Augmentation of Endothelium-Independent Flow Constriction in Pial Arteries at High Intravascular Pressures

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The effects of an increase in intraluminal pressure and flow on the diameter and active smooth muscle tone of pial arteries was studied in perfused segments. Resistance arteries (approximately 250–300 μm i.d.) were perfused under controlled pressure and flow conditions, and changes in arterial diameter registered with an automated video device. In any particular segment, diameter measurements were normalized to that observed at 5 mm Hg. Changes in active wall force were determined by relating the observed diameter under a particular set of conditions to the diameter at the same intramural pressure when smooth muscle tone was inhibited (calcium-free physiological saline solution) and to the diameter when smooth muscle cells were activated close to maximum (KCl; 89 mM). At 60 mm Hg, the diameter decrease of 21% in the absence of flow represented stretch-induced tone. No additional changes in diameter were encountered with a flow of 20 μl/min. Diameter decreased a further 7% at 100 μl/min. When intraluminal pressure was 90 mm Hg, diameter decreased 39% without flow. Additional constriction of 10% and 19% occurred at flows of 20 and 100 μl/min, respectively. At the higher pressure, the vasoconstriction occasioned by flow was significantly greater than that at the lower pressure. After endothelium inactivation by passing hypo-osmotic Krebs’ solution followed by air through the segment, mean diameter was less at each combination of pressure and flow, although this difference did not reach statistical significance. The diameter reductions to increases in pressure from 60 to 90 mm Hg and to flow at 40 μl/min were not altered by endothelium inactivation. We conclude that the vasoconstrictor effects of flow as well as pressure are augmented at high intravascular pressures and that these constrictor effects are independent of the endothelium. (Hypertension 1991;17:870–874)
transducer for the flow contraction is a sodium-
dependent system in the vascular smooth muscle cell
or its extracellular matrix. The experiments described
in this article suggest that this system is influenced by
intravascular pressure.

Methods

The perfusion technique used was similar to that
previously described.15 Rabbit pial artery segments
were taken from the middle cerebral artery bed. They
varied in diameter from approximately 250 to 300 \( \mu \)m
i.d. determined at 5 mm Hg intraluminal pressure.
They were mounted in the automated video perfu-
sion system of Halpern et al.17 modified to allow
independent control and registration of pressure and
flow. Arteries were taken from New Zealand White
rabbits (2.5–3.5 kg) of both sexes killed by exsanc-
guination after intravenous sodium pentobarbital (29
mg/kg) and heparin (1,000 units/kg). Arteries were
removed quickly using microsurgical instrumentation
and a dissection microscope, were maintained in cold
physiological saline solution (PSS), and were cannu-
lased at both ends for perfusion of Krebs’ bicarbo-
nate solution equilibrated with 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \) at
37°C. They were immersed in a bath solution of
identical composition. The unstretched length of the
segments was 1.5±0.45 mm (SEM), and they were
mounted with a longitudinal stretch of 20–30%. Diameter was monitored over the middle third of the
segment. Flow was made in the direction of normal
blood flow.

Pressure recordings inside the proximal and distal
cannulas were made using pressure transducers and of arterial diameter with the video monitoring system
on a strip chart recorder.

After an initial equilibration period of 1 hour,
intravascular pressure was set at 60 mm Hg using a
servo-perfusion system connected to the proximal
cannula. Responses were recorded to the addition of
standardizing drugs (histamine, 10\(^{-7}\) M; potassium, 9,
17, 33 mM; acetylcholine, 10\(^{-7}\) M) designed to test
the reactivity of the artery segment. In the absence of
flow, pressure was raised to 90 mm Hg by resetting
the servo-perfusion system, and the new equilibrium
diameter was noted. Measurements of diameter at 60
and 90 mm Hg were repeated to show constancy
(within 10%) of response.

Flow of preheated, preequilibrated PSS was
achieved by using two servo-perfusion systems to
control both the absolute levels of pressure and the
transarterial pressure gradient. By this means, a
specific flow could be achieved at different mean
pressures. At both 60 and 90 mm Hg, equilibrium
responses to flow were recorded first at 20 \( \mu \)l/min and
then after flow was discontinued and a rest period at
100 \( \mu \)l/min. Preliminary experiments had shown that
the order in which these flows were made did not
influence equilibrium response. Mean values of re-
sponses for each experimental condition were de-
lected for each segment and used in subsequent
analyses.

All diameter measurements were normalized to
the internal diameter measured at 5 mm Hg within
the first 30 minutes after setup, and this is referred to
as basal internal diameter. This pressure is the lowest
pressure at which the corrugations in the artery wall
disappear and also one at which there is no active
myogenic tone. The diameter achieved by a close-to-
maximum level of active smooth muscle tone was
determined at a particular pressure in the absence of
flow during the experiment by changing the bath
solution to one containing KCl (89 mM) obtained by
iso-osmotic substitution for NaCl. In separate experi-
ments, we found that this value represented approx-
imately 90% of the maximum tone that this artery
could achieve when exposed to histamine (10\(^{-3}\) M)
and KCl (89 mM). These latter conditions could not
be used during an experiment because of poor re-
versibility. At the termination of each experiment,
the perfusate and bath solution was changed to
calcium-free PSS containing EGTA (1 mM). The
diameter measurement in calcium-free PSS was con-
sidered the passive diameter of the vessel at a given
pressure. Thus, a change in artery diameter could be
reexpressed as a change in active smooth muscle tone
by relating the observed diameter to the diameter
range found between calcium-free PSS and KCl (89
mM) PSS. This provides a measurement of passive
change in vascular smooth muscle tone independent
of passive change in the artery wall.

