Differential Inhibition of Functional Dilation of Small Arterioles by Indomethacin and Glibenclamide

Leah W. Hammer, Alison L. Ligon, Robert L. Hester

Abstract—Indomethacin or glibenclamide treatments attenuate functional dilation of larger-diameter “feed” arterioles paired with venules in hamster cremaster muscle. We tested the hypothesis that release of cyclooxygenase products from venules is important for functional dilation of third- and fourth-order arterioles. We also tested whether ATP-sensitive potassium channels are important during functional dilation of smaller arterioles. The microcirculation of hamster cremaster muscle was visualized with in vivo video microscopy. We measured diameter responses of third- and fourth-order arterioles paired and unpaired with venules in response to 2 minutes of muscle field stimulation (40 μs, 10 V, 1 Hz). Control diameters of vessels were 31±2 (n=19), 13±1 (n=12), 12±2 (n=12), and 10±1 (n=12) for paired and unpaired third-order and paired and unpaired fourth-order arterioles, respectively. In all groups, field stimulation resulted in increases in mean control diameter of >80%. Indomethacin (28 μmol/L) superfused on the preparation was used to inhibit cyclooxygenase metabolism, or glibenclamide (10 μmol/L) was used to block ATP-sensitive potassium channels. Indomethacin attenuated arteriolar vasodilations to electrical stimulation in paired third-order vessels only, whereas glibenclamide attenuated this vasodilation in all 4 groups. These results support a role for ATP-sensitive potassium channels in functional dilation of arterioles of all sizes regardless of whether or not they are paired with venules. Conversely, a role for cyclooxygenase products is limited to larger “feed arterioles” paired with venules. This study provides further evidence that venules may be the source of prostaglandin release during functional hyperemia. (Hypertension. 2001;37[part 2]:599-603.)

Key Words: indomethacin ■ microcirculation ■ arterioles ■ potassium channels ■ cyclooxygenase

One of the remarkable characteristics of the microcirculation is the ability of the tissue to “control” local blood flow in such a manner that the metabolic requirements of the tissue are adequately met. For instance, during periods of increased muscle metabolism such as during exercise, blood flow to skeletal muscle increases. In hamster cremaster muscle, functional dilation of larger “feed” arterioles can be attenuated by disruption of the endothelium of venules running parallel to these arterioles, by inhibition of ATP-sensitive potassium (KATP) channels, and by inhibiting the production of arachidonic acid metabolites. Collectively, these studies suggest that during periods of increased muscle metabolism, a metabolite of arachidonic acid is released from the venular endothelium that subsequently diffuses to and dilates the adjacent arteriole.

To achieve a maximal increase in blood flow to the tissue, all arterioles within the vascular tree must dilate. As vessels approach the capillary bed, they tend to lose their paired arrangement with venules, and in hamster cremaster muscle, many third-order and the majority of fourth-order arterioles do not have an adjacent venule (see Figure 1). Therefore, an additional mechanism(s) must be responsible for communication between the tissue and terminal arterioles. In hamster cremaster muscle, the KATP channel blocker glibenclamide can partially block the arteriolar response to muscle stimulation in larger-order arterioles, suggesting that the opening of these ion channels is important during functional hyperemia. Activation of KATP channels is modulated by hydrogen ions, hypoxia, adenosine, and lactate. As tissue levels of all of these factors are increased during increases in muscle metabolism, KATP channels may play an important role during the initiation of functional dilation in small, terminal arterioles. The present study was undertaken to determine whether cyclooxygenase products and KATP channels are important in the functional arteriolar dilation of small third- and fourth-order arterioles, paired and unpaired with venules.

Methods

Animal Preparation
The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out according to both the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act.

Fifty-three male Golden hamsters (weight, 120 to 200 g; Charles River) were anesthetized with an intraperitoneal injection of sodium...
Experimental Measurements

The microcirculation of the cremaster muscle was transilluminated and observed with a Leitz Laborlux 12 FS microscope fitted with a ×32 long-working-distance objective (numerical aperture=0.40). The microscopic image was televised with a Dage closed-circuit television camera and displayed on a Sony monitor. The magnification of the image was ×900 from the tissue to the monitor screen. Vessel diameter was measured by a Colorado Video 321 analyzer modified to function as a video micrometer. With the use of this device, two movable lines were positioned on the inside walls of the vessel, and a DC voltage proportional to the line separation was recorded with a computerized data collecting system. The resolution of this system was ±1 μm.

