Increased flow precedes remote arteriolar dilations for some microapplied agonists

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Frame, Mary D. Increased flow precedes remote arteriolar dilations for some microapplied agonists. Am J Physiol Heart Circ Physiol 278: H1186–H1195, 2000.—This study asks which occurs first in time for remote responses: a dilation or a remote change in flow. Arteriolar diameter (~20 μm) and fluorescently labeled red blood cell (RBC) velocity were measured in the cremaster muscle of anesthetized (pentobarbital sodium, 70 mg/kg) hamsters (n = 51). Arterioles were locally stimulated for 60 s with micropipette-applied 10 μg/ml LM-609 (αvβ3-integrin agonist), 10−3 M adenosine, or 10−3 M 3-morpholinosydnonimine (SIN-1, nitric oxide donor) as remote response agonists or with 10−3 M papaverine, which dilates only locally. Observations were made at a remote site 1,200 μm upstream. With LM-609 or adenosine, the RBC velocity increased first (within 5 s), and the remote dilation followed 5–7 s later. N-nitro-L-arginine (100 μM) blocked the LM-609 (100%) and adenosine (60%) remote dilations. SIN-1 induced a concurrent remote dilation and decrease in RBC velocity (~10 s), suggesting the primary signal was to dilate. Papaverine had no remote effects. This study suggests that, although remote responses to some agonists are induced by primary signals to dilate, additionally, network changes in flow can stimulate extensive remote changes in diameter.

remote dilatory responses can be initiated by a localized (micropipette) stimulus. This induces the vasculature to dilate at a remote location, which is, by definition, always out of range of the direct effects of the stimulus. Remote responses have been demonstrated by many stimulation modes, including agonists, such as ACh (7), L-arginine or nitric oxide (NO) donors (7, 10, 24), integrin (9), or ATP (17); they have also been initiated physiologically by localized muscle stimulation (1) and by sudden pressure changes accompanying a localized myogenic response (22). The stimulation sites are typically either arterioles or capillaries along the same flow path (e.g., see Refs. 6 and 9). However, adenosine can elicit a remote dilation from tissue stimulation, along a different flow path (26). Finally, there is increasingly a lack of supportive evidence for a single common remote response mechanism (e.g., see Refs. 7, 10, 24); only some stimuli appear to induce an electrotonic signal for transmission along the vascular wall (e.g., see Refs. 31 and 32). Thus vascular communication is a multimodal classification of vasoactive responses that is dependent on the stimulus type, stimulus duration, and stimulus location.

Most studies report remote responses as diameter changes; however, the remote response also consists of a remote change in flow, at least with some agents (15, 18). We have therefore considered that a localized stimulus may induce other changes, such as a shift in pressure distribution within the vascular network, leading to an altered resistance that could alter flow. Potentially, the flow change could alter the diameter of the remote site by a less direct mechanism (e.g., flow-dependent dilation). To begin to address whether elevated flow could directly induce a remote dilation, we have asked a simple question. With agonist-induced remote responses, which occurs first in time, the remote dilation or the remote change in flow? In the present study, we have chosen the following three remote response agents: 3-morpholinosydnonimine (SIN-1, NO donor), adenosine, and LM-609 (αvβ3-integrin agonist; see Ref. 2). Additionally, we have used papaverine, which does not induce a remote dilation and which stimulates only a local dilation.

METHODS

With University approval, adult male Golden hamsters [HSD:SYr, 80 ± 2 days, 123 ± 9 g (mean ± SD), n = 51] were anesthetized with pentobarbital sodium (70 mg/kg ip), tracheostomized, and maintained on a constant infusion of pentobarbital sodium (10 mg/ml at 0.56 ml/h) via a femoral venous catheter. Systemic hematocrit (54 ± 4%) did not change during the experiment. Body temperature was maintained between 37 and 38°C by a conductive heat source. Mean arterial pressure was monitored via a left femoral arterial catheter (100 ± 11 mmHg) and was constant (±10 mmHg) for each preparation. Red blood cells from age- and weight-matched donor animals (n = 21, 83 ± 2 days, 123 ± 8 g) were labeled with a fluorescent dye (substituted tetramethylrhodamine isothiocyanate, XRTC; Molecular Probes, Eugene, OR) using an established protocol (28). The fluorescently labeled red blood cells were injected in tracer quantities via a right jugular catheter and were used to quantitate blood flow changes. The right cremaster was prepared for in vivo microcirculatory observations. The preparation was continuously superfused with bicarbonate-buffered saline containing (in mmol/l) 132 NaCl, 4.7 KCl, 2.0 CaCl2, 1.2 MgSO4, and 20 NaHCO3 (equilibrated with gas containing 5% CO2 and 95%
N₂ gas, pH 7.4 at 34°C). All chemicals were obtained from Sigma Chemical (St. Louis, MO), unless otherwise noted.

