On the role of mechanosensitive mechanisms eliciting reactive hyperemia

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Koller, Akos and Zsolt Bagi. On the role of mechanosensitive mechanisms eliciting reactive hyperemia. Am J Physiol Heart Circ Physiol 283: H2250–H2259, 2002; 10.1152/ajpheart.00545.2002.—We hypothesized that changes in hemodynamic forces such as pressure (P) and flow (F) contribute importantly to the development of reactive hyperemia. To exclude the effects of vivo factors, isolated rat skeletal muscle arterioles (~130 μm) were utilized. We found that changes in P or P + F following occlusions elicited reactive dilations (RD). The peak of RD (up to ~45 μm), but not the duration of RD, increased to changes in P (80 to 10, then back to 80 mmHg) as a function of the length of occlusions (30, 60, and 120 s). However, changes in P + F (80–10-80 mmHg + 25–25 μl/min) increased both the peak and duration of RD (from ~25 to 90 s) with longer occlusions. When only P changed, inhibition of nitric oxide synthesis or endothelium removal (E−) reduced only the peak of RD, whereas when P + F were changed, both the peak and duration of RD became reduced. Inhibition of stretch-activated cation channels by gadolinium reduced the peak but enhanced the duration of RD (both to P or P + F) that was unaffected by Nω-nitro-l-arginine methyl ester (l-NNAME) or by E−. When only P changed, inhibition of tyrosine kinases by genistein reduced peak RD but did not affect the RD duration. However, when P + F changed, genistein reduced both the peak and the duration of RD, additional l-NNAME reduced the peak RD, but did not affect the duration of RD. Thus in isolated arterioles an RD resembling the characteristics of reactive hyperemia can be generated that is elicited by deformation, stretch, pressure, and flow/shear stress-sensitive mechanisms and is, in part, mediated by nitric oxide.

isolated arteriole; stretch; pressure; flow; endothelium; nitric oxide; tyrosine kinase

Perhaps one of the first physiological responses of the circulation that has been observed, already in the middle of the nineteenth century, was reactive hyperemia. This observation initiated many studies, among them Bayliss (5), who suggested that a pressure-sensitive myogenic response of vessels elicits the changes in blood volume of the hindlimb of dogs after a brief period of occlusions of the femoral artery. Soon thereafter, the role of the myogenic response in the development of reactive hyperemia was questioned by Anrep (3) in 1912, who suggested that tissue-derived metabolic factors contribute primarily to the reduction in resistance following occlusions. Although the existence of myogenic and metabolic mechanisms and their role in various circulatory responses have been well substantiated (13, 20), their specific contribution and the possible contribution of other mechanisms to the development of reactive hyperemia have not yet been established.

The magnitude of reactive hyperemia varies from one tissue to another, and it seems to be less developed in the liver (16) and kidney (13) and more pronounced in the myocardium (7, 18, 35, 36) and skeletal muscle (19, 22, 26, 29). Because the magnitude of reactive hyperemia has been shown to depend on the length of the occlusion, the role of tissue-derived metabolic factors was assumed to be the primary cause of the maintenance of the dilation of arterioles after the release of longer occlusions (19, 21, 22, 26). Whereas a number of metabolic factors have been suggested to mediate reactive hyperemia (13), such as arterial Po2 (8), adenine (22, 37), ATP-sensitive potassium channels (4), prostaglandins (1), and nitric oxide (NO) (27), less is known regarding the role of mechanosensitive mechanisms intrinsic to the vascular wall (23). The pressure-sensitive myogenic response may be important in the development of reactive hyperemia. Yet no direct evidence exists to support this idea. In this context, Eikens and Wilcken (10) have demonstrated that extremely short periods of occlusions (<1 s) resulted in reactive hyperemia, whereas Schwartz et al. (39) found that diastolic coronary occlusion of a duration >100 ms resulted in reactive hyperemia, conditions in which the role of metabolic factors is likely to be negligible, reasoning in favor of the involvement of mechanosensitive mechanisms in reactive hyperemia.

In addition to pressure changes, after release of an occlusion, a sudden increase of blood flow could also activate shear stress-sensitive mechanisms (24, 25, 28, 42) eliciting the release of endothelium-derived dilator factors (such as NO) that may limit myogenic constriction and modify the duration of reactive hyperemia.

