis, we show that the pathway provides a mechanism for coordinating response patterns in the multiple vessel segments that comprise the arteriolar network and that such coordination can have important physiological consequences.

METHODS

Experimental Preparation and Video Microscopy

Male golden hamsters (120 g) were anesthetized with pentobarbital sodium (Nembutal, Abbott; 70 mg/kg ip) and tracheotomized to maintain a patent airway. The left femoral vein was cannulated for infusion to replace respiratory fluid losses and for maintenance of a stable level of anesthesia [Nembutal in isotonic saline (10 mg/ml) was infused at 420 μl/h]. The cheek pouch was exteriorized on a Plexiglas pedestal for observation of microvessels (4). Esophageal temperature was maintained at 37°C with conducted heat. Cheek pouch preparations were superfused continuously with a bicarbonate-buffered physiological salt solution (37°C, pH 7.4) of the following composition (in mM): 131.9 NaCl, 4.7 KCl, 1.2 MgSO4, 2 CaCl2, 20 NaHCO3. Superfusion solutions were initially gassed with 5% CO2-95% N2.

Microvessels were observed with a Zeiss microscope (model ACM) using Koehler illumination (condenser NA = 0.33; Leitz objectives: UM 20, NA = 0.33; or UMK 50, NA = 0.60). All data were recorded using the UMK 50 objective. Observation sites were chosen at the tip of the iontophoresis pipette (“local”), and at selected distances along the vessel as determined with an eyepiece reticle. At each observation site, the response amplitude (i.e., change in diameter) was calculated as the absolute difference between the peak response diameter and the resting diameter before stimulation. Dimensional measurements were calibrated with a stage micrometer (Graticules, Tonbridge Kent, England).

The microscope image was projected to a video camera (series 68; Dage-MTI; see Ref. 21) and displayed on a video monitor (model BH15R; Ball Electronic Display). For data collection, the final magnification was ~1200x. Vessel diameters were recorded on-line with a modified video analyzer (model 321; Colorado Video), which was...
operated as a video caliper. The output of the analyzer was recorded on a Brush recorder (model 260, Gould).

Cheek pouch preparations were allowed to stabilize for 45–60 min after surgery, and arterioles were then tested for vasodilation in response to topical application of 10⁻⁴ M adenosine (ADO). To increase tone in preparations that displayed moderate responses to ADO, the oxygen content of the superfusion solution was increased to either 10 or 21%, with 5% CO₂ and the balance N₂. This maneuver was performed in ~30% of the preparations used in the present experiments. As results were similar regardless of the oxygen content of the superfusate, data were pooled. Note that elevation of superfusate PO₂ does not affect propagation (20).

At the end of each day’s experiments, the hamster was given an overdose of pentobarbital through the femoral venous catheter.

Micropipettes and Iontophoresis

Glass capillary tubes (no. 1B120F-4; World Precision Instruments) were flushed sequentially with several volumes each of 50% HNO₃, distilled water, and acetone. Pipettes were then pulled (model 700C; David Kopf Instruments), mounted on a balance arm (3), and beveled at a 30° angle (0.3 μm abrasive film, A. H. Thomas) to produce 1–2 μm tips (ID) as measured with brightfield microscopy.

Pipettes were backfilled with filtered solutions (0.2 μm Acrodisc no. 4192; Gelman Sciences) of either 1.0 M acetylcholine chloride (ACh) or 0.5 M l-norepinephrine bitartrate (NE) dissolved in distilled water. All chemicals were obtained from Sigma Chemical unless indicated otherwise.

The pipette tip was positioned adjacent to the vessel using a Leitz micromanipulator, and the pipette was connected via a Ag-AgCl wire to an iontophoresis programmer (model 160; World Precision Instruments). An Ag-AgCl reference electrode was secured at the edge of the preparation. A retaining current, typically 100–120 nA, was adjusted to be just sufficient to prevent local effects of ACh or NE on the arteriole. Ejection currents ranged from 50 to 1,000 nA in amplitude and from 200 to 750 ms in duration. The iontophoresis programmer was gated externally by a physiological stimulator (model S4; Grass Instrument).