The endothelium was inactivated by the perfusion
through the segment for 5 minutes of PSS containing
NaCl (50 mM) followed by air. Complete loss of
dilation to acetylcholine (10\(^{-7}\) M) was taken as
evidence that the endothelium had been inactivated.
Only those tissues in which this response had been
completely abolished were included in the experi-
mental series. The perfusate then was restored to
normal, and the artery rested at 5 mm Hg without
flow for 30 minutes. During this whole process, the
artery was submerged in normal PSS at 37°C equili-
brated with 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \).

Statistics

All results are reported as mean±SEM, with the
number of observations reported in parentheses. Tests
of significance were made using the Student’s \( t \) test for
grouped single and paired data as appropriate. A val-
ue of \( p<0.05 \) was considered statistically significant.

Results

Relation Between Flow Rate and Arterial Diameter at
90 mm Hg

In an initial series of experiments, the relation
between flow rate and decrease in diameter was
studied in pial arteries perfused at a mean pressure
of 90 mm Hg. Flow was changed in random sequence.
The results of a single experiment are shown in
Figure 1. There was little difference between the
contractions observed at 60 and 100 \( \mu \)l/min. In all
experiments, flow at 20 \( \mu \)l/min caused a response that
RESPONSES OF PIAL ARTERIES TO FLOW (200–300 μm BID)

FIGURE 1. Line graph showing relation between change in diameter expressed as percent of maximum change and intravascular flow from a single experiment carried out on rabbit pial artery. BID, basal internal diameter of the segment; the diameter at 5 mm Hg without flow was 280 μm (for further details, see text).

fell within the range of 30–70% maximum flow response. On the basis of these trial observations, the studies included in this paper were undertaken at flow rates of 20 and 100 μl/min.

Effect of Intraluminal Flow on Arterial Diameter at 60 and 90 mm Hg

As the branching of the middle cerebral artery was not consistent and there was variation in the diameter of the segments used, change in diameter is expressed as a percent of the basal internal diameter. This allowed normalization of responses, facilitating comparison of arteries of differing diameters. The two pressures studied were 60 and 90 mm Hg.

In the absence of flow (Figure 2); arterial diameters at 60 and 90 mm Hg were 119±5.2 (11) and 94±4.3 (11). These values were significantly different from those determined in calcium-free PSS, which were 150±5.7 (11) and 153±4.0 (11), respectively.

At an intraluminal pressure of 60 mm Hg, flow at 20 μl/min had no effect on diameter. At the same pressure, the maximum effect of flow was to reduce the mean diameter further by approximately 7%.

When flow was made at a mean pressure of 90 mm Hg, significant changes in diameter occurred at both the lower and higher flow rates. These were 10% and 19%, respectively, and represent significant decreases. The effect of flow at 100 μl/min was greater than that at 20 μl/min.

**Effect of Intraluminal Flow on Vascular Smooth Muscle Tone at 60 and 90 mm Hg**

Our experimental protocol was designed to separate passive from active changes in diameter. For this reason, at each pressure in each animal, the experimentally observed diameter was related to the diameter range between that in calcium-free PSS when there was no active tone, and that on exposure to KCl (89 mM) when close-to-maximum activation of the smooth muscle cells occurs.

At the two pressures studied in these experiments, neither the diameters attained in calcium-free PSS (mean values, 148% and 150%, respectively) nor those attained in high-potassium PSS were significantly different from each other. For this reason, the histogram showing diameter changes (Figure 2) adequately reflects changes in active wall tone.

**Effect of Endothelium Removal**

The pattern of diameter change with alteration in pressure and flow was similar after endothelium removal. Segments were only included in this series when, after the denudation procedure, there was complete loss of the dilation seen previously in the intact arteries to acetylcholine (10⁻⁷ M). Data from a second series of experiments (Figure 3) show the changes in diameter expressed as changes in basal internal diameter before and after endothelium removal, at 60 and 90 mm Hg, with and without flow at 40 μl/min. In each circumstance, endothelium removal resulted in a decrease in mean diameter, but in no instance was the change statistically significant.

