Intraluminal Flow Increases Vascular Tone and 45Ca2+ Influx in the Rabbit Facial Vein

Daniel Henrion, Ismail Laher, and John A. Bevan

The buccal segment of the rabbit facial vein exhibits a high level of myogenic tone in vitro that develops only in stretched vessel segments between 33° and 44°C. The infusion of physiological salt solution into the lumen of 2-mm-long rabbit facial vein segments induced a flow rate–dependent increase in wall tone, both in the presence (37°C) and absence (30°C) of myogenic tone. In calcium-free physiological solution with EGTA, neither flow nor stretch-induced tone was observed. This flow-induced contraction was associated with an increase in 45Ca2+ unidirectional influx and net uptake. These measurements correlated positively with the level of the associated constrictor responses, both in the presence or absence of myogenic tone. The mean contractile responses to flow (10 and 40 μl/min), stretch, and histamine (1 μM) were 13%, 28%, 24%, and 33% of the tissue maximal response, respectively. When 45Ca2+ influx was expressed in relation to the force development (45Ca2+ influx per milligram), the amount of calcium entry was dependent on the stimulus. Values for 45Ca2+ influx per milligram in response to flow (10 and 40 μl/min) and to histamine (1 μM) were not significantly different. The value was significantly lower for the response to stretch. On the other hand, 45Ca2+ net uptake, when expressed per unit force, was similar in response to flow (10 and 40 μl/min), histamine (1 μM), and stretch. We conclude that flow-induced contraction in the rabbit facial vein is associated with Ca2+ entry into the smooth muscle cells through a pathway that may be different from that associated with stretch-activated contraction. (Circulation Research 1992;71:339–345)

Key Words • flow • veins • blood vessels • contraction • calcium influx • calcium uptake

Changes in intraluminal flow may induce either dilation or constriction in vitro, depending on the level of tone of the smooth muscle cells in the vessels; this phenomenon suggests that the effect of flow is the result of a balance between those forces. Flow-induced contraction occurs in a variety of isometrically mounted arteries and in the perfused pial and femoral arteries. It is dependent on the presence of extracellular calcium but not the integrity of the endothelium. The findings of previous studies indicate that the flow-induced contraction mechanism might be expected to be different from that required by stretch-induced myogenic contraction.

To gain insight into the mechanisms involved in flow-induced contraction and to compare these with those related to stretch, we studied 45Ca2+ influx and net uptake in the rabbit facial vein (RFV). This is an isolated vascular segment that responds by contraction in vitro to both flow and stretch. The RFV possesses a high level of myogenic tone in vitro that is temperature dependent. It is present at 37°C and absent at 30°C. Tone that is due to other stimuli, including flow (the present study), is not temperature dependent. Thus, this preparation allows a study of the constrictor response to flow in either the presence or the absence of myogenic tone. This allows for the comparison of the responses of the RFV to flow and stretch in the same preparation and the opportunity to assess whether stretch and flow-induced contraction involve the same pathway. Moreover, because only minimal levels of flow-induced dilation have been observed in the RFV in the conditions we used, the response to flow in this vessel is predominantly one of flow-induced constriction.

Flow was induced by the infusion of physiological salt solution (PSS) at different rates into the lumen of myograph-mounted RFV segments. PSS was infused when myogenic tone was present (at 37°C) or absent (at 30°C). The resultant changes in wall force were recorded isometrically. The experimental evidence suggests that flow-induced contraction is associated with calcium influx and uptake that is related to the magnitude of the response, through a pathway different from that for myogenic (stretch) tone.

