Mesenteric afferent nerves are sensitive to vascular perfusion in a novel preparation of rat ileum in vitro

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A DENSE NEURAL NETWORK is present around gastrointestinal blood vessels. This “perivascular nerve plexus” is comprised of both sympathetic postganglionic fibers and primary sensory (afferent) neurons (see Ref. 15). Sensory nerves, originating from cell bodies in the dorsal root ganglia, project to the intestine following the mesenteric vasculature (12) sending collateral projections predominantly to small arteries and arterioles (3, 11, 37) where they form varicose or smooth terminals either in the adventitia or at the medial-adventitial border (22). These terminals colocalize the neuropeptide substance P (SP) and calcitonin gene-related peptide (12), both of which are known to dilate mesenteric vessels (15, 38). In addition, SP may modulate transmitter release from perivascular sympathetic nerves (7), suggesting a complex interplay between both sympathetic efferents and perivascular afferents in the regulation of vessel tone.

Extrinsic afferents, i.e., sensory nerves originating outside the gut, are implicated in the local control of gastrointestinal blood flow (17, 37, 40). In addition, by regulating blood flow in response to potential noxious stimuli such as H⁺, these afferent nerves can also serve a protective function. Being highly sensitive to capsaicin, an invaluable experimental tool that selectively activates extrinsic afferent nerves (14, 23), many reports support that these afferents have a dual sensory and motor function (24). Activation of these so-called “sensory-motor” nerves can lead to release of vasoactive peptides from axon collaterals after antidromic invasion of the terminals (24). This antidromic axon reflex is thought to mediate the vasodilatory “flare” reaction in the skin in response to injury, and similar reflexes may underlie hyperemia in response to various stimuli in the gut lumen including nutrients (32) and H⁺ (1, 34). In this way, sensory-motor nerves can mediate a number of homeostatic functions at a local level. In contrast to the “efferent” (motor) actions, it is not known whether these sensory nerves also have an “afferent” (sensory) function at the level of the blood vessel. Furthermore, could these nerves provide a role in the moment-moment regulation of local circulation?

Clearly to understand the physiological role for these extrinsic afferents in sensory signaling, we need more information about the range of stimuli that can activate them. We hypothesized that perivascular afferents can actually sense changes in blood flow in addition to their efferent actions on vessel tone. The physical qualities of blood flow (forces associated with flow and pressure), the chemical changes (O₂/CO₂/pH) associated with changes in perfusion, or even the contractile state of the encompassing vascular smooth muscle could all act as potential stimuli. In this way, extrinsic afferents with terminations near blood vessels could be envisaged to have a role in the fine control of local blood flow. The aim of the present study was to investigate this potential sensory role by developing a novel in vitro preparation of vascularly perfused ileum from the rat. To this end, we recorded vascular perfu-
mesenteric artery was cannulated to permit intravascular perfusion and the system was connected to a pressure transducer to allow monitoring of VPP. VPP was used as an index of vascular tone/blood flow. The artery was perfused with a modified KBS (+3% Dextran, 10 mM glucose, 0.6 mM glutamine) vigorously gassed with 95% O₂-5% CO₂ and perfused with a peristaltic pump. Pulsatile flow did not result, however, because vascular fluid was also passed through a bubble trap. To prevent fluid build-up in the recording chamber, the venous effluent was allowed to drain into the gut chamber by puncturing the small veins draining the segment. The aperture linking the two compartments was then sealed with grease, and the recording compartment was filled with heavy liquid paraffin. The preparation was allowed to warm slowly, reaching a working temperature of 32–34°C before a mesenteric nerve recording was obtained.

Nerve Recording

Under a stereo microscope, one of the two paravascular nerve bundles was teased out from between the small artery and vein supplying the segment and wrapped around one arm of a bipolar platinum recording electrode with a length of connective tissue attached to the second electrode. The electrodes were connected to a neurelog headstage (NL 100), and the signal was amplified (NL 104, ×20,000) and filtered (NL 125 band width 100–1,000 Hz) then relayed to a spike processor (Digitimer D130) to allow discrimination of action potentials from noise using a manually set amplitude and polarity window. The whole nerve recording was displayed on a storage oscilloscope (Tektronix 5111A) and digitized (PCM-2 A/D VCR adapter, Medical Systems) to allow recording on VHS video tape for future off-line analysis. Whole nerve activity was continually monitored as spike discharge [impulses (imp)/s] and stored on a personal computer using Spike-2 software (Cambridge Electronic Design).