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Although it has been proposed that NO release contribute to the modulation of coronary blood flow during reactive hyperemia in guinea pig heart (27) and also in human forearm vessels (30, 42), the mechanisms responsible for the release of NO have not yet been clarified. One may propose however that changes in hemodynamic forces (intraluminal pressure and flow/shear stress) themselves (23) and/or changes in the shape of vascular cells due to a change in diameter (41) may lead to the release of vasoactive factors.

On the basis of the aforementioned, we hypothesized that during and after brief occlusion of vessels, signaling pathways of mechanotransduction are activated by changes in intraluminal pressure and flow, eliciting reactive dilation that would contribute to the development of reactive hyperemia. To test this hypothesis we investigated whether brief occlusions of isolated arterioles in vitro could elicit reactive dilations, in a condition, in which the neuronal, humoral, and tissue-derived metabolic or other in vivo factors are not present. In addition, we aimed to characterize the sole role of changes in intraluminal pressure and/or flow/shear stress, as well as some of the cellular pathways responsible for the development of reactive dilation/hyperemia.

METHODS

Isolation of Arterioles

Male Wistar rats (n = 50; 300–350 g, Charles River) were housed separately and had free access to water and standard rat chow. All procedures were in accordance with guidelines set by the Institutional Animal Care and Use Committees. Experiments were conducted on isolated gracilis muscle arterioles (diameter: ~130 μm) as described previously (25, 28). In brief, after overnight fasting, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The gracilis muscle was excised and placed in a siliconized petri dish containing cold (0–4°C) physiological salt (PS) solution composed of (in mmol/l) 110 NaCl, 5.0 KCl, 2.5 CaCl2, 1.0 MgSO4, 1.0 KH2PO4, 5.0 glucose, and 24.0 NaHCO3 equilibrated with a gas mixture of 10% O2–5% CO2, balanced with nitrogen, at pH 7.4. With the use of microsurgery instruments and an operating microscope, a branch (~1.5 mm in length) of the gracilis arteriole running intramuscularly was isolated and transferred into an organ chamber containing two glass micropipettes filled with PS solution. Arterioles were cannulated on both sides (Fig. 1B), and both micropipettes were connected with silicone tubing to an adjustable PS solution reservoir. Inflow and outflow pressures were continuously measured by an electromagnetic flowmeter (Omega; Stamford, CT). Perfusion flow was measured with a ball flowmeter (Omega; Stamford, CT).

Experimental Protocols

First, arteriolar responses were obtained to changes only in intraluminal pressure (Pressure protocol) at a zero-flow condition (Fig. 1A). During “occlusion” of perfusion tubes (both input and output) intraluminal pressure was decreased from 80 to 10 mmHg for 30 s and then after the release of the occlusion, it was increased back to 80 mmHg (within 1–2 s). Changes in arteriolar diameter were continuously recorded. Responses were also obtained after 60- or 120-s occlusions. Between interventions, 10-min equilibration periods (to reach arteriolar steady-state diameter again) were utilized. In one group of experiments all procedures were repeated after 30 min to obtain time controls.

In the presence of constant intravascular pressure (80 mmHg) ~30 μl/min flow was then established by changing the inflow (100 mmHg) and outflow (60 mmHg) pressure to an equal degree, but in opposite directions, to keep midpoint luminal pressure constant (80 mmHg). Arteriolar responses to brief occlusions of the inflow cannula were then obtained. In this condition both intraluminal pressure and flow were changed (Fig. 1A, Pressure + Flow protocol). The inflow cannula was occluded for 30, 60, or 120 s, while the output pressure was maintained at 10 mmHg. After the occlusion was released and flow reestablished, the output pressure was brought back to 80 mmHg. Changes in diameter were continuously recorded. In these protocols 10 min was kept between occlusions and time controls were obtained as well. Arteriolar responses in the same protocols (Pressure and Pressure + Flow) were obtained in the presence of Nω-nitro-L-arginine methyl ester (L-NAME, 10-4 mol/l, for 20 min under zero-flow conditions), an inhibitor of endothelial NO synthase (eNOS) or after endothelium denudation. The endothelium of the arterioles was removed by perfusion of the vessel with air, as described previously (25). Endothelium denudation was assessed by the loss of dilation to acetylcholine and by the maintained dilation to the NO donor sodium nitroprusside.