Materials and Methods

Rabbit Facial Vein

Buccal segments of the RFV were isolated as previously described and mounted as 2-mm-long ring segments between parallel stainless-steel wires (0.2 mm in diameter) in 3-ml organ baths. The average separation of the wires was 4 mm (after stretching the segment). One wire was attached to a fixed support; the second wire was connected to a movable holder supporting a tension transducer (model FT.03, Grass Instrument Co., Quincy, Mass.) so that isometric force measurement could be recorded on a myograph (model SD 90,
Grass). The vein segments were maintained at either 30° or 37°C and equilibrated with 95% O₂–5% CO₂ in PSS with the following composition (mM): NaCl 130, NaHCO₃ 14.9, KCl 4.7, CaCl₂ 1.6, KH₂PO₄ 1.2, MgSO₄ 1.2, and glucose 11. The PSS was bubbled with 95% O₂–5% CO₂, and the pH was 7.4. After a 30-minute equilibration period, the segments were subjected to a 500-mg force (stretch) when at 30°C. This is the optimum length for the development of agonist tone for this vein. It produces a maximum level of stretch-induced myogenic tone in the RFV when temperature is increased to 37°C. Myogenic tone is taken as the level of maintained equilibrium response.

PSS (maintained at 37°C and bubbled as described previously) was infused into the RFV lumen through a glass micropipette whose tip (0.2 mm i.d. and 0.35 mm o.d.) was placed within one of the open ends of the ring without occluding it. The PSS was infused by means of a Masterflex peristaltic pump (model 7550-60, Cole-Parmer Instrument Co., Chicago, Ill.) with Tygon tubing (0.2 mm i.d.). A length of the infusion pipette (3 cm), which contained a 10-μl volume of PSS, three to four times that of the internal volume of the mounted RFV, lay beneath the bath fluid level. This ensured that the temperature of the PSS infusion was the same as the solution bathing the vein segment. Flow-induced contraction is relatively insensitive to temperature. The transit time for the PSS through the submerged pipette, at 10 μl/min, is 80 seconds. At all times the infusate had the same composition as the bath solution. PSS was infused into the RFV lumen every 5 minutes for 2 minutes, until stabilization of the size of the response. The equilibrium level of myogenic tone was taken as the difference in baseline recorded after decreasing the bath temperature to 30°C. Responses to intraluminal flow or histamine (added to the bath solution) were determined as increases in wall force over baseline (see Figure 1). In some experiments the endothelium was removed by infusion of air bubbles into the lumen of the RFV segments (10 μl/min for 15 minutes). The acetylcholine (1 μM)–induced relaxation of histamine (10 μM)–precontracted RFV segments was determined both before and after this procedure. Loss of relaxation at this concentration of acetylcholine was taken as an indicator of functional loss of the endothelium.

\[ 4^{53}Ca^{2+} \] Net Uptake

The RFV segments were bathed in PSS containing \( 4^{53}Ca^{2+} \) (0.67 μCi/ml) for 90 minutes in order to label the exchangeable calcium. This was followed by either intraluminal flow (10 or 40 μl/min) or histamine (1 μM) for 3 minutes. Control segments of the RFV, those not subject to intraluminal flow or histamine, were exposed to \( 4^{53}Ca^{2+} \) for 93 minutes. The net cellular \( 4^{53}Ca^{2+} \) content was then determined (see below).

\[ 4^{53}Ca^{2+} \] Unidirectional Influx

\( 4^{53}Ca^{2+} \) unidirectional influx was determined during constriction induced by intraluminal flow (10 or 40 μl/min), stretch, or histamine (1 μM). After equilibration, \( 4^{53}Ca^{2+} \) was added to the bath solution (0.67 μCi/ml) 15 seconds before intraluminal flow or exposure to histamine (1 μM), and the bath solution was vigorously bubbled to ensure a homogeneous distribution of the \( 4^{53}Ca^{2+} \) in the PSS. This was continued for a further 90-second period, during which the RFV segment was submitted to flow, histamine (1 μM), or stretch. The amount of \( 4^{53}Ca^{2+} \) entering the tissues during such a short period (90 seconds) can be assumed to be primarily due to calcium influx from the extracellular space to the intracellular medium. Control segments of the RFV were exposed to \( 4^{53}Ca^{2+} \) for a 15- plus 90-second period, without flow or drug addition. At the end of this period, the net cellular \( 4^{53}Ca^{2+} \) content was determined (see below).