The microcirculation was observed with transillumination using a modified Nikon upright microscope (Nikon) with a \( \times 25 \) (Nikon) or a \( \times 40 \) (SWI; Olympus) objective at 1 or 1.5 times magnification. Epi-illumination was used to visualize the XRITC-labeled red blood cells using a Chroma 22A filter (Chroma, Brattleboro, VT). Video images were produced using a charge-coupled device \( 72s \) video camera and a Gen/Sys II video intensifier (Dage-MTI, Michigan City, IN). During a 60-min stabilization period, the XRITC-labeled cells were administered, and arteriolar tone was verified by dilation to topically applied 10⁻⁴ M sodium nitroprusside and constriction to 5% oxygen added to the superfusate. Observations were made along a transverse arteriole (feed vessel) located in each preparation, as described previously (11).

Micropipette administration of stimuli. Concentrated stock solutions of the test agents were added to control suffusate to obtain the desired final concentrations. The micropipette tip diameters were 20 ± 5 µm. Micropipettes were placed within 25 µm of the vessel wall. All agents were applied using a pressure delivery system with \( -2 \) cmH₂O holding pressure and +30 cmH₂O delivery pressure. Each micropipette contained 30 µM fluorescein-labeled dextran (4,000 mol wt) as a flow marker to verify the region of the arteriole (feed vessel) that was exposed to pipette contents and was observed using a B1E filter (Chroma). For each exposure, the FITC-dextran from the micropipette was always verified to expose only the local arteriolar site or the designated alternative site and then to convectively flow in a stream \( ~100–200 \) µm wide, away from the local site and off of the dissection board with the superfusate (5 ml/min; see Ref. 26). FITC-dextran in control suffusate does not alone induce a remote response (10).

Protocol 1: Simultaneous determination of remote diameter and velocity changes. One protocol was used for each of the following four test agents: adenosine (\( n = 7, 10^{-3} \) M), SIN-1 (\( n = 7, 10^{-3} \) M), LM-609 (\( n = 7, 10^{-4} \) µg/ml, anti-\( \alpha_\beta_3 \) monoclonal antibody; Chemicon International), and papaverine (\( n = 6, 10^{-3} \) M). These concentrations have been shown in previous experiments (26), or in the present study, to provide a maximal remote dilation in the cremaster muscle preparation. Some animals were exposed multiple times to the same test agent (5 exposures/animal, see Protocol 2). Stable arteriolar diameters (no vasomotion) were verified for 2–5 min before application of a test agent. Baseline red blood cell velocities were videotaped for the feed vessel proximal to the first branch for 30 s before application of test agents. Remote responses were obtained by applying the test agent at the local site downstream, via micropipette, for 1 min while observing a remote location upstream. Four to five randomized concentrations were studied in each animal, using a 10-min waiting period between exposures; only one antibody was studied per animal. In three experiments with LM-609, the red blood cell velocity was determined at the remote site for the 30-s baseline and for the 15-s period after the onset of stimulation.

Protocol 2: Concentration-response to LM-609 and P1F6. The maximal remote dilation was determined in six of the experiments with LM-609 (0.33–33 µg/ml) and in six additional animals with 0.33–10 µg/ml P1F6 (anti-\( \alpha_\beta_3 \) monoclonal antibody; Chemicon International). Remote responses were obtained by applying the test agent at the local site downstream, via micropipette, for 1 min while observing a remote location upstream. Four to five randomized concentrations were studied in each animal, using a 10-min waiting period between exposures; only one antibody was studied per animal. In three experiments with P1F6, the red blood cell velocity was determined at the remote site for the 30-s baseline and for the 15-s period after the onset of stimulation.

Protocol 3: Stimulus duration with SIN-1 and LM-609. The effect of stimulus duration on the peak remote dilation was determined with LM-609 (10 µg/ml, \( n = 3 \)) and with SIN-1 (100 µM, \( n = 3 \)). In random fashion, the stimulus duration was 0.5, 5, 15, 30, 60, or 120 s, with a 10-min recovery period between exposures. Only diameter and flow was determined by video microscopy.