Responses to changes in Pressure or Pressure + Flow were obtained in the intraluminal presence of the stretch-activated cation channel blocker gadolinium (10-5 mol/l) after incubation for 30 min (under zero-flow condition) and after simultaneous administration of gadolinium and L-NAME in endothelium-intact and endothelium-denuded arterioles. In separate experiments, arteriolar responses to occlusions were also obtained after intraluminal administration of the tyrosine kinase inhibitor genistein (5 × 10-6 mol/l) after

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**Fig. 1.** A: changes in intraluminal pressure (Pressure) or pressure and flow (Pressure + Flow) during occlusion (O) and after release (R) of tubes used for cannulation of an isolated skeletal muscle arteriole. B: P1 and P2 indicate inflow and outflow pressure, respectively.
incubation for 30 min (under zero-flow condition) and after simultaneous administration of genistein and L-NAME.

Data Analysis and Statistics

The reactive dilation of arterioles that developed after release of occlusions were characterized by the peak and the duration of the increase in diameter. Duration was assessed by the time necessary for the diameter to return to 90% of the baseline diameter. We have also calculated “repayment” by the reactive dilation obtained in vitro analogous to the in vivo flow debt repayment formula of the reactive hyperemic response (22, 36). The areas under the occlusion and reactive dilation were measured from the original records, and the ratio was expressed as a percentage, based on the following equation

\[
\frac{[(PD - BD) \times T_{od} \times (1 - \frac{1}{e})]}{(BD - OD) \times T_{od}} \times 100
\]

where PD is peak diameter, BD is basal diameter, OD is diameter during occlusion, \(T_{od}\) is duration of dilation, and \(T_{od}\) is duration of occlusion.

At the conclusion of each experiment, to obtain the maximum passive diameter, the suffusion solution was changed to a Ca\(^{2+}\)-free PS solution, which contained EGTA (10^{-3} mol/l), and the vessel was incubated for 10 min. All drugs were added to the vessel chamber, and final concentrations are reported. All salts and chemicals were obtained from Sigma-Aldrich. Solutions were prepared on the day of the experiment. Data are expressed as means \(\pm\) SE. Statistical analysis was performed by two-way analysis of variance (ANOVA) for repeated measure. Accordingly during comparison of several groups of experimental data ANOVA was applied, followed by a post hoc test, whereas Student’s t-test was applied for paired data. \(P < 0.05\) was considered statistically significant.

RESULTS

In the presence of 80-mmHg intraluminal pressure, the active diameter of arterioles was 127 \(\pm\) 5 \(\mu\)m, whereas the passive diameter (in the absence of extracellular Ca\(^{2+}\)) was 241 \(\pm\) 22 \(\mu\)m. Thus arterioles isolated from gracilis muscle developed a pressure-induced active tone (44 \(\pm\) 3\% of passive diameter), without the use of any vasoactive agent. Endothelium removal or inhibition of NOS did not significantly affect the vessel diameters (to 115 \(\pm\) 5 and 118 \(\pm\) 7 \(\mu\)m, respectively). Also arteriolar tone was not significantly altered by intraluminal administration of gadolinium or genistein (to 124 \(\pm\) 10 and 135 \(\pm\) 9 \(\mu\)m, respectively).

Reactive Dilation Induced by Occlusions When Only Intraluminal Pressure Changes

In the first series of experiments, we obtained changes in diameter when only intraluminal pressure had changed during occlusions (Pressure). Original figures show that arteriolar diameter decreased by (41 \(\pm\) 6 \(\mu\)m) when intraluminal pressure was decreased from 80 to 10 mmHg (Fig. 2A). Then after the release of 30-, 60- or 120-s occlusions, intraluminal pressure increased to 80 mmHg (within 1–2 s) followed by a marked increase in arteriolar diameter above the initial value. The arteriole then slowly constricted reaching the initial diameter (Fig. 2A). We designated this diameter response as “reactive dilation” because it resembles the shape and time course of reactive hyperemia observed in vivo (7, 18, 22, 26, 35, 36). Summary data show that the peak of reactive dilation of arterioles significantly increased as a function of the length of occlusion (Fig. 2B), whereas the duration of dilation did not change significantly (~30 s, Fig. 2B).