Net Cellular \( 4^{53}Ca^{2+} \) Content

At the end of the influx or uptake experiments, tissues were bathed in ice-cold \( 4^{53}Ca^{2+} \)-free PSS containing EGTA (2 mM) for 45 minutes to remove extracellular \( 4^{53}Ca^{2+} \). The tissues were then blotted, weighed, and incubated overnight in 1.6 ml of 5 mM EDTA solution at room temperature. Finally, 5 ml scintillation cocktail was added, and the vials were analyzed for \( 4^{53}Ca^{2+} \) in a liquid scintillation counter (model LS 7800, Beckman Instruments, Inc., Fullerton, Calif.). Data are expressed as micromoles per kilogram wet weight for the \( 4^{53}Ca^{2+} \) net uptake or micromoles per kilogram wet weight per minute for the \( 4^{53}Ca^{2+} \) influx. The ratio of the \( 4^{53}Ca^{2+} \) net uptake or influx to the corresponding increase in wall force was determined for each experiment and was expressed as \( 4^{53}Ca^{2+} \) net uptake (micromoles per kilogram) or influx (micromoles per kilogram per minute) per unit force (milligram). The correlation between the \( 4^{53}Ca^{2+} \) net uptake or influx and the corresponding increase in wall force in response to intraluminal flow was determined at both 30° and 37°C.
The PSS in the infusion pipette had the same composition as the bath solution (the same concentration of $^{45}\text{Ca}^{2+}$ as in the $^{46}\text{Ca}^{2+}$ net uptake or influx experiments and the same concentration of histamine when histamine was used).

In some experiments, $^{45}\text{Ca}^{2+}$ net uptake and influx were measured in unstretched segments of the RFV at either 30°C or 37°C. The segments were mounted slackly, as described above, between the two wires of the myograph. No force (stretch) was applied.

**Statistical Analysis**

Results are expressed as mean±SEM (n is the number of observations). Significance of the differences between means of the different groups was determined by one-factor analysis of variance, followed by a Student-Newman-Keuls test when significant. Values of $p<0.05$ were considered to be significant.

**Results**

**Effect of Temperature on Myogenic Tone and Intraluminal Flow**

After stretching, myogenic tone developed after 3–5 minutes in the RFV at 37°C (274±22 mg [n=15]). It reached a plateau within 15–30 minutes, which was maintained throughout the experiment (Figure 1). Stretch-induced myogenic tone was dependent on the presence of extracellular calcium and on bath temperature. It was absent in RFV segments bathed in $\text{Ca}^{2+}$-free PSS plus EGTA (2 mM) (baseline as defined in Figure 1, 201.5±39.0 mg [n=6]) or when temperature was decreased to 30°C (baseline, 213.6±22.7 mg [n=15]). Decrease in the temperature to 30°C reduced measured wall force to a level similar to that observed when segments were bathed in a $\text{Ca}^{2+}$-free PSS plus EGTA (2 mM) or when papaverine (10 μM) was added to the bath solution (baseline, 209.4±19.6 mg [n=15]). In RFV segments maintained in PSS at 30°C, removal of calcium by placing tissues in $\text{Ca}^{2+}$-free PSS plus EGTA (2 mM) did not cause further loss of myogenic tone (baseline, 196.2±34.6 mg [n=5]). Moreover, both in segments maintained at 30°C and segments bathed in a $\text{Ca}^{2+}$-free PSS plus EGTA (2 mM), the addition of papaverine (10 μM) did not change the baseline (199.1±26.7 mg [n=6] and 206.8±31.0 mg [n=5], respectively).

A dose–response curve was obtained from the cumulative addition of histamine (from 1 μM to 1 mM). The contractile response to histamine was not influenced by the temperature (maximum, 831.8±103.2 mg [n=11] at 37°C and 716.7±80.3 mg [n=6] at 30°C; ED$_{50}$, 3.8±1.6 μM at 37°C and 3.2±1.4 μM at 30°C). Similar results have been obtained for potassium.

Variation in the intraluminal flow rate of PSS into the lumen of the RFV between 5 and 60 μl/min induced a rate-dependent increase in wall force at both 37°C and 30°C. A maximum increase was reached at 40 μl/min (Figure 2). The maximum response to flow corresponded to 28% of the maximum response to histamine at 37°C and 13% at 30°C. No response to flow was detected in $\text{Ca}^{2+}$-free PSS plus EGTA (2 mM) or in the presence of papaverine (10 μM) at either 30° or 37°C.