Experimental Protocols

Sensory-motor function. To test the ability of afferent nerves to influence vascular tone, capsaicin was used to specifically stimulate extrinsic primary afferents. Capsaicin was applied into the gut lumen in intact gut-on preparations and intra-arterially in gut-off preparations. In the former, vascular flow rate was set at 1 ml/min and the vasculature was constricted with l-phenylephrine (LPE; 100 μM) in the serosal bathing media. LPE increased VPP, which plateaued after 15–20 min. Preparations were discarded if this plateau was not achieved after 30 min. Once VPP was stable, a 2-ml solution of the vehicle control (0.1% DMSO in saline) was rapidly instilled into the lumen of the gut segment, followed after 15 min with an equal volume of capsaicin (100 μM, DMSO 0.1% in saline). In gut-off preparations, a constant infusion of capsaicin (100 nM) was applied intra-arterially once a stable plateau in VPP was reached using LPE (10 μM), also applied intra-arterially.

Afferent responses to changing vascular perfusion rate. We examined whether changes in the vasculature (changes in flow with associated pressure changes) could, in turn, alter afferent discharge. The effects of increasing vascular perfusion rate and the response to stopping perfusion were examined in both intact and gut-off preparations. After a stabilization period of 20–30 min, vascular perfusion rate was increased stepwise for 5-min intervals from a rate of 0.6 ml/min to 1.0, 1.4, and 1.8 ml/min in gut-on preparations, and from 1 ml/min to 1.4, 1.8, and 2.2 ml/min, in gut-off preparations. Vascular flow was then returned to basal and main-
tained for 15 min, after which flow was stopped for 5 min before returning again to basal. In preliminary studies with gut-on preparations, increasing vascular perfusion rate often increased motility, which in turn, influenced AD. In the present studies, the gut tube was routinely stretched between the two cannulas in the bathing chamber to minimize motility. The mean discharge rate was measured over the last minute of each 5-min period of increased flow and compared with basal discharge 1 min before the onset of the stepwise changes.

Neuraminidase treatment. The effects of flow rate were also investigated in some gut-off experiments after pretreatment (20 min) with the enzyme neuraminidase (0.2 U/ml). This was added to the vascular perfusion fluid (intra-arterial) in one set and perfused directly into the bath in another set, such that the final concentration/rate of delivery to the bath were similar in both sets of experiments. Neuraminidase is also known as sialidase and is thought to cleave sialic acid residues from the endothelial glycocalyx (20). However, because the vascular fluid also drains into the bath, the enzyme could also have extracellular actions. This was examined using the set of experiments with bath perfusion of neuraminidase. Preparations were perfused for a 20- to 30-min equilibration period before neuraminidase was added and then the effects of changing flow rate were examined. Neuraminidase was present throughout the experiment. Separate tissues were used for each experiment.

Functional viability tests after neuraminidase. The effects of intra-arterial neuraminidase treatment on the functional viability of both vascular muscle and endothelium were also examined in a separate set of experiments. Here, responses to the muscarinic agonist bethanechol (BCh; 5 μM in 3 ml vascular perfusion fluid) were examined in tissues preconstricted with LPE (10 μM ia). Response to LPE and BCh were compared in preparations treated with intra-arterial neuraminidase (0.2 U/ml, 20 min before and present throughout the experiment) and compared with time-controlled experiments. Vasconstriction induced by LPE and vasodilatation induced by BCh were used as indexes of vascular smooth muscle and endothelial viability, respectively. In addition, in some experiments, the effects of capsaicin (100 nM ia) were also investigated to examine whether capsaicin-induced vasodilatation was affected by the enzyme treatment.

**Data Analysis**

Data are expressed as means ± SE. Two means were compared using unpaired t-tests or Mann-Whitney rank sum tests where appropriate. Multiple comparisons against control were conducted using Dunn’s method after repeated-measures ANOVA on ranks. Values of \( P < 0.05 \) were considered significant.

