Extracellular Calcium and Vascular Responses After Forearm Ischemia

Virginia A. Imadojemu, MD; Kenneth Mooney, MD; Cindy Hogeman, BA, BSN, RN; Mary E.J. Lott, PhD; Allen Kunselman, BS, MA; Lawrence I. Sinoway, MD

Background—The myogenic response is a phenomenon in which blood vessels respond to increases and decreases in transmural pressure with constriction and dilation, respectively. Despite intense investigation into the signaling mechanisms underlying this response, the precise mechanisms remain unclear. It has been suggested that the myogenic response occurs when pressure or stretch evokes increases in vessel wall tension that results in vessel smooth muscle cell depolarization. This causes Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels. Of note, in vitro studies demonstrate that the magnitude of the myogenic response is dependent on the extracellular Ca\(^{2+}\). We tested the hypothesis that in conscious humans, physiological changes in extracellular Ca\(^{2+}\) concentrations would be an important determinant of the myogenic response.

Methods and Results—Venous blood ionized calcium was used as an index of interstitial calcium and was measured 5, 15, and then every 15 seconds for 75 seconds, then every 30 seconds for 90 seconds, then finally at the 300-second mark. Forearm blood pressure and flow velocity were determined after 10 minutes of forearm ischemia. We found that the rate of change in serum calcium levels varied as a function of transmural pressure (r=0.96). Moreover, the calcium concentration was inversely proportional to forearm blood velocity (r=0.99).

Conclusions—We hypothesize that muscle stretch caused by a rise in transmural pressure raises interstitial calcium by unknown mechanisms and this in turn acts to lower limb flow velocity. (Circulation. 2004;110:79-83.)

Key Words: blood flow ■ calcium ■ vasoconstriction

The myogenic response is an autoregulatory process in which blood vessels respond to transmural pressure elevation with constriction and to pressure reduction with dilation. It is inherent to smooth muscle and independent of neural, metabolic, and hormonal influences.\(^1\)\(^2\) The mechanical stimulus for this response is a pressure-induced change in the tension of the vessel wall.\(^3\) It has been suggested that the myogenic response contributes significantly to autoregulation in several vascular beds. Despite intense investigation into the signaling mechanisms underlying the vascular myogenic response,\(^4\) the precise mechanism remains unclear. It has been suggested that a myogenic response occurs when pressure or stretch through increases in wall tension results in vascular smooth muscle depolarization. This causes Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels.\(^5\)-\(^7\) Despite some evidence suggesting that the myogenic response is due to changes in cellular Ca\(^{2+}\) sensitivity, the majority of evidence supports the concept that calcium concentrations within smooth muscle cells must rise for myogenic constriction to occur.\(^8\) This rise in intracellular calcium requires a net movement of the ion from the interstitium to the smooth muscle cells. Indeed, in vitro studies demonstrate that the magnitude of the myogenic response is dependent on the extracellular Ca\(^{2+}\) concentration.\(^9\)

The purpose of the present study was to test the hypothesis that in conscious humans, changes in extracellular Ca\(^{2+}\) concentrations would be an important determinant of the myogenic response.

We used venous blood ionized calcium as our index of interstitial calcium. We measured pressure and flow velocity in the forearm as flow was restored after 10 minutes of forearm ischemia and used the changes in forearm blood pressure as an index of transmural pressure. In these experiments, changes in serum calcium levels varied as a function of transmural pressure. Moreover, changes in this calcium concentration were inversely proportional to forearm blood flow velocity. These findings are consistent with the hypothesis that changes in transmural pressure lead to changes in extracellular Ca\(^{2+}\), which leads to directionally opposite changes in flow velocity.

Methods

Subjects
We studied 8 healthy subjects, 4 men and 4 women (Table 1). The studies were performed in a quiet and dimly lit clinical research environment.

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laboratory with the subjects in the supine positions. The protocol was approved by the Institutional Review Board at the Milton S. Hershey Medical Center, and written informed consent was obtained from all subjects.