Statistics

One or two vessels were studied in a typical cheek pouch preparation. Each vessel was treated as a separate observation. Summary data are presented as means ± SE. Data were analyzed using t tests, linear regression, and analysis of variance. Differences were considered to be of statistical significance if P < 0.05.

**RESULTS**

**Iontophoresis Controls**

Iontophoretic application of ACh or NE directly onto an arteriole typically produced both local and propagated responses after a brief delay (1–2 s). This was not a diffusional spread, since ACh or NE iontophoresed into the tissue parenchyma at distances of 215–1075 μm from an arteriole usually induced no observable vasomotor response. In those few cases where responses were observed, the time lag was greater (6–8 s) and the amplitude was reduced compared with direct application onto arterioles.

To eliminate any contribution of convective transport of ACh or NE in arteriolar blood, sites upstream from the pipette tip were typically observed. In several experiments, possible countercurrent transport of ACh or NE in adjacent venules was circumvented either by observing arteriolar segments devoid of adjacent venules or by simultaneous occlusion of adjacent venules. Propagated vasomotor responses under these conditions were not noticeably different from those where such measures were not undertaken. Iontophoresis of 1.0 M NaCl at currents and durations identical to those used for ACh and NE had no effect on vessel diameter at any location, intensity, or duration tested.

Characterization of Propagated Responses

Comparison of propagated vasodilation and vasoconstriction. Propagated vasoconstriction in response to NE was somewhat unreliable, being observed in ~60% of the vessels tested. Also, repeated applications of NE often induced pronounced vasomotion thereby making subsequent observations of propagated vasomotor responses unreliable. Neither the lack of propagation nor the induction of vasomotion appeared to be associated with a change in the sensitivity of the arteriole to NE at the site of stimulation. Propagated vasodilation with ACh was much more stable, being observed in over 90% of the arterioles tested; this response typically consisted of a smooth, transient vasodilation that was highly repeatable. Therefore, the propagated vasomotor responses were studied in greatest detail using ACh. Selective additional experiments were then performed with NE to determine whether an observed characteristic was common to both constriction and dilation. For example, the amplitude of propagated vasoconstriction (4 ± 1 μm; observed at 1,042 ± 22 μm upstream from a NE pipette; n = 3) was not affected by elimination of blood flow in the observed segment with microocclusion. This independence of propagation from blood flow was shown previously using ACh-induced propagated vasodilation (17).

Dose-response to iontophoretic stimuli. The magnitude of arteriolar dilation observed at the tip of the ACh pipette (local) and at selected distances upstream increased in proportion to the iontophoresic current (Fig. 1). The dilation spread both upstream and downstream and decayed with distance from the point of ACh application. Iontophoresis of NE resulted in local and propagated vasoconstrictions that, analogous to responses obtained with ACh, declined with distance (Fig. 2). Responses were dose dependent as increasing the stimulus current from 350 to 600 nA (at constant pulse durations) resulted in the amplitude of propagated vasoconstriction (observed at 1,050 ± 20 μm upstream from the NE pipette) increasing from 3 ± 1 to 6 ± 1 μm (n = 3).

Mechanical length constant. A “mechanical” length
constant was estimated by observing the decay in the amplitude of the propagated vasomotor response with distance from the stimulus origin (Fig. 2). Responses within the diffusion field of the iontophoresis pipette are a combination of direct and propagated effects. Accordingly, responses at distances <215 μm from the site of stimulation were excluded from the analysis. By analogy with the cable properties of excitable membranes (12), length constants were calculated from the slopes obtained from regression analysis that related the logarithm of the response to distance along the vessel. Length constants of 1.9 and 1.8 mm were obtained for ACh and NE, respectively. A Student's t test (8) indicated that these values were not significantly different.

Coordination of Microvessel Responses

Propagation into parent vessels. If propagation is to be effective in coordinating responses among different regions of the microvasculature, then the propagated response must cross branch points. Behavior of the propagated response in relation to branch points was examined in a sample of arteriolar branches chosen such that the resting diameter of parent vessels (43 ± 5 μm) was approximately twice that of the daughter branch (22 ± μm) to which a stimulus was applied (n = 7). When ACh was iontophoresed (1,000 nA, 500 or 750 ms) onto daughter branches at 1,753 ± 223 μm distal to the branch point, vasodilation propagated into the parent vessels and increased diameters significantly (to 47 ± 5 μm; P < 0.05; paired t test). In two of these experiments it was observed that there was an apparent “threshold” for inducing propagation into the parent vessel; subthreshold stimuli would propagate up to, but not through, the branch origin. Vasoconstriction also propagated into parent vessels in a similar fashion (see below).