Silver–silver chloride electrodes were placed across the narrow proximal portion and the wider distal portion of the cremaster muscle. Muscle contraction was elicited by a Grass S44 stimulator with a square-wave-pulse of 40 μs in duration, at 10 V, and a frequency of 1 Hz for 2 minutes. A 30-minute recovery period was allowed after surgery for the vessels to reach a steady-state baseline tone. The main arteriole supplying the cremaster muscle is considered the first-order arteriole. Arteriole branches are subsequently numbered in descending order of size (see Figure 1). A third- or fourth-order arteriole, paired or unpaired with a venule, was selected for study, and in all experiments, the diameter of the arteriole was measured immediately before stimulation, 1 minute into the stimulation period and immediately after the cessation of the 2-minute stimulation period. In all experiments described in this study, the cremaster was subjected to 2 stimulation periods, a control stimulation period followed 30 minutes later by an experimental stimulation period. In all experiments described in this study, the diameter of the arteriole was measured immediately before stimulation, 1 minute into the stimulation period and immediately after the cessation of the 2-minute stimulation period.

Results

The Table summarizes the mean diameters of arterioles observed in this study at rest and during maximum dilation in

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>n</th>
<th>Resting Diameter, μm</th>
<th>Diameter During Sodium Nitroprusside (10 μmol/L) Superfusion, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third order, paired</td>
<td>19</td>
<td>31±2*</td>
<td>65±3*</td>
</tr>
<tr>
<td>Third order, unpaired</td>
<td>12</td>
<td>13±1</td>
<td>29±3</td>
</tr>
<tr>
<td>Fourth order, paired</td>
<td>12</td>
<td>12±2</td>
<td>30±3</td>
</tr>
<tr>
<td>Fourth order, unpaired</td>
<td>12</td>
<td>10±1</td>
<td>17±1†</td>
</tr>
</tbody>
</table>

Data are mean±SEM; n is number vessels studied.

*Significantly different from all other arterioles; †significantly different from paired fourth-order arterioles.
response to sodium nitroprusside (SNP, 10 μmol/L). The mean diameter of paired third-order arterioles (n=19) was significantly greater than that of unpaired third-order arterioles (n=12). In addition, SNP resulted in a significantly larger dilation of the paired third-order arterioles compared with the unpaired third-order arterioles (P<0.05). No difference was observed between the mean resting diameters of the paired (n=12) and unpaired (n=12) fourth-order arterioles, whereas the response to SNP was significantly greater in the paired vessels (P<0.05). The unpaired third-order arterioles had resting diameters similar to the paired fourth-order arterioles, and these vessels exhibited similar responses to superfused SNP.

**Effect of Indomethacin on Functional Dilation**

Figure 2A shows that electrical stimulation of the hamster cremaster muscle for 2 minutes resulted in significant dilation of paired third-order arterioles (from 32±3 to 57±5 μm, n=6, P<0.05). Although indomethacin had no effect on the resting (control) diameter of these arterioles, it significantly attenuated the response to electrical stimulation (from 29±4 to 38±5 μm, P<0.05). Indomethacin vehicle (sodium carbonate) had no effect on responses to electrical stimulation in paired third-order arterioles (Figure 2B, n=3). Unpaired third-order arterioles diluted from 14±1 to 26±2 μmol/L during muscle stimulation (Figure 2C, n=6). Neither the control diameter or the response to muscle stimulation of unpaired third-order arterioles was affected by the presence of indomethacin.

Paired fourth-order arterioles diluted from 18±4 to 35±5 μm after 2 minutes of electrical stimulation (Figure 3A, n=6). Neither the control diameter or the response to stimulation was altered by the presence of indomethacin. Similar results were obtained with the unpaired fourth-order vessels (Figure 3B, n=6). In the absence of indomethacin, electrical stimulation resulted in an increase in arteriolar diameter from 11±2 to 21±2 μm. Indomethacin had no effect on control diameter or on the response to muscle stimulation.

**Effect of Glibenclamide on Functional Dilation**

Figure 4A shows that electrical stimulation of the hamster cremaster muscle for 2 minutes resulted in significant dilation of paired third-order arterioles (from 27±5 to 48±6 μm, n=6, P<0.05). Although glibenclamide had no effect on the control diameter of these arterioles, it significantly attenuated the response to electrical stimulation (from 26±4 to 31±5 μm, P<0.05). The vehicle for glibenclamide, DMSO, had no effect on control diameter or responses to muscle stimulation in third-order arterioles (Figure 4B, n=4). Figures 4C and 5, A and B, show that muscle stimulation caused functional dilation of the remaining 3 groups of arterioles that was significantly attenuated by glibenclamide in all cases (P<0.05, n=6).
Discussion

This study tested the general hypothesis that the mechanisms underlying functional dilation differ, depending on the size of the arteriole examined and on its orientation within the microcirculatory vascular bed. To this end, we examined the role of cyclooxygenase products and KATP channels in arteriolar dilation mediated by electrical field stimulation of the hamster cremaster muscle in small (third- and fourth-order) arterioles paired or unpaired with venules. Our results suggest that regulation of functional hyperemia is multifactorial. We have demonstrated that cyclooxygenase products are important in functional dilation of larger arterioles, which are paired with a venule, and are less important in smaller vessels. In contrast, KATP channels were found to be important in functional dilation of all arterioles, regardless of size or relation to venules.