Blood Pressure and Heart Rate Measurements
Arterial pressure was measured on a beat-by-beat basis by finger photoplethysmography, using a Finapres device (Ohmeda). A Finapres device was placed on a finger of each hand. This device has been shown to reliably register blood pressure changes. Care was taken to fit the finger with an appropriate cuff and to stabilize the hand at the level of the heart. Baseline arterial pressure measurements obtained with the Finapres device were validated with an automated sphygmomanometer (Dinamap, Critikon). The heart rate was derived from the ECG or from the arterial pressure curve.

Limb Blood Flow and Velocity
Brachial blood flow velocity was determined with the use of the Doppler technique, as described previously. This technique permits a beat-by-beat measurement of mean blood velocity (MBV) and thereby allows the detection of rapid and transient changes in flow velocity. The Doppler recordings were later analyzed with a Macintosh computer equipped with a MacLab analysis system (ADInstruments).

Venous Blood
Antecubital venous catheters were inserted in both arms to obtain blood for the measurement of ionized serum calcium.

Experimental Protocol
Blood pressure was measured with finger cuffs (Finapres device) on both hands, and ECG electrodes were placed on the chest to monitor the heart rate and rhythm (Figure 1). MBV was determined by ultrasound probes positioned over the brachial arteries just below the elbows on both arms. The reactive hyperemic blood velocity (RHBV) protocol was performed on one of the subject’s arms; the other arm was used as a control (for both velocity and venous calcium measurements). To measure RHBV, a large pneumatic cuff was positioned on the upper arm as far from the antecubital fossa as possible. During baseline, blood pressure and velocity were measured continuously, and two blood samples were obtained from each arm. The arm cuff was inflated to 250 mm Hg for 1 minute and then released. After a period of rest, the arm cuff was reinflated for 10 minutes. During minute 10 of ischemia, a venous blood sample was obtained from the ischemic forearm and from the opposite freely perfused forearm. On release of the arm cuff, blood pressure and MBV were continuously measured in both arms. Blood samples (1 mL each) were drawn during the last minute of arterial occlusion and at 5, 15, and every 15 seconds after release of occlusion for 90 seconds, then every 30 seconds until the 180-second time period, and then again at the 300 second-mark. After a period of rest to allow the blood flow to return to baseline levels, the RHBV procedure was performed for a second time. The results of the two trials were averaged.

Statistical and Data Analysis
Beat-by-beat Doppler waveforms of brachial blood flow as well as MAP values were averaged over cardiac cycles in the last 10 to 15 seconds of the period of interest, for example, baseline. With the data obtained, we determined the correlation between changes in serum calcium, transmural pressure, and MBV.

If one envisions a “control component” for the relation between calcium and flow velocity consistent with classical control theory, the calcium control component probably would be related to the integrated change in calcium. This type of physiological response system has several advantages. Perhaps the most important advantage is that the calcium signal would be inherently smoothed,
delivering a stable control signal. Accordingly, we postulated that if extracellular Ca\(^{2+}\) was an important determinant of the myogenic response, then venous Ca\(^{2+}\) would correlate with the fall in MBV after peak MBV was achieved.\(^\text{14}\) Moreover, if one envisions that extracellular Ca\(^{2+}\) is an important physiological determinant of the myogenic response, then changes in integrated Ca\(^{2+}\) should correlate with transmural pressure during the period after transmural pressure was changing most dramatically (ie, approximately the first minute after the release of forearm ischemia).

The timing relation between calcium and MBV were examined by determining the breakpoints of the integrated Ca\(^{2+}\) and MBV that followed the release of forearm ischemia. Each curve exhibited a steep, linear early phase (0 to 75 seconds after occlusion) and a shallow, linear late phase (120 to 300 seconds after occlusion). For each parameter, the early- and late-phase data were selected and plotted separately, and linear regression line equations and \(r^2\) values were determined for both phases. Each breakpoint was easily determined by solving the early- and late-phase regression equations for their points of intersection. In the data to follow, we concentrate on the steep early phase of changes in extracellular calcium and flow velocity. We also compared the responses of venous sodium with those of calcium by using multivariate repeated-measures analyses.