The absence of a functional endothelium had no significant effect on either stretch-induced myogenic tone (352±42 mg [n=4] with endothelium and 358±48 mg [n=4] without endothelium) or flow-induced contraction (153.3±17.0 mg [n=4] with endothelium and 186.3±27.0 mg [n=4] without endothelium). In the presence of endothelium, acetylcholine (1 μM) induced a 52±12% relaxation of histamine (10 μM)–precontracted vessels (550±34 mg [n=4]). In the absence of endothelium, acetylcholine (1 μM) did not induce relaxation (−6±2%) of histamine (10 μM)–precontracted vessels (588±45 mg [n=4]).

**Changes in $^{45}\text{Ca}^{2+}$ Uptake and Influx Associated With Stretch-, Histamine-, and Flow-Induced Tone**

In the RFV maintained at 37°C, stretch-induced myogenic tone (stretched segments, 274±22 mg [n=15]; unstretched (slack) segments, 0 mg [n=8]; p<0.05) was associated with an increase in both unidirectional $^{45}\text{Ca}^{2+}$ influx (stretched segments, 35.7±2.5 μmol/kg/min [n=8]; unstretched segments, 22.6±4.4 μmol/kg/min [n=8]; p<0.05) and $^{46}\text{Ca}^{2+}$ net uptake (stretched segments, 262.7±26.0 μmol/kg [n=6]; unstretched segments, 113.0±5.8 μmol/kg [n=6]; p<0.05). At 30°C, no myogenic tone was detectable, and stretch of the RFV segments was not associated with a significant change in either unidirectional $^{45}\text{Ca}^{2+}$ influx (stretched segments, 28.3±5.4 μmol/kg/min [n=7]; unstretched segments, 27.0±1.9 μmol/kg/min [n=9]) or $^{46}\text{Ca}^{2+}$ net uptake (stretched segments, 95.8±7.6 μmol/kg [n=6]; unstretched segments, 94.0±11.4 μmol/kg [n=6]).

Both intraluminal flow and histamine (1 μM) increased $^{45}\text{Ca}^{2+}$ uptake (Figure 3) and $^{46}\text{Ca}^{2+}$ influx (Figure 4) in the presence or in the absence of myogenic tone. The increases in $^{45}\text{Ca}^{2+}$ uptake and influx were flow rate dependent. The increase in wall force paralleled the increase in $^{45}\text{Ca}^{2+}$ uptake (Figure 3) and influx (Figure 4) when the flow rate was increased from 10 to 40 μl/min. In the absence of myogenic tone (30°C), $^{45}\text{Ca}^{2+}$ uptake and influx and the corresponding increases in wall force induced by flow were lower than those measured at 37°C. No change in $^{45}\text{Ca}^{2+}$ uptake and influx or in the corresponding increases in wall force...
induced by histamine (1 μM) were observed between 30° and 37°C.

The expression of the results as 45Ca2+ uptake or influx per unit force (Table 1), allows for a comparison of 45Ca2+ fluxes elicited by stretch, flow, or histamine, independent of differences in the amplitude of responses. Values for 45Ca2+ uptake per unit force at 37°C in response to flow (10 or 40 μl/min), stretch, or histamine (1 μM) were the same and not different from the value measured at 30°C for flow and histamine (1 μM). At 30°C there is no response to stretch. Values for 45Ca2+ influx per unit force at 37°C in response to flow (10 or 40 μl/min) and histamine (1 μM) were the same; the values obtained in response to these conditions did not differ from each other, nor from those at 30°C. However, stretch-induced 45Ca2+ influx per unit force at 37°C was lower than flow or histamine-induced 45Ca2+ influx per unit force (Table 1).

**Correlation of Flow-Induced Contraction and 45Ca2+ Uptake and Influx**

A positive linear correlation was found between flow-induced contraction and 45Ca2+ uptake (Figure 5) and influx (Figure 6) at both 37° and 30°C. For both 45Ca2+ uptake and influx values at 30° and 37°C, the regression line crossed the intercept at a measurement different from zero. This value is close to the control value (for stretched RFV segments without flow or histamine, 45Ca2+ uptake was 95.8±7.6 μmol/kg at 30°C and 262.7±26.0 μmol/kg at 37°C, and 45Ca2+ influx was 28.3±5.4 μmol/kg per minute at 30°C and 35.7±2.5 μmol/kg per minute at 37°C). This control value is located within the 95% confidence interval of the regression line.