Measurement of hemodynamic parameters. Red blood cell flux (F, cells/s) is calculated by \( F = (m/p) t \), where \( m \) is the number of fluorescent cells crossing a specified vessel plane in time \( t \), and \( p \) is the fraction of fluorescent cells in the total red blood cell population (0.35 ± 0.02%, mean ± SD). Individual velocities (µm/s) were measured as the distance traveled in one video field (11/60 s) for all fluorescent cells crossing the specified sampling plane during the 30-s control period and during the 60-s test period (during downstream application of the test agent). There was an average of 25 ± 4 (mean ± SD) fluorescent cells per 5-s time interval. Thus, for one exposure, an average of 450 fluorescent red blood cells was analyzed over 90 s (e.g., see Fig. 2). The harmonic mean of the individual red blood cell velocities for each 5-s interval was calculated (mean axial cell velocity, \( \bar{v} \)). To evaluate the entire baseline period, the harmonic mean velocity was determined for all cells in the 30-s baseline period. Hematocrit (H), as the time-averaged volume fraction of cells in the vessel, was calculated as \( H = F \cdot V_c / V_t \cdot \pi^3 \), where \( r \) is the vessel radius, and \( V_t \) is the mean corpuscular volume. The apparent viscosity (\( \eta_{app} \)) was calculated from the relationship between vessel hematocrit, vessel diameter (D), and the relative viscosity (21). The shear rate (\( \gamma \), s⁻¹) was calculated as \( \gamma = 8 \cdot V_c / D \). Wall shear stress (\( T_w \), dyn/cm²) was calculated as \( T_w = \eta_{app} \cdot \gamma \).

Statistics. The response curves were constructed by averaging the remote diameter change at each concentration or stimulus duration (protocols 2 and 3). With data from protocol 1, the baseline diameters and red blood cell velocities were examined for each experiment for trends over time by linear regression. Trends would have indicated a nonsteady baseline state; 2 of 29 experiments were excluded for a nonsteady baseline period (F-test, slope > 0). With data from protocol 1, the wall shear stress for the entire baseline period was compared with the subsequent peak of the remote dilation, using linear regression, to determine the relationship between baseline wall shear stress and the immediately subsequent remote dilation.

To evaluate the temporal relationship between change in velocity over time, the hemodynamic values for each 5-s time interval were first determined for individual experiments and...
RESULTS

Characterization of remote dilation to integrin stimulation. Figure 1A shows that the remote dilation induced by extraluminal stimulation of $\alpha_\nu\beta_3$ or $\alpha_\nu\beta_5$ integrins is dose dependent. A maximal remote response was seen with a 60-s exposure to 10 $\mu$g/ml LM-609 (stimulates $\alpha_\nu\beta_3$) or 10 $\mu$g/ml P1F6 (stimulates $\alpha_\nu\beta_5$). For LM-609, a higher dose of 33 $\mu$g/ml stimulated a concentration dependence of the remote increase in diameter (Fig. 1A) and velocity (Fig. 1B). Figure 1B shows the associated concentration dependence of the remote increase in blood flow velocity during local LM-609 stimulation.

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<th>Table 1. Baseline and final values for diameter and velocity</th>
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Values are means ± SE of the pooled baseline values for the entire 30-s baseline period and pooled final values obtained between 50 and 60 s of continuous downstream exposure. For local diameter, "final" indicates diameter at local site obtained between 60 and 90 seconds. *Significant change from baseline.

This illustrates that changes in both the velocity and diameter were stimulus strength dependent.

With LM-609, an alternate exposure site was used in the capillary network downstream from the local arteriolar site to test whether the remote dilations were due to exposure of a large area of the tissue. We have previously defined the tissue exposure area using this means of micropipette stimulation (26). Stimulation with LM-609 for 60 s within the downstream capillaries did not induce a remote dilation (0.21 ± 0.19 $\mu$m, n = 15 capillary exposure sites in 4 animals). Thus the LM-609-induced remote dilation is not due to exposure of an unspecified tissue area, but instead the stimulus site includes a downstream arteriole.