**Results**

**Effect of Forearm Occlusion on Serum Ca\(^{2+}\), MBV, Transmural Pressure, Vascular Conductance, and Serum Ca\(^{2+}\) in Active and Control Arms**

The age, body mass index, and gender of the volunteers are shown in Table 1. Data for flow velocity, transmural pressure, and venous calcium (control and ischemic arm) for the entire study are shown in Table 2. Peak velocity after the release of forearm ischemia was noted at 5 seconds and then fell rapidly and in a linear fashion over the next 70 seconds.

On the release of circulatory arrest, venous Ca\(^{2+}\) rose by \(~0.06\) mmol/L during the first 75 seconds past the release of forearm occlusion. The change in integrated Ca\(^{2+}\) from the nadir noted during forearm ischemia and the change in MBV appeared to be inverse of each other (Figure 2A). Of note, Ca\(^{2+}\) tracked forearm pressure (our index of transmural pressure) and integrated Ca\(^{2+}\) paralleled changes in transmural pressure (Figure 2B). The relation between integrated Ca\(^{2+}\) and change in flow velocity is shown in Figure 3A. The relationship between change in transmural pressure and integrated Ca\(^{2+}\) is shown in Figure 3B. Integrated Ca\(^{2+}\) correlated inversely with velocity and directly with transmural pressure.

To exclude the possibility of a dilutional phenomenon as an explanation for the calcium response, we examined the responses of venous sodium obtained simultaneously with calcium in all the subjects. Using multivariate repeated-measures analysis, we compared integrated sodium and integrated calcium responses and noted that the ions behaved differently from each other (main effect, \(P=0.0001\)).

**Discussion**

In the present study, we examined the relation between ionized serum Ca\(^{2+}\) and flow velocity after the release of 10 minutes of forearm ischemia. Forearm ischemia evokes large changes in transmural pressure, and this serves as an excellent model to examine potential relations between transmural pressure and flow velocity, and in the process, examine the myogenic response. The main findings in this report are that there is a strong association between venous Ca\(^{2+}\) levels, transmural pressure, and the fall in forearm reactive hyperemic flow velocity that follows the postischemic peak flow. We believe that these findings are consistent with the hypothesis that extracellular Ca\(^{2+}\) may play an important role in mediating the effects of transmural pressure on flow during the myogenic response.

Previous work has examined the effects of the fall in transmural pressure on the peak flow response seen after ischemia, and others have examined the potential role that

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**Table 2. Responses of Transmural Pressure, Calcium, and MBV at Baseline and Forearm Ischemia Across the Paradigm**