Summation of vasomotor stimuli. In parent arterioles, interactions between stimuli arising from two daughter vessels were also investigated. For these experiments, we chose vascular configurations in which two daughter vessels of similar diameter originated from a bifurcation of the parent vessel (Fig. 3A). Micropipettes were positioned on each of the two daughter vessels at distances 500–1,100 μm downstream from the bifurcation. Vasomotor responses were observed 500–1,100 μm upstream from the bifurcation (total distance for propagation was 1,000–2,200 μm). Propagated responses of similar amplitude were induced at the observation site in the parent vessel by separately controlling the currents passed through each of the two pipettes. Simultaneous application of ACh onto the two branches resulted in linear summation of responses in the parent vessel (Fig. 3C).

Vasomotor responses crossing the bifurcation were not confined to the parent vessel. With stimulation of one daughter branch, a propagated response (amplitude, 2–3 μm) was typically observed in the contralateral daughter branch, as well as in the parent vessel.

Summation also occurred with simultaneous application of dilator and constrictor stimuli to the two daughter branches (Fig. 4). The ACh and NE stimuli were individually adjusted so as to produce propagated responses in the parent vessel that were identical in magnitude but...
FIG. 3. Summation of propagated vasodilation in parent arterioles. A: illustration of experimental paradigm. Parent arteriole bifurcates into two daughter arterioles, with similar diameter. Locations of acetylcholine (ACh) iontophoresis pipettes are indicated on daughter vessels. "Observe" arrow indicates site for recording propagated vasomotor responses in parent vessel. B: representative illustration of changes in diameter in parent arteriole during stimulation of daughter vessels. First arrow, ACh stimulus given at one daughter branch; second arrow, similar ACh stimulus given at other daughter branch; third arrow, simultaneous stimulation of both branches. C: summary data (means ± SE) from 6 experiments for amplitude (peak response minus control diameter) of propagated vasodilation in parent arterioles (resting diameter, 29 ± 5 μm). Total distance for propagation from either pipette, 1,260 ± 164 μm. Iontophoretic current was 500 or 1,000 nA; pulse durations were 200, 500, or 750 ms. * Responses to simultaneous delivery of both stimuli were significantly different from responses to individual stimuli, P < 0.05 (analysis of variance).

In some experiments, stimulus intensities were selected such that responses triggered on either daughter vessel did not propagate into the parent vessel. When such subthreshold stimuli were applied simultaneously, propagated responses often summed to produce a detectable response in the parent vessel; this was true with both dilator and constrictor stimuli.

DISCUSSION

We have demonstrated that an arteriolar conduction system exists that possesses a number of characteristics appropriate for communicating vasomotor responses over distances of several millimeters. Vasomotor responses to both ACh and NE propagated upstream into parent arterioles, demonstrating a functional connection between series resistance elements in the microcirculation. When separate stimuli were applied to paired daughter vessels of a branch, both the amplitude and direction of the propagated vasomotor responses in parent arterioles could be affected by varying the relative intensity of ACh and NE stimuli on respective daughter branches. This observation supports the idea that more proximal feed vessels in the microvascular network can serve as a locus for integration of diverse vasoactive stimuli originating from distant vascular locations.

Physiological Significance of Propagated Vasomotor Responses

Folkow et al. (7) recognized the need for coordination among vessel elements and reported an "ascending dilation" during exercise, a finding that led to the concept that the locus of flow control "shifts" upstream with an increase in metabolic demand (7, 9, 17). Furthermore, it is now well established that both metabolic and pharmacological stimuli applied downstream from major feed vessels can induce dilations that ascend into these feed vessels (10, 13, 16, 18).