Baseline diameter with P1F6 was 13 ± 1.8 $\mu$m at the local site of stimulation and 18 ± 1.3 $\mu$m at the remote site; the baseline diameter values with LM-609 are given in Table 1. With 10 $\mu$g/ml LM-609, the local diameter change was a dilation of 4 ± 1 $\mu$m, and with 10 $\mu$g/ml P1F6 there was a significant local diameter change (−0.8 ± 1.2 $\mu$m). Thus with P1F6 there was only a remote response and no local diameter change.

Temporal changes in remote diameter vs. velocity. The baseline conditions were not different between treatments; no trends over time were observed during baseline for the diameter or velocity (n = 27). The baseline diameter for all animals was 18.9 ± 1.8 $\mu$m (mean ± SD) at the remote site and 14 ± 1.0 $\mu$m at the local drug application site. The distance between the local site of drug application and the remote observation point was 1,200 ± 360 $\mu$m (Fig. 2A).

Downstream, localized exposure to LM-609, adenosine, or SIN-1 each induced a significant local dilation and a significant remote dilation (Table 1). Although papaverine significantly dilated the local stimulation site, there were no remote diameter changes with papaverine.

Figure 2B shows, as an example, the velocity of individual red blood cells measured over time for one experiment with adenosine. Within seconds of adeno-
sine exposure (begun at t = 0), the red blood cell velocity increased and was maintained for the duration of exposure. Figure 2C shows that the diameter change during that same experiment occurred after the velocity change.

Figure 3 shows the normalized velocity and diameter before and during exposure to LM-609 (n = 7 experiments), adenosine (n = 7), SIN-1 (n = 7), and papaverine (n = 6). The baseline and final values are given in Table 1. Velocity change preceded diameter change for LM-609 and for adenosine; red blood cell velocity was significantly greater than baseline during the entire test period with LM-609 or with adenosine. With LM-609, the onset of the velocity change was 3 ± 2 s (mean ± SD), which was significantly earlier than the onset of the diameter change (13 ± 3 s). Likewise, with adenosine, the onset of the velocity change was 3 ± 2 s, and the onset of the diameter change was 13 ± 2 s. With SIN-1, there is a significant decrease in velocity (11 ± 5 s) that occurred with the onset of remote dilation (10 ± 4 s); however, velocity did not change further after dilation had begun. Although the remote diameter changes occurred with roughly the same onset times, and were roughly of the same magnitude, the corresponding changes in red blood cell velocity were quite different for these three agents. In three experiments with P1F6, the remote velocity was determined during the initial 15 s of P1F6 exposure and was compared with the velocity in the 30-s baseline period. Remote velocity did not change (baseline of 1,458 ± 34 to 1,327 ± 98 µm/s) with P1F6 stimulation. No remote changes occurred with papaverine.

To evaluate whether the differences in remote responses to LM-609 vs. SIN-1 were related to the total tissue area exposed, the duration of exposure was varied (0.5–120 s). Figure 4 shows that a maximal remote dilation is stimulated with a brief 5-s exposure to LM-609 (10 µg/ml, n = 3); importantly, the remote dilation occurred with an onset of ~10 s, which is well after the end of downstream exposure. In contrast, with SIN-1 (10⁻³ M, n = 3), a 15-s exposure stimulated a submaximal remote dilation. Only with a longer exposure time (30 s) was there a maximal remote dilation to SIN-1. Thus a minimum exposure time of >15 s was required for maximal remote dilation to SIN-1.

In vivo flow-dependent dilation vs. conducted dilation. Figure 5 shows the changes in the shear rate. With LM-609, the shear rate peaked and then declined toward baseline due to the remote dilation. With adenosine, the same pattern is suggested; however, the values are not significantly different from baseline. With SIN-1, the shear rate progressively declined with dilation and was significantly lower than baseline by 30 s of exposure. Again, no changes occurred with papaverine.

Figure 6 shows the changes in wall shear stress over time during remote stimulation. The wall shear stress peaked within seconds of downstream exposure with LM-609 followed by a rapid decline. With SIN-1, there was progressive decline in wall shear stress, which was partially compensated (returning toward baseline) after 30 s by the increase in cell flux (see Fig. 8). There were no significant changes in wall shear stress with adenosine, nor with papaverine.