**Discussion**

This study is the first showing an increase in vessel wall calcium influx in response to intraluminal flow. The main findings of our study are that 1) flow of PSS into the lumen of the RFV induces an increase in wall force, 2) flow-induced contraction occurs when myogenic tone is present or absent, and 3) it is dependent on extracellular calcium and is associated with an increase in 45Ca2+ net uptake and influx. Both 45Ca2+ net uptake and influx are correlated positively with the contractile response of the RFV to flow both in the presence and the absence of myogenic tone.

The RFV was chosen for use in the present study because of its useful experimental properties, in that it develops in vitro a high level of myogenic tone in response to stretch. The RFV serves as a temperature-sensitive sphincter contributing to cranial thermoregulation.8,9,13 The level of myogenic tone developed depends on the temperature. It is maximal at 44°C and absent below 33°C.9,10 This allows flow-induced contraction that is little influenced by temperature to be studied in either the presence (37°C) or the absence (30°C) of myogenic tone and to be compared with myogenic tone. Myogenic tone in the RFV is dependent on extracellular calcium, probably reflecting the relatively small amount of sarcoplasmic reticulum in its smooth muscle cells.16 It has been previously shown that stretch-induced myogenic tone in the RFV was associated with an increase in 45Ca2+ influx.12 Thus, it represents an isolated preparation in which 45Ca2+ influx and wall force can be measured quantitatively at the same time to both pressure (stretch) and flow. Finally, the RFV responds to flow by an increase in wall force. Only minimal flow-induced dilation could be obtained in this vessel under the conditions of these experiments. This permits the study of flow-induced contraction without the complication of significant concomitant flow-induced dilation. The response to flow in most arteries may represent a balance between flow-induced contraction and dilation.5
Flow-induced dilation was first described in vivo,17 and this phenomenon has been found in vivo in organ systems of various species.6,18–23 Flow-induced dilation has also been observed in vitro in different vessels and species.4,24,25 On the other hand, flow-induced vasoconstriction has been only recently described in vitro in isometrically mounted resistance arteries and veins.1,2 In perfused resistance arteries,4,26 flow-induced contraction has also been observed. It also occurs after endothelium removal in the perfused and superfused rabbit femoral artery.5 Flow-induced contraction in the rabbit ear artery is abolished in calcium-free PSS plus EGTA and by Mn2+ or Ca2+−3.

In our experiments, the RFV responded to flow by an increase in wall force (Figure 2). The flow-induced contraction increased as flow rate increased until 40 μl/min. When the flow rate was over 40 μl/min, the contractile response to flow reached a plateau; when the flow rate was over 60 μl/min, the amplitude of the response decreased (result not shown). Finally, we cannot exclude the possibility that part of the contractile response to flow is due to the washout of a dilator agent present either in the lumen or in the wall of the vessel. We have previously argued that this is an unlikely explanation of flow-induced contraction,1 although recent evidence in support of a role of ATP in this phenomenon has been brought out.27,28

The RFV responded to flow by an increase in wall force that correlated positively with an increase in 45Ca2+ net uptake and unidirectional influx. Both influx and net uptake were measured to quantify the different phases of Ca2+ mobilization during the flow-induced increase in wall tone. The initial 45Ca2+ influx through the plasma membrane is measured after a short exposure (90 seconds in this study) to avoid the complication of back fluxes and exclude the complication of intracellular 45Ca2+ release. These effects have been shown to occur with the action of constrictor agonists.14 The maintenance of contraction is associated with a steady state involving Ca2+ influx and back flux,29 whose equilibrium is quantified by the measurement of net uptake.14 The levels of 45Ca2+ influx and net uptake found in our control experiments were close to those found previously in the rabbit aorta14,30,31 and the RFV.12 The levels of 45Ca2+ influx and net uptake evoked by flow and histamine in our experiments were similar to those found for potassium and for norepinephrine.14,30