<table>
<thead>
<tr>
<th>Time in Seconds</th>
<th>Base</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>300</th>
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<tbody>
<tr>
<td><strong>Active arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmural pressure</td>
<td>91.9±4.3</td>
<td>56.8±4.2</td>
<td>63.9±3.8</td>
<td>75.0±3.5</td>
<td>80.0±3.1</td>
<td>85.1±3.6</td>
<td>84.8±3.5</td>
<td>89.9±3.1</td>
<td>92.5±4.4</td>
<td>92.6±4.7</td>
<td>92.5±4.6</td>
<td>92.2±4.8</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>1.25±0.01</td>
<td>1.23±0.01</td>
<td>1.26±0.02</td>
<td>1.28±0.01</td>
<td>1.28±0.01</td>
<td>1.29±0.01</td>
<td>1.28±0.01</td>
<td>1.28±0.01</td>
<td>1.27±0.01</td>
<td>1.22±0.01</td>
<td>1.22±0.01</td>
<td>1.21±0.01</td>
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</tr>
<tr>
<td>MBV</td>
<td>4.0±0.5</td>
<td>51.3±5.8</td>
<td>47.7±5.1</td>
<td>38.1±5.4</td>
<td>30.0±4.7</td>
<td>21.5±3.8</td>
<td>15.6±3.3</td>
<td>11.6±2.2</td>
<td>6.7±1.2</td>
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<td>5.5±0.9</td>
<td>4.5±0.6</td>
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<tr>
<td><strong>Control arm</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Transmural pressure</td>
<td>92.2±3.0</td>
<td>91.7±3.4</td>
<td>88.3±3.7</td>
<td>88.7±3.3</td>
<td>90.6±3.7</td>
<td>89.8±3.1</td>
<td>90.8±3.6</td>
<td>90.7±3.1</td>
<td>88.9±2.9</td>
<td>90.5±3.5</td>
<td>90.1±2.7</td>
<td>89.7±3.4</td>
<td>88.3±2.8</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.25±0.01</td>
<td>1.24±0.01</td>
<td>1.26±0.01</td>
<td>1.26±0.01</td>
<td>1.26±0.01</td>
<td>1.27±0.01</td>
<td>1.26±0.01</td>
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<td>1.26±0.01</td>
<td>1.21±0.01</td>
<td>1.22±0.01</td>
<td>1.21±0.01</td>
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<tr>
<td>MBV</td>
<td>2.64±0.40</td>
<td>2.77±0.42</td>
<td>2.38±0.37</td>
<td>2.42±0.44</td>
<td>2.39±0.41</td>
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<td>2.50±0.36</td>
<td>2.20±0.37</td>
<td>2.46±0.37</td>
<td>2.30±0.34</td>
<td>2.45±0.39</td>
<td>2.81±0.46</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 2.** A, Relation between integrated ionized calcium and change in MBV from the point of release of forearm ischemia. B, Relation between transmural pressure and integrated ionized calcium. As transmural pressure increases, calcium also increases.
The signaling and transduction mechanisms underlying the myogenic phenomenon are not well understood. It has been established by numerous animal studies that an increase in transmural pressure causes membrane depolarization and an increase in intracellular Ca\textsuperscript{2+} concentration in smooth muscle cells, which causes vasoconstriction.\(^4,19\)

What is the source of the increased extracellular calcium observed in our report? Interstitial or extracellular calcium may be released from localized calcium stores such as the mitochondria, sarcoplasmic reticulum, and the extracellular matrix.

The extracellular matrix and cytoskeleton have been implicated in mechanotransduction.\(^4\) Recent work has shown that integrins, which are heterodimeric proteins that have large extracellular domains for binding matrix proteins and short cytoplasmic tails,\(^4\) can also facilitate mechanotransduction.\(^24\) It is also possible that conformational changes in integrins alter extracellular Ca\textsuperscript{2+} levels.\(^20\) Whether this contributes to the ability of integrins to activate the myogenic response is unclear.\(^21–23\) Hofer et al\(^25\) have demonstrated the presence of extracellular calcium-sensing receptors that propagate intercellular communication during calcium signaling events within the cell. It is clear that extracellular Ca\textsuperscript{2+} is necessary for the myogenic response because the removal of extracellular Ca\textsuperscript{2+} abolishes the myogenic response.\(^19\) Extracellular calcium enters the cell through voltage-gated calcium channels (VGCCs). VGCCs have been identified in many types of vascular smooth muscle. The L-type channels are believed to be more important in arterial smooth muscle than the T-type.\(^26\) There is a significant amount of evidence that suggests that VGCCs play an important role in determining the myogenic response.\(^4\)

**Limitations**

We realize that our findings do not establish a cause-and-effect relation between serum Ca\textsuperscript{2+} and postischemic flow. To establish this relation, additional studies will be necessary.

We did not examine the role that nitric oxide or other endothelial products might play in modulating the observed responses.\(^15–17\) Additional work will be necessary to examine the interaction between endothelial products and the myogenic response.

The present report suggests that transmural pressure may lead to a rise in plasma Ca\textsuperscript{2+}, which in turn is linked to the myogenic response in the human forearm.

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**References**


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