To test whether the remote diameter changes required intact endothelial cell-dependent release of nitric oxide, remote dilatory responses were tested during suffusate exposure to 100 µM L-NNA (n = 6). Figure 7A shows that, with L-NNA exposure, the remote dilation to LM-609 is completely blocked, and the remote dilation to adenosine is 60% blocked. The remote dilatory response to SIN-1 is unaffected by L-NNA. To determine whether the velocity increase with LM-609 still had occurred in the absence of a remote dilation, the
red blood cell velocity during the initial 15 s of LM-609 exposure was compared with the velocity in the baseline period. Before L-NNA, the remote dilation to LM-609 was preceded by an increase in remote blood velocity of 306 ± 697 µm/s (baseline velocity: 1,176 ± 48 µm/s). With L-NNA exposure, the remote velocity with LM-609 still increased significantly by 230 ± 33 µm/s (baseline velocity: 805 ± 57 µm/s), despite the fact that the diameter did not change. Thus LM-609 stimulation still caused a remote change in flow velocity without a remote change in diameter with L-NNA.

To test whether the remote responses involved sympathetic nerve stimulation, remote dilatory responses were determined in the presence and absence of tissue-wide exposure to TTX (1 µM, n = 6). TTX had no effect on the baseline diameter (25.8 ± 1.7 µm before, 25.0 ± 1.8 µm after 20-min exposure to TTX). Three agents were tested in each animal. Remote responses were tested during local arteriolar exposure to 10 µM LM-609, 10⁻³ M adenosine, and 10⁻³ M SIN-1. Figure 7B shows that none of the remote dilatory responses were affected by 20-min tissue-wide exposure to TTX. The response to adenosine was tested using an alternate stimulation site with TTX to further ensure that components of the remote response were not due to a larger tissue area exposed to the stimuli. This is based on our previous finding of tissue-stimulated remote dilation with adenosine (26). The alternate stimulation site was a comparable distance from the remote site but was along a different flow path, as defined previously (26). TTX did not alter the remote dilatory response to adenosine with exposure of a downstream arteriole (3.28 ± 0.3 µm) or with exposure of the alternate tissue site in a different flow path (3.1 ± 0.5 µm). Thus these remote dilatory responses are not TTX sensitive.

Agonist-dependent changes in red blood cell flux. Red blood cell flux was examined separately to determine whether the remote responses had a sustained impact on the apparent oxygen supply capacity to the arteriolar network. Figure 8 shows that the red blood cell flux increased over time with each remote response agent, but both the magnitude and time course were agonist specific. With LM-609, flux increased progressively over 30 s of stimulation, and this was maintained. With adenosine, there were two distinct peaks in red blood cell flux, consistent, once again, with the idea that more than one process may be involved in the remote changes. With SIN-1, red blood cell flux began to increase only...
after 30 s of downstream exposure and then rapidly increased to >80% above baseline by 45 s. Thus each remote response agent caused an increase in red blood cell supply to the arteriolar network, and the temporal changes in flux were different for each agent. There were no changes in cell flux with papaverine.

We also examined the relative apparent viscosity and vessel (tube) hematocrit. For all animals combined, the systemic hematocrit was 54 ± 4%; in the feed arteriole, the baseline hematocrit was 26 ± 8% (mean ± SD), and the baseline viscosity was 1.82 ± 0.14 cP. After 45 s of stimulation with LM-609 or with adenosine there was a

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**Fig. 5.** Shear rate (s⁻¹) values (mean ± SE) for each 5-s interval during baseline and during downstream exposure to 10 µg/ml LM-609 or 10⁻³ M (each) adenosine, SIN-1, or papaverine. *Different from the pooled baseline value for that treatment.

**Fig. 6.** Wall shear stress (dyn/cm²) values (mean ± SE) for each 5-s interval during baseline and during downstream exposure to 10 µg/ml LM-609 or 10⁻³ M (each) adenosine, SIN-1, or papaverine. *Different from the pooled baseline value for that treatment.
one-third reduction in feed vessel hematocrit (to ~15%) and an 8% drop in viscosity (to 1.6 cP). In contrast, with SIN-1, there was no significant change in the vessel hematocrit, yet viscosity initially decreased to 10% below baseline and then increased to 12% (to 2.33 cP) above baseline by 60 s of stimulation. With papaverine, neither the hematocrit nor the viscosity changed.