Recently, the mechanism by which the metabolic needs of the tissue are communicated to the vasculature has been a topic of interest in our laboratory and other laboratories. Lash and Bohlen11 proposed that larger arterioles play a significantly larger role in functional hyperemia than smaller arterioles because they contribute a much greater fraction of the total resistance than do small arterioles. Indeed, these authors demonstrated that only major venules paired with intermediate- and large-diameter arterioles had a large and sustained decrease in perivenular P\textsubscript{O\textsubscript{2}} during contraction of the rat spinotrapezius muscle and thus proposed the concept of cross-talk between large venules and arterioles. In 1990, Falcone and Bohlen12 reported that acetylcholine could stimulate the release of nitric oxide from the venular endothelium of rat intestinal and spinotrapezius muscle, which subsequently dilated the adjacent arteriole. Similarly, studies from our laboratory have suggested that arteriolar dilation of large hamster cremaster arterioles, in response to electrical field stimulation, may be due to arachidonic acid metabolites released from the endothelium of adjacent venules.1,3,4 However, as vessels approach the capillary bed, they lose their paired arrangement and thus an alternative explanation for the arteriolar dilation must be sought.

Consistent with previous findings in our laboratory in which first- or second-order arterioles were observed,3 we showed that inhibition of cyclooxygenase with indomethacin attenuated functional dilation of third-order arterioles paired with a venule (Figure 2A). These results support the hypothesis that during increased muscle metabolism, a metabolite of cyclooxygenase, such as prostacyclin, is released from the venular endothelium, which diffuses to and dilates the adjacent arteriole. This hypothesis is further supported by the observation that indomethacin was without effect on functional dilation of third-order arterioles that did not have an adjacent venule (Figure 2C).
In contrast to the results observed with the third-order arterioles, inhibition of cyclooxygenase did not affect functional dilation of paired fourth-order arterioles (Figure 3A). One possible explanation for this may lie with the method in which we classified the arterioles in this study. Classifying vessel branch orders from largest to smallest may not correlate well with the physiological function of these vessels. Others have classified arterioles according to their orientation relative to capillary modules. Using this method of classification, it is very likely that the unpaired third-order arterioles and the paired fourth-order arterioles observed in the present study are functionally similar. Consistent with this idea is the fact that these vessels were observed to have similar diameters at rest and at maximal dilation in response to SNP (Table). We also observed that at the level of fourth-order arterioles, pairing with venules became less well defined, with many small-venule branches running in many directions. These venules were not as close to the arterioles as were the higher-order vessels and in fact, in some preparations, it was not possible to find paired fourth-order vessels at all. Thus, we hypothesize that at this level of the microcirculation, the relation between arterioles and venules is not as functionally important as it appears to be at the higher levels.

In our second set of experiments, we examined functional dilation in the absence and presence of the $K_{ATP}$ channel blocker glibenclamide. In these experiments, we observed an almost complete abolition of functional hyperemia in the presence of glibenclamide in all 4 vessel groups (Figures 4 and 5). These results are consistent with previous studies from our laboratory in which glibenclamide was observed to significantly attenuate functional dilation of paired first- and third-order vessels. However, in the present study, we were able to demonstrate that the effect of glibenclamide was not dependent on the presence of a paired venule. These results suggest that during periods of increased muscle metabolism, $K_{ATP}$ channels are activated throughout the microcirculation. Activation of $K_{ATP}$ channels results in hyperpolarization of vascular smooth muscle cells, which reduces entry of calcium through voltage-dependent calcium channels, resulting in vasodilation. It is possible that tissue factors such as increases in hydrogen ions, adenosine, lactate, or decreases in oxygen are responsible for activating $K_{ATP}$ channels during muscle stimulation at the terminal arterioles. The resultant hyperpolarization may then be conducted several arteriolar generations upstream. Because conducted vasodilation appears to decay with distance in small arterioles, it is likely that in the larger arterioles, the signal for hyperpolarization and thus dilation may arise from cyclooxygenase products released from the adjacent venules.

**Summary**

Our results suggest that functional vasodilation is mediated by at least two different mechanisms. Cyclooxygenase products from venular endothelium appear to be important in larger feed arterioles, whereas $K_{ATP}$ channels appear to be important throughout the entire vascular tree. Together, these mechanisms may be responsible for initiating and maintaining vasodilation, as required, during periods of increased muscle metabolism.

**Acknowledgments**

This work was supported by grants from the National Institutes of Health (HL-51971) and the American Heart Association (a grant-in-aid).

**References**

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Hypertension. 2001;37:599-603
doi: 10.1161/01.HYP.37.2.599

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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