Reactive Dilation Induced by Occlusions When Both Intraluminal Pressure and Flow Change

In the second series of experiments, we examined the effect of occlusions on arteriolar diameter, when both intraluminal pressure and flow were changed (Pressure + Flow). First, 30 \(\mu\)l/min intraluminal flow was initiated, which increased the basal diameter (by 32 \(\pm\)
4 μm) confirming previous findings (16). In the presence of the new steady-state diameter, the inflow cannula was occluded for 30, 60, or 120 s. The flow became zero, while the outflow pressure was decreased to 10 mmHg. During occlusion, arteriolar diameter decreased similar to that obtained in the Pressure protocols (Fig. 2A) and then after the release of occlusion reactive dilations were again observed. The peak reactive dilations increased as a function of the length of the occlusions and were not significantly different from those obtained when only pressure changed (Fig. 2B). However, in contrast to the Pressure protocol, in the presence of Pressure + Flow the duration of reactive dilation significantly increased as a function of the length of the occlusion (Fig. 2B).

Calculation of Flow-Dependent Dilation and “Repayment” by Reactive Dilation

Data obtained in the Pressure and Pressure + Flow protocols were used to calculate the sole contribution of “Flow” in eliciting reactive dilation. We have found that subtraction of the Pressure curve from the Pressure + Flow curve provided a slowly developing diameter curve following occlusions that shows the characteristics of flow-dependent dilation (25) of isolated arterioles (Fig. 3A).

To facilitate further the comparison of in vitro and in vivo findings and analogous to the analysis of reactive hyperemic responses observed in vivo (36), we calculated the ratio of areas of occlusion and reactive dilation (called “flow debt repayment” in vivo) in the present experimental conditions. We found that in the Pressure protocols, the repayment significantly decreased, whereas in the protocols of Pressure + Flow, the repayment significantly increased as a function of the length of the occlusions (Fig. 3B).

Cellular Pathways Mediating Reactive Dilation of Isolated Arterioles

Effects of l-NAME or endothelium removal on the reactive dilation. In the Pressure protocols, inhibition of NO synthesis or endothelium removal significantly reduced the peak (~ by 35%) but did not affect the duration of reactive dilation (Fig. 4, A and B). In the Pressure + Flow protocols, inhibition of NO synthesis or endothelium removal significantly decreased not only the peak dilation, but also the duration of reactive dilation of arterioles (Fig. 4, A and B).

Effects of gadolinium on reactive dilation of isolated arterioles. Arteriolar dilations were obtained in the Pressure protocol after intraluminal application of gadolinium (a blocker of stretch-activated cation channels) and after simultaneous administration of gadolinium and l-NAME. In the presence of endothelium in the Pressure protocol, gadolinium significantly reduced the peak reactive dilation, whereas it enhanced the duration of dilation (Fig. 5, A and B). The remaining dilations were unaffected by additional l-NAME or by endothelium removal (Fig. 5, A and B). In the Pressure + Flow protocol gadolinium treatment reduced the peak reactive dilation and enhanced the duration of dilation of arterioles. Additional administration of l-NAME or endothelium removal did not affect the peak but significantly reduced the duration of reactive dilations (Fig. 5, A and B).

Effects of genistein on reactive dilation of arterioles. Arteriolar reactive dilations were also obtained in the Pressure protocol after intraluminal application of genistein (a tyrosine kinase inhibitor). Genistein significantly reduced the peak of reactive dilations but did not affect the duration of reactive dilations (Fig. 6, A and B). In the Pressure + Flow protocol, genistein treatment reduced both the peak and the duration of reactive dilations of arterioles (Fig. 6, A and B). Additional l-NAME further significantly reduced the peak dilation of arterioles, whereas it did not affect the duration of dilations.