In the absence of flow, the increase in intraluminal pressure from 60 to 90 mm Hg caused a significant narrowing of arteries both with and without endothelium. At 60 mm Hg, flow at 40 μl/min had

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FIGURE 2. Bar graph showing diameter of rabbit pial artery expressed as percent of basal diameter (determined at 5 mm Hg) under different conditions of pressure and flow. Measurements were made at 60 and 90 mm Hg without flow, with flow at 20 and 100 μl/min, and in calcium-free physiological saline solution. The latter represents the arterial diameter in the absence of smooth muscle tone.
only a small insignificant effect; however, at 90 mm Hg, this flow rate caused significant vasoconstriction in both intact and denuded pial arteries. At this pressure, reductions in diameter with flow in arteries with and without endothelium were not significantly different in magnitude.

**Discussion**

Intravascular flow can cause vasoconstriction,13,14,18 This effect can be distinguished from the myogenic (stretch-related) constriction that follows a rise in intravascular pressure19 (see below). The phenomenon has been observed only in isolated artery experiments. In vivo, increases in pressure and flow often occur at the same time in the same vascular bed; however, no attempt has yet been made to separate the consequences of these two stimuli on vascular wall tone.

In this study, flow at 20 µl/min, when the pressure was 60 mm Hg, caused no additional constriction. However, a decrease in diameter did occur when flow was raised to 100 µl/min. When pressure was raised to 90 mm Hg, significant flow constriction was observed at 20 µl/min and a further significant constriction at 100 µl/min. Of the active constriction observed at a pressure of 60 mm Hg and with a flow of 100 µl/min, 76% is pressure-dependent and 24% flow-dependent. At the higher pressure studied, 67% of the total diameter change is pressure-related.

The data suggest that an increase in cerebrovascular tone, occasioned by a rise in pressure or flow, is not simply additive. This is to be expected, because the effect of flow is not simply due to an increase in the sensitivity of the artery to pressure but depends on different cellular mechanisms. Flow constriction is sodium-dependent,16 and the stretch-induced effect is calcium-dependent.20 Opinions differ regarding the mechanism of stretch-induced constriction of resistance arteries.21-23 Both endothelium-dependent and -independent hypotheses have been proposed, and different conclusions may result from the method used to destroy or remove the endothelium. After chemical methods have been used, the myogenic response disappears, and after mechanical means it tends to be preserved.8,24,25 Flow constriction has been claimed to be only endothelium-independent.13-15 Researchers have shown flow-dependent dilation in small arteries supplying the cremaster muscle to be prevented by endothelium impairment,11,26 a conclusion confirmed by others.6,8 In this study, the changes in artery diameter to both pressure and flow occurred after endothelium removal. The mean diameters of the perfused artery at a particular pressure and flow were invariably less after endothelium destruction, although the effect was not statistically different. However, the changes due to pressure and flow on arterial diameter were essentially the same after destruction of the inner tunica of the blood vessel wall.

It is interesting to speculate why flow constriction should be augmented at high intramural pressures. One suggestion depends on the observation that flow probably elicits simultaneously both constrictor and dilator changes. The final level of tone results from the algebraic interaction of flow constriction and flow dilation.27 In the perfused artery, flow dilation is the dominant effect at lower intraluminal pressures and flow constriction at higher pressures.12 Thus, an increase in flow could result from a smaller flow dilation, a greater flow constriction, or both. Without a specific antagonist of one response, this cannot be resolved. Flow contraction may be associated with membrane depolarization (G.C. Wellman and J.A. Bevan, unpublished observations, 1991), which would lead to calcium entry through potential-sensitive calcium channels.28 Several investigators29,30 have shown that myogenic tone can be negatively correlated with the membrane potential. Thus, at higher pressure, the membrane potential is lower and closer to the level at which an additional depolarization, for example, resulting from flow might result in a larger tone increase than at lower pressures.

It has been suggested that the vascular response to flow is to maintain wall shear stress.7,29 However, our data are not consistent with this conclusion. At 20 µl/min, flow did not alter vascular smooth muscle tone at 60 mm Hg; it was the same as "no flow." This
flow rate only resulted in altered diameter at 90 mm Hg. The data also show that the response to flow is not to maintain the level of intrinsic tone, that is, tangential wall stress.

The lower pressure was chosen because in preliminary experiments flow at 20 μl/min did not cause a significant change in diameter. It was presumed to represent an approximate null point at which the constrictor and dilator effects of flow were balanced.22 It also is a value that probably is close to the physiological pressure in this artery.31 The higher level of 90 mm Hg was arbitrarily selected. It represents a 50% increase in pressure, and experimentally repeated shifts of intraluminal pressure between 60 and 90 mm Hg resulted in reproducible changes in arterial diameter, that is, in levels of myogenic tone and of the effects of flow.

The cerebral circulation is known to maintain a constant cerebral blood flow in the face of changes in perfusion pressure. A number of factors are recognized to contribute to this regulation, such as constrictor and dilator innervation, metabolite formation, and intravascular pressure.32 The results of these experiments suggest a role for flow. At higher pressures, flow-induced constriction may contribute to autoregulation. If flow is a regulated vascular parameter of the brain circulation and contributes directly to the homeostasis of hemodynamics, it would seem preferable that flow itself be monitored.

References


KEY WORDS: endothelium • blood flow • constriction • cerebral arteries • blood pressure • myogenic tone
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