Flow-linked ability to respond. Different from a dilation caused by an acute change in flow is the concept that the baseline wall shear stress, “set point,” may influence the ability of a blood vessel to respond to a quantum remote stimulus. We tested this by evaluating whether the baseline wall shear stress conditions affected the subsequent remote dilation. Figure 9 shows the baseline wall shear stress calculated for the entire pooled 30-s baseline period plotted against the immediately subsequent remote dilation (peak diameter change during a 60-s exposure). For animals with a higher baseline wall shear stress, there was a subsequent greater remote dilation with 10 µg/ml LM-609 (n = 12 exposures in 7 animals), or with 10⁻³ M SIN-1 (n = 7 exposures in 7 animals), but not with 10⁻³ M adenosine (n = 11 exposures in 7 animals). The regression lines were significant with LM-609 or with SIN-1 but not with adenosine.

**DISCUSSION**

This study shows that integrin stimulation induces an ascending flow-dependent dilation. The data demonstrate that a localized stimulation can first change flow within an arteriolar network and that the subsequent remote dilation can be caused by the flow change. Importantly, not all remote response agonists work this way.
way. Exogenous NO stimulation induces a change in diameter first. The response to adenosine is more complex, with <60% of the response being related to flow-dependent dilatory pathways.

There is strong evidence that there are multiple avenues for initiation and transmission of remote responses. As an example, using ACh as the “classic” stimulus for remote dilation, it is now clear that the remote response to a very brief (<5 s) controlled application is fundamentally different from a longer (30 s) application in a number of ways. These include the muscarinic receptor subtypes involved (25), summation characteristics of the response (23), the ability of halothane to block the remote dilation (10, 29), and the ability of NO donors to mimic the responses (7, 10, 24). The complexity of remote responses is further illustrated by the variety of agents and stimuli that induce a remote response (6, 9, 10, 17, 22, 31, 32). There does not appear to be a common second messenger system nor a common electrotonic signal for transmission of all remote dilations.

Flow dependent network responses. The studies mentioned above, including ours, have followed the premise that the primary remote signal is the signal to dilate. An alternative possibility exists, namely that the localized stimulus has initiated a network change in flow and that changes in diameter are subsequent to the flow changes. This study approaches the remote dilatory mechanism from two separate views. In the first view, where the initial signal would be to dilate, flow changes would occur concurrently or after the remote diameter change in an intact closed system. At least initially, the velocity would decrease with the onset of dilation (i.e., before compensation). In the second view, the initial signal may involve a network change in pressureflow distributions, which would alter the conditions at our observation point. [Note: this study does not address whether the remote dilation is due to a remote change in transmural pressure (e.g., see Refs. 4 and 22).] The ensuing dilation would be due to a secondary signal, such as a local flow-dependent response. For this second view, we would expect flow changes to occur first and dilation to follow.

No remote changes were seen with papaverine. This is an important control since papaverine only initiates a local dilation. The lack of change in hemodynamic flow parameters over time at the remote location indicates that a localized dilation within a network does not impact the flow into this arteriolar network. Additionally, this suggests that the method of stimulation is not causing the remote responses, thus ruling out recirculation or venoarterial transfer of drugs. This means that, for the remote response agents tested, other mechanisms must be responsible for the flow changes.

Both adenosine and integrin stimulation clearly show that the remote change in velocity precedes the remote change in diameter (cf., Fig. 3). This immediately raises a number of questions to be examined in greater depth. How can the velocity increase before an increase in diameter? We speculate that a shift in network resistance has occurred that is independent of the local dilation at the stimulus site and that this resistance shift leads to a velocity increase at our vantage point. Although we do not have direct evidence for a mechanism, it is not difficult to propose multiple ways by which flow might be altered at one vantage point within a closed network system (e.g., Refs. 8, 12, 21).

We asked whether the remote dilation to integrin or adenosine stimulation was flow dependent. With extraluminal integrin stimulation, there is an immediate transient increase in shear rate and in wall shear stress that was totally blocked by L-NNA (cf., Figs. 5, 6, and 7A and RESULTS). This is consistent with previous studies that have shown, for isolated arterioles, that flow-dependent dilation serves to maintain the shear forces (13, 14). Thus it is likely that the remote dilation to extraluminal integrin stimulation is a flow-dependent network response. As an interesting potential mechanism of vascular control of tone, this is opposite to the effect of intraluminal stimulation of the integrins (19). With intraluminal stimulation/occupation of αβ3 integrin receptors, local flow-dependent dilation is inhibited. This is an example of a common theme in which different vasoactive effects are seen from intra- vs. extraluminal stimulation. For example, this has been shown for remote responses with ATP in the microcirculation, where intraluminal ATP induces a remote dilation and extraluminal ATP induces a remote constriction (17).