DISCUSSION

The new findings of this study are that in isolated skeletal muscle arterioles changes in intraluminal pressure and flow, as a result of brief occlusions, elicit “reactive dilation,” which resembles the characteristics of in vivo reactive hyperemia. This reactive dilation develops as a result of sequentially activated mechanosensitive mechanisms that are sensitive to 1) cell des-
Fig. 4. A: original records show changes in diameter of isolated gracilis arterioles to changes in pressure (from 80–10 back to 80 mmHg; Pressure) or to changes in Pressure + Flow in response to 30-, 60-, 120-s occlusions or before and after administration of L-NAME in the presence or in the absence of endothelium (Endo−). B: summarized data of peak (top) and duration (bottom) of reactive dilations in both protocols to 30-, 60-, and 120-s period of occlusions. (*n = 10–10). *Significant differences between responses developed to various lengths of occlusions. #Significant differences from control responses.

formation, 2) stretch, 3) pressure, and 4) flow/shear stress. These mechanisms elicit a diameter response that causes reactive dilation. On the basis of these findings, we propose that changes in hemodynamic forces during and after occlusion of blood flow contribute substantially to the development of reactive hyperemia in vivo by activating mechanosensitive mechanisms intrinsic to the arteriolar wall.

Reactive hyperemia has been intensively studied in the past 100 years (7, 13, 18, 21, 22, 26, 35, 36). Initially, Bayliss (5) proposed that a pressure-sensitive “myogenic” mechanism may contribute importantly to the development of reactive hyperemia. His idea has been further supported by observations of many other investigators (10, 39). But because others have found that longer occlusions prolonged the duration of reactive hyperemia, a role for tissue-derived metabolic factors in mediating reactive hyperemia was also proposed (7, 13, 18, 21, 22, 26, 35, 36). Furthermore, recent reports (27, 30, 42) have suggested that in addition to metabolic and myogenic mechanisms, endothelium-mediated, flow/shear stress-sensitive responses may also contribute to the development of reactive hyperemia (24). Although the contribution of all of these mechanisms seems plausible, there are no reports showing clear evidence for the role of mechanosensitive mechanisms eliciting reactive hyperemia. This is due to the fact that all previous investigations of reactive hyperemia were conducted in vivo, a condition in which one could not separate with any confidence the effects of neural, humoral, and tissue metabolic or other in vivo factors. Thus the specific role and magnitude of the contribution of pressure- and flow/shear stress-dependent vascular mechanisms in the development of reactive hyperemia could not be clearly elucidated.

Mechanosensitive mechanisms are activated by the presence of and changes in hemodynamic forces (9, 17, 20, 23, 33, 38). Microvessels, such as arterioles, unlike the larger, conduit vessels, have the unique property to respond with acute and substan-
tial changes in diameter in response to changes in pressure and flow/shear stress (23, 25). This is relevant because when their diameter changes, this, in turn, will change, in a feedback manner, the magnitude of forces that act on them (23). Thus we hypothesized that changes in hemodynamic forces, during and after release of an occlusion, will result in "reactive dilation" of isolated arterioles. If such a dilation occurs in vivo, then the large number of arterioles together is able to reduce the resistance of the arteriolar network and hence elicit reactive hyperemia. We have chosen to study gracilis skeletal muscle arterioles because our previous work has shown that they exhibit substantial myogenic and flow/shear stress-dependent diameter responses (40).

**Role of Endothelial Cell Deformation During Occlusion (First Phase of Reactive Dilation)**

In the absence of intraluminal flow, changes in pressure (from 80 to 10 and back to 80 mmHg), (Pressure protocol) as the result of a brief occlusion elicited reactive dilation. The peak but not the duration of the reactive dilation increased significantly as a function of the length of the occlusion (Fig. 2, A and B). Because the peak reactive dilation developed regardless of whether or not flow was present (Fig. 2A), we conclude that mechanisms sensitive to flow/shear stress do not contribute to the magnitude of the peak of reactive dilation.