**RESULTS**

**Effect of Capsaicin Applied to the Gut Lumen**

To test the link between extrinsic afferent nerves and blood vessel tone in our gut-on preparation, the effects of instilling capsaicin into the gut lumen were investigated and compared with vehicle controls. In four of five intact preparations, luminal capsaicin caused a transient (73 ± 14 s) activation of afferent discharge (Fig. 2). This was accompanied after a delay of 51 ± 22 s by a fall in VPP that reached a maximum after 5.5 ± 0.4 min. As seen in Fig. 2, these effects of capsaicin were independent of changes in gut perfusion pressure caused by either distension with vehicle or capsaicin. Vehicle controls (0.01% DMSO, saline) had no effect on vascular perfusion pressure. This confirmed that selective stimulation of afferent nerves could influence vessel tone, thus supporting a functional “intimacy” between afferent terminal and blood vessels in our model. Although this exemplifies the motor action of these sensory nerves, the close association between nerve and blood vessels also prompts the question of whether physiological stimuli within the vessel could in turn influence the afferent terminal. Our further

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**Fig. 2.** Example of the effects of capsaicin and vehicle instilled into the gut lumen. Effects on VPP, AD, and GPP are shown. Group data (\( n = 4 \)) are shown in bar form on right. Falls in VPP represented as %change, while AD and GPP after administration are expressed as area under the response curve. Means ± SE, \(^*\) \( P < 0.05 \) vs. vehicle.
studies focused on the sensory (afferent) function of these extrinsic afferent nerves.

**Effects of Increasing Vascular Perfusion Rate**

In all six experiments, stepwise increases in vascular flow rate caused a concomitant reduction in afferent discharge as illustrated in Fig. 3. The fall in AD usually occurred gradually over the 5 min, with maximal effect in the last minute. Data are summarized in Fig. 4A, where reductions in AD were statistically significant at vascular flow rates of 1.4 and 1.8 ml/min. In addition, AD returned to basal levels when flow was returned to baseline. Because gut perfusion pressure (GPP) was not significantly affected by changes in vascular flow rate, AD appeared to respond to changes in the flow rate (or vascular pressure) itself.

**Effects of Stopping Flow**

In direct contrast to the effects of increased flow, stopping flow caused an abrupt increase in AD as shown in Fig. 3. By measuring average discharge in 1-min intervals (and first 30 s), the profile shown in Fig. 4B demonstrates how the maximum level of discharge was reached within 30 s of stopping flow and was sustained for the 5-min period of “flow-off.”

**Dependence on Vascular Oxygen?**

To test whether the afferent response to increased flow and to terminating flow were the result of flow-dependent changes in vascular oxygen concentration, the O2-CO2 gassing the vascular perfusion fluid was replaced with N2-CO2. The change to N2-CO2 had no obvious effect on AD (data not shown).
addition, when flow rate was increased stepwise after the original protocol, similar falls in AD still occurred (Fig. 5A). These falls in AD were statistically significant at flow rates of 1.4 and 1.8 ml/min, as was seen in controls. In addition, the afferent response profile to stopping flow was also similar to controls as shown in Fig. 5B. The absolute increase in AD [area under curve (AUC)] was therefore, unaffected (control AUC, 2,106 vs. 2,349, N2-CO2, P < 0.05). Thus both the flow-associated falls in AD and increases in AD after flow-off appear to be independent of vascular oxygen delivery.

Dependence on “Gut Factors”? To eliminate the potential for mediators released from the gut wall to influence the afferent responses, a gut-off preparation was used by carefully removing the gut body as described earlier. In these preparations, preconstricted with LPE (10 μM ia), intra-arterial capsaicin (100 nM, constant perfusion) still activated AD and caused a sustained reduction in VPP (37.5 ± 2.5%, n = 4), confirming that afferents can still influence blood vessel tone in these preparations (Fig. 6). In addition, similar falls in VPP (39.5 ± 7.9%, n = 4) were observed in separate experiments pretreated with TTX (1 μM ia), despite a marked reduction in AD induced by capsaicin (impulses in first minute: control, 417 ± 58 vs. 27 ± 16, after TTX, P < 0.05).

Effects of Increasing Flow Rate in Gut-Off Preparations

Although basal afferent discharge was reduced in most preparations once the gut body was cut away, only a minor difference in mean baseline firing resulted (gut-on, 11.4 ± 2.2 vs. 8.25 ± 1.3 imp/s, gut-off). A fall in AD was observed in six of seven gut-off preparations when flow rate was increased stepwise from 1 to 1.4, 1.8, and 2.2 ml/min, as shown in Fig. 7A. Significant reductions in AD occurred at flow rates of 1.8 and 2.2 ml/min (Fig. 7B). Afferent discharge returned to levels that were not significantly different than baseline discharge when vascular flow returned to 1 ml/min.