The present work is focused on the coordination of vasomotor responses among microvascular elements and shows that propagation may serve as a means of coupling a highly localized metabolic stimulus (e.g., the contraction of a single muscle fiber or fiber bundle) to the dilation of a longer segment of the arteriole. Without such spreading dilation the local change in resistance of...
TABLE 1. Length constants for electrotonic conduction in mammalian tissues

<table>
<thead>
<tr>
<th>Tissue Preparation</th>
<th>Species</th>
<th>Length Constant, mm</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Isolated Purkinje fibers</td>
<td>Goat</td>
<td>1.9</td>
<td>23</td>
</tr>
<tr>
<td>Taenia coli strips</td>
<td>Guinea pig</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Aorta smooth muscle strips</td>
<td>Rabbit</td>
<td>2.1</td>
<td>14</td>
</tr>
<tr>
<td>Submucosa arterioles</td>
<td>Guinea pig</td>
<td>1.6</td>
<td>11</td>
</tr>
<tr>
<td>Uterine smooth muscle strips</td>
<td>Rat</td>
<td>2.6</td>
<td>22</td>
</tr>
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Tabulated values were determined with electrophysiological techniques. Length constant is defined typically as distance required for stimulus to decline to 1/e of value determined at site of stimulation (12).

A single arteriole segment would likely be too small to produce a significant increase in tissue blood flow. By inducing a spread of dilation into the parent vessel, propagation reduces the resistance of more proximal vessels that otherwise could restrict flow, even when local arteriolar dilation is maximal (18). Thus the propagation of vasodilation across branch points and into the parent vessel (Figs. 3 and 4) will ensure that local perfusion increases concomitant with the metabolic requirements of parenchymal cells. In two experiments, a threshold of stimulus intensity was required to induce propagation from smaller (12-15 μm diameter) daughter vessels into parent arterioles. This observation is consistent with the graded amplitude of propagated responses (Fig. 1) and suggests that the magnitude of a local stimulus (e.g., metabolic demand) may dictate not only the amplitude of propagation but in fact whether or not a proximal feed vessel may be "recruited" into the collective vasomotor response.

By analogy to the analysis of the phenomenon of coronary steal (6), we can infer that propagation may also serve to produce a more homogeneous flow distribution during functional hyperemia. If vasodilation were to occur in one of two daughter branches arising from a bifurcation, flow would increase in the parent vessel, thereby increasing the pressure drop across the parent vessel. This would reduce the pressure at the entrance to the bifurcation and thereby reduce flow through the nondilated daughter branch and this would, in principle, lead to an imbalance between flow and metabolism (6). A mechanism for coordinating changes in resistance in vessel segments across branch points would minimize such effects and ensure that tissue perfusion remains uniform in the face of changing metabolic needs in localized regions of the tissue.

The exact relationships between the two agonists that we have chosen for study and the factors active in metabolic control of tissue blood flow remain to be explored, since it is unlikely that either ACh or NE is a mediator in the local control processes. We presume that the propagated responses that we have studied with these two agents reflect an inherent property of the vessel wall and that these responses can be invoked during such processes as functional hyperemia (18). The specific metabolite(s) that may induce such a response remain to be determined.

Nature of Propagation

Electrotonic conduction is a common denominator for the coordination of function in smooth muscle cells of the gastrointestinal tract (2), the myometrium (22), the aorta (14), and arterioles (11, 15). We therefore surmise that the propagated vasomotor responses reflect an underlying spread of electrical activity, although no direct measurement of such electrical activity in our preparation has yet been performed. Our demonstration that mechanical length constants are on the order of 2 mm (Fig. 2) is quite consistent with the values obtained for electrotonic spread of hyperpolarizing and depolarizing currents in arterioles of the guinea pig mucosa (11, 15) and also consistent with length constants measured electrophysiologically in a variety of isolated mammalian tissue preparations (Table 1).

In conclusion, responses to multiple vasomotor stimuli were found to interact additively so that inputs from paired daughter vessels sum in their effects on proximal feed arterioles. Arterioles appear to function as a syncytium, and the propagation of vasomotor responses is one mechanism for precisely coordinating the magnitude and distribution of tissue blood flow in accord with local metabolic demand. Propagation in cheek pouch arterioles is consistent with electrotonic conduction of electrical responses along the vessel wall. The relationships between the membrane potential of either arteriolar smooth muscle or endothelial cells and the changes in microvessel diameter remain to be defined, as does the cellular basis of the conduction pathway.

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