The exact mechanisms by which the longer occlusions elicit greater peak dilation of arterioles are not completely understood. Nevertheless, it seems that the release of endothelium-derived NO is involved because inhibition of eNOS or endothelium removal significantly reduced the peak dilation (Fig. 4, A and B). Previous electron microscopic studies by Carlson et al. (6) and Greensmith and Duling (14) showed that during decreases in vessel diameter, endothelial cells undergo substantial folding and shape changes. In this context we recently (41) demonstrated that the defor-
mation of endothelium that occurs when the lumen of arterioles is decreased passively in response to increases in extraluminal pressure or constrictor agents induces NO release. In addition, deformation of endothelial cells in culture also leads to release of NO (41). Figure 2A shows that during occlusions, arteriolar diameter decreased, and thus it is likely that endothelial cells have undergone deformation and shape changes. On the basis of present and previous findings (41), we propose that deformation of endothelial cells during occlusion activates eNOS.

Cell deformation may activate eNOS via different mechanisms, including integrins and protein kinases (2, 15, 44). Recent reports (11, 12, 32, 34, 43) suggest that nonreceptor-activated tyrosine kinases play a central role in endothelial cell signaling and might functionally link deformation to the classical signal transduction pathways. Because we have found that inhibition of tyrosine kinases by genistein significantly reduced the peak dilation of arterioles, which was then further significantly reduced by additional inhibition of eNOS (both in the Pressure or Pressure + Flow protocols), we propose that both tyrosine kinase-dependent and -independent mechanisms are involved in the deformation-induced NO release from arteriolar endothelium. Summarized data of Fig. 6B, showing the peak dilation of arterioles, demonstrate that the tyrosine kinase-dependent part of NO production increased, whereas the tyrosine kinase-independent NO part did not change as a function of the length of occlusion. The assumption of the activation of tyrosine kinase-dependent pathways allows us to speculate how endothelial cells “remember” the length of occlusions. It is likely that during longer occlusions the longer deformation of endothelial cells causes a time-dependent increase in the activation of eNOS by tyrosine kinases, eliciting a greater NO production and thus a greater peak increase in diameter after reestablishing the original conditions.

Fig. 6. A: original records show changes in diameter of isolated gracilis arterioles to changes in pressure (from 80–10 back to 80 mmHg; Pressure) or to changes in intraluminal Pressure + Flow in response to 120-s occlusions before and after intraluminal application of genistein or genistein + L-NAME. B: summarized data of peak (top) and duration (bottom) of reactive dilations in both to 30-, 60-, and 120-s period of occlusions (n = 10–10). *Significant differences between responses developed to various lengths of occlusion. #Significant differences from control responses. $Significant differences from genistein-treated vessel.
Role of Pressure Eliciting Stretch and Myogenic Constriction (Second and Third Phases of Reactive Dilation)

After release of occlusions a sudden increase in pressure or pressure plus flow occurs. In the presence of the stretch-activated cation channel blocker gadolinium, when only pressure has changed, the peak dilation was significantly reduced and the duration of dilation was enhanced. Inhibition of eNOS or endothelium removal did not affect the reduced peak dilation or the prolonged duration of dilation (Fig. 5, A and B). When, in the presence of gadolinium, pressure and flow had changed, the peak dilations were also significantly reduced and were not affected by additional inhibition of eNOS or endothelium removal. However, inhibition of eNOS or endothelium removal significantly reduced the enhanced duration of reactive dilations (Fig. 5, A and B) due to the lack of an NO-mediated flow-dependent part of the dilation (see the next paragraph).

Collectively, these findings suggest that the sudden increases in pressure activate stretch receptors located in both endothelium and smooth muscle cells. Increases in pressure seem to elicit NO release from the endothelial cells, which contributes to the development of the peak reactive dilation. At the same time, increases in pressure activate cation channels in smooth muscle cells, which via mechanotransduction elicits myogenic constrictions.