The response to adenosine is more complex. Clearly, the red blood cell velocity increase preceded dilation; however, only 60% of the dilation was blocked by L-NNA, and therefore only this amount of the dilation can potentially be attributed to a flow-dependent mechanism. Yet, we have shown that, when flow is occluded, the remote dilation to adenosine persists, unchanged in magnitude (26), thus strongly indicating that some compensating component of the remote dilation to adenosine is not dependent on flow. There is a further observation with adenosine that is important here. A
remote dilation to adenosine does not require stimulation of the downstream vasculature, per se (26). Stimulation within the tissue, along a different flow path, induces a remote dilation of the same magnitude as does stimulation along the common flow path. This suggests that at least part of the remote dilatory signal stimulated by adenosine is likely to be nonvascular in origin. We do not think venoarteriolar transfer of adenosine can explain these responses. The concentration and time dependence of the diameter and velocity changes we report with adenosine (present study and Ref. 26) are much faster than that reported for venoarteriolar transfer of adenosine (12). Furthermore, in the present study, the arteriolar observation site is downstream from the arteriovenular pairs, making this a less likely mechanism. Although transmural pressure changes have not been ruled out in this response, our experiments with TTX (cf., Fig. 7B) show that sympathetic nerve activity is not involved. The remote response to adenosine is thus very likely to be multimodal. Distinguishing the mechanisms will require further study.

In contrast to integrin or adenosine stimulation, with SIN-1 the remote diameter and velocity changes were initially concurrent. We therefore cannot conclude that the remote dilation was flow dependent, i.e., initiated by a change in flow. This is supported by our recent finding that acute occlusion of flow has no effect on the remote dilation to NO donors (26); a flow change is not required for the remote dilation.

Wall shear stress set point. Flow dependence of the remote response can also be examined from another view (that of the response capability for different baseline flow states). This is different from a flow-dependent dilation wherein a dilation is caused by an acute change in flow. Separately from a flow-induced response is the suggestion from our data that the baseline flow state within an arteriolar network comprises a set point for a dilatory response capability (present study and Ref. 9). We found that, with extraluminal integrin stimulation, the magnitude of the remote dilation is tightly linked to the prevailing (baseline) flow state. For a higher baseline wall shear stress, the subsequent remote dilation is greater (cf., Fig. 9). For integrin stimulation, the remote dilation is also a flow-dependent dilation (cf., Fig. 7A). For SIN-1, the magnitude of the remote dilation was linked to the wall shear stress set point; however, the dilation itself appeared to be initiated through a primary signal to dilate with SIN-1 and did not appear to be flow dependent. In comparison, the remote dilation to adenosine was unrelated to the baseline wall shear stress set point and was not an entirely flow-dependent type of response. Thus, for these three remote response agonists, only two (integrin and SIN-1) stimulate remote responses linked to the baseline wall shear stress. Furthermore, the presence of a relationship between response and baseline shear is not a predictor of flow dependence of the remote response.

Network regulation of red blood cell content. There is evidence from other studies that, in the peripheral circulation, cell flux is regulated independently of other hemodynamic parameters (5, 8, 16, 20, 27). The present study supports the suggestion that recruitment of red blood cells and regulation of cell content in the microcirculation is stimulus specific (cf., Fig. 8). We found that the red blood cell flux did not always increase passively with changes in velocity and in diameter. Instead, the changes in the red blood cell content, or vessel hematocrit, were a function of the agonist itself for these arterioles. The red blood cell flux was progressively elevated by 40% with integrin stimulation and was elevated by 80% (after a 30-s delay) with SIN-1 stimulation. With adenosine, there was a multiphase increase in cell flux. Both the amount and the time course of the red blood cell changes were entirely stimulus specific. At present, no mechanistic insight into cell content regulation is afforded by this study. The data add to the growing body of information that red blood cell content is a regulated parameter.

In summary, this study examines which occurs first in time, a remote dilation or a remote change in flow during stimulation by three remote response agonists. This study demonstrates that, in intact arteriolar networks, the initial signal with integrin or adenosine stimulation is a remote change in red blood cell velocity (e.g., a network flow effect), with the remote dilation involving a separate or secondary mechanism. In contrast, the initial signal with exogenous NO involves a dilatory mechanism. This study introduces the concept that a remote change to a localized stimulus may in some cases be due to an induced change in the prevailing flow.

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