Our results suggest differences between flow-induced contraction and stretch-induced myogenic tone (corresponding to differences in temperature sensitivity and in 45Ca2+ influx per unit force). These observations have to be taken together with results of previous studies showing a difference in susceptibility of flow and stretch-induced tone to reduction in the extracellular calcium and a difference in their susceptibility to calcium entry blockers.32 Flow-induced contraction is resistant, and stretch-induced tone is inhibited by verapamil.32 On the other hand, flow-induced contraction is more susceptible to nimodipine than stretch-induced tone.32 The sensitivity to different calcium entry blockers has also

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**Table 1.** 45Ca2+ Influx and Net Uptake Into the Rabbit Facial Vein in Relation to the Level of Developed Wall Force Induced by Stretch, Intraluminal Flow, or Histamine

<table>
<thead>
<tr>
<th></th>
<th>45Ca2+ influx per unit force (μmol·kg⁻¹·min⁻¹·mg⁻¹)</th>
<th>45Ca2+ net uptake per unit force (μmol·kg⁻¹·mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37°C (n)</td>
<td>30°C (n)</td>
</tr>
<tr>
<td>Stretch-induced myogenic tone</td>
<td>0.13±0.02† (7)</td>
<td>NA</td>
</tr>
<tr>
<td>Flow (10 μl/min)</td>
<td>1.04±0.13† (6)</td>
<td>ND</td>
</tr>
<tr>
<td>Flow (40 μl/min)</td>
<td>0.93±0.10† (6)</td>
<td>1.02±0.09† (11)</td>
</tr>
<tr>
<td>Histamine (1 μM)</td>
<td>0.78±0.10† (6)</td>
<td>1.36±0.70† (4)</td>
</tr>
</tbody>
</table>

n, Number of observations; NA, not applicable (no force development); ND, not determined. Values are mean±SEM.

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**Figure 5.** Graphs showing the relation between 45Ca2+ uptake and the increase in wall tone resulting from the intraluminal flow of physiological salt solution in rabbit facial vein segments at 37°C (left panel) or 30°C (right panel). The 95% confidence band of the mean of y (45Ca2+ uptake) is represented.
been used to differentiate stretch-induced and agonist-induced tone. In the RFV, stretch-induced tone was resistant to concentrations of nifedipine, diltiazem, and verapamil, which inhibit the response to potassium.32,33 Thus, stretch-induced tone is different from agonist and flow-induced tone. Finally, whether flow-induced contraction is different from agonist-induced tone (histamine in the present study) cannot be concluded from our results. However, previous observations that flow-induced contraction is resistant to verapamil at a dose that inhibits potassium-induced constriction suggest two different mechanisms.32 Thus, the three types of stimuli studied in the RFV (stretch, flow, and histamine) involve different pathways for calcium entry and/or for the activation of the contractile proteins. The difference in the calcium influx required by flow and stretch to obtain a similar level of constriction is consistent with the observation of calcium-independent processes contributing to the regulation of vascular tone.34 Moreover, the relation between the intracellular calcium concentration and the level of phosphorylation of the myosin light chains, which is related to the calcium-stimulated contraction,35,36 differs with the nature of the contracting stimulus.34 Stretch-induced myogenic tone has been shown to depend on the activation of protein kinase C,37,38 and the calcium sensitivity of myosin light chains is lowest when stimulated by the protein kinase C activator phorbol ester.39 This is consistent with our finding of a relatively low calcium influx requirement by stretch-induced myogenic tone.

It has been proposed that the sensitivity of flow effects to changes in sodium concentration reflects the importance of this ion at a site common to both flow-induced contraction and dilation. This could be a flow sensor located on the smooth muscle cell or its extracellular matrix.40,41 Flow-induced contraction might result from subsequent vascular smooth muscle activation of a calcium entry pathway that is at least different from that associated with myogenic tone and may be different from that associated with potassium and agonist action.

We conclude that the RFV, which exhibits a high level of myogenic tone in vitro responds to intraluminal flow by an increase in wall force. This is associated with calcium influx into the smooth muscle cells that is proportional to the magnitude of flow-induced response. This pathway may be different from that associated with stretch-induced contraction.

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