Role of Flow/Shear Stress (Fourth Phase of Reactive Dilation)

This phase of reactive dilation is present only when in addition to pressure, intraluminal flow is present after the release of the occlusion (Fig. 2A). Because the peak dilation of arterioles was not significantly greater than when only pressure has changed, the development of peak reactive dilation is unlikely to be modulated by flow-dependent mechanism. The duration of reactive dilation, however, greatly increased when both pressure and flow changed, suggesting that activation of eNOS by increases in flow/shear stress prolongs the duration of the reactive dilation. This conclusion is supported by the findings that subtracting the diameter response developed to Pressure from that of Pressure + Flow results in a continuous increase in diameter, which clearly shows the characteristic of flow-dependent dilation (Fig. 3A). Furthermore, endothelium removal or inhibition of eNOS also significantly decreased the duration of reactive dilation. We assume that the flow/shear stress-dependent mechanism has an even more substantial role in the development of reactive hyperemia in vivo, because the dilation of the distal arteriolar network allows further increases in blood flow velocity hence wall shear stress. (In the present experiments after release of occlusions intraluminal flow only returned to the control flow rate.)

Because inhibition of stretch-activated cation channels by gadolinium (both in Pressure and Pressure + Flow protocols) significantly enhanced the duration of reactive dilation, we suggest that activation of these channels is not involved in the flow-induced release of NO, but rather they are primarily responsible for reducing the duration of reactive dilation by the myogenic mechanism.

Intraluminal administration of genistein, an inhibitor of tyrosine kinases (32), reduced the peak dilation as discussed above, but also reduced the duration of dilation when pressure and flow had changed (Fig. 6, A and B). The finding that additional administration of L-NAME did not reduce further the duration of dilation of genistein-treated arterioles supports the hypothesis that in Pressure + Flow protocols the duration of dilations is affected by the flow-dependent release of NO, primarily due to the activation of eNOS by tyrosine kinase pathways. Indeed, this pathway has been shown to mediate flow/shear stress activation of eNOS (32, 43).

![Fig. 7. A: proposed sequential and parallel phases of reactive dilation-hyperemia and their relative contribution, elicited by deformation, pressure-stretch, flow-shear stress, and tissue-derived metabolic, and other in vivo factors. B: proposed mechanosensitive signaling pathways contributing to the development of reactive dilation hyperemia.](https://www.ajpheart.org/content/283/6/H2257)
Reactive Dilation Versus Reactive Hyperemia

To assess the importance and relative contributions of mechanosensitive mechanisms of arterioles in the in vivo development of reactive hyperemia, we have compared the areas under the diameter curves observed during occlusions and during reactive dilations. We refer this value as repayment, analogous to the values used in in vivo studies for analyzing the magnitude of reactive hyperemia (see METHODS). Figure 3B demonstrates that when only the pressure changed (Pressure), the “repayment” decreased as a function of the length of occlusions. However, when both pressure and flow (Pressure + Flow) changed, the repayment increased as a function of the length of the occlusion (maximum: ~110%). In vivo investigations demonstrated that the flow debt repayment is ~200% in response to brief occlusion of skeletal muscle arteries in vivo (31). Comparison of these findings suggests that there is a substantial role for flow/shear stress and in general mechanosensitive mechanisms in the development of reactive hyperemia of skeletal muscle. It is of note, however, that in vivo, other mechanisms, among them metabolic, originating in parenchymal tissue (19, 29), have additional roles in augmenting the reactive hyperemia.

On the basis of previous and the present findings, we propose a new model describing the sequence of events and the mechanisms responsible for the development of reactive dilation/hyperemia (Fig. 7, A and B): during occlusion, deformation of endothelial cells activates the synthesis of NO via tyrosine kinase dependent- and -independent pathways, contributing to the development of peak reactive hyperemia; after release of the occlusion, the pressure-induced stretch elicits NO release from the endothelium but also activates myogenic constrictor mechanisms by stimulation of stretch-activated cation channels in the endothelium and smooth muscle, respectively; and then when flow/shear stress increases, NO synthesis is activated via the tyrosine kinase pathway, which then primarily determines the duration of reactive dilation.

Collectively, these findings indicate that in isolated skeletal muscle arterioles changes in cell shape, pressure, stretch, and flow/shear stress, as a result of occlusions activate mechanosensitive-signaling mechanisms in the arteriolar wall, which elicit reactive dilation that resembles the characteristics of reactive hyperemia. Thus, in addition to previously described metabolic factors, mechanosensitive mechanisms, via activation of endothelial and smooth muscle cell stretch receptors and endothelial NO synthesis, are likely to contribute significantly to the in vivo development of reactive hyperemia.

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