Effects of Stopping Vascular Flow in Gut-Off Preparations

Even in these simplified gut-off preparations, afferent discharge was markedly increased when vascular perfusion was stopped. In six experiments, the absolute increase in discharge (area under response) was 1,416 ± 365 and the profile of the response, in Fig. 7C, is similar to that in gut-on studies. Again, discharge increased rapidly within the first 30 s and was maintained. Furthermore, the absolute increase in discharge caused by terminating flow was not significantly smaller in gut-off compared with intact preparations (gut-off, 1,416 ± 365 vs. 2,106 ± 646, gut-on, P > 0.05) representing 72 ± 21 and 70.5 ± 13% increases above basal, respectively.

Effects of Neuraminidase (Sialidase)

In six gut-off preparations, intra-arterial neuraminidase treatment had no effect on baseline discharge (mean discharge 5 min before, 4.3 ± 1.5 vs. 5.1 ± 1.3 imp/s, last 5 min neuraminidase treatment, P > 0.05).
Increases in VPP after increased flow rate as shown in Fig. 8A, were also unaffected by the enzyme. However, the falls in AD associated with these increases in flow/pressure were abolished (Fig. 8B). Similar inhibition (4 of 7 experiments) also occurred when neuraminidase was perfused directly into the bathing solution (data not shown). Despite these dramatic effects on flow-induced falls in AD, intra-arterial neuraminidase had no effect on the total AD induced by terminating flow (control, 1,416 ± 365 vs. 1,561 ± 438, neuraminidase, *P > 0.05) or the profile of response, which remains remarkably similar (Fig. 8C). Similarly, bath application of the enzyme had no effect on the afferent response to stopping vascular flow (not shown).

Fig. 7. A: example of the effects of changing vascular flow rate on VPP and AD in a gut-off preparation. Group data for effects of changing vascular flow rate (B) and stopping vascular flow (C). Means ± SE, n = 6, *P < 0.05 compared with first flow rate of 1 ml/min (B) and compared with time (5 min) (C).

Fig. 8. Effect of intra-arterial neuraminidase (0.2 U/ml) on changes in VPP (A) and AD (B) induced by increased vascular flow rate and AD (C) induced by stopping vascular flow. Data obtained from gut-off preparations, n = 6, *P < 0.05 vs. corresponding control values at each flow rate in B.
Effects of Intra-Arterial Neuraminidase on Functional Viability of Vascular Smooth Muscle and Endothelium

Vasoconstriction (maximum increase in VPP) induced by LPE (10 \( \mu \)M ia) was not significantly different after neuraminidase (Fig. 9A). A 3-min pulse of BCh (5 \( \mu \)M in 3 ml vascular perfusing fluid) caused a transient increase in VPP in four of six control and five of six neuraminidase-treated preparations, followed in all experiments by a marked fall in VPP (Fig. 9B). The magnitude of the increase (constriction) and decrease (dilatation) in VPP was unchanged after treatment with neuraminidase (Fig. 9C). Capsaicin (100 \( n \)M ia) caused a sustained fall in VPP in both control \((n = 3)\) and neuraminidase-treated \((n = 3)\) preparations (maximum fall in VPP control, 25 \( \pm \) 4 vs. 20 \( \pm \) 3\%, neuraminidase, \( P > 0.05 \)).

DISCUSSION

The purpose of this study was to examine the functional interaction between extrinsic afferent nerves and the vasculature in the rat ileum. Although it is well established that nonadrenergic noncholinergic nerves are involved in modulating vascular tone/blood flow in the gut, it is not known whether these extrinsic sensory nerves have an afferent function at the level of the blood vessel. By developing a novel in vitro technique where afferent discharge and vascular perfusion pressure are recorded simultaneously, we showed for the first time that afferent discharge is influenced by vascular perfusion, which in turn can be modified when these extrinsic afferents are stimulated by capsaicin.

Effects of Capsaicin Applied to the Gut Lumen (Gut-On)

In contrast to a putative afferent function at the blood vessel, evidence already supports an efferent role for extrinsic afferents. This has largely stemmed from studies involving selective stimulation of extrinsic afferent nerves using capsaicin. Similarly, in our present study, application of capsaicin to the gut lumen increased AD and reduced VPP in intact preparations preconstricted with LPE. It is likely that capsaicin acts through the classical axon reflex whereby impulses are also conducted antidromically invading axon collaterals and releasing vasoactive peptides from afferent terminals near blood vessels. This reflex arrangement has been proposed to mediate mesenteric hyperemia induced by luminal capsaicin in the dog (33). More direct evidence for a TTX-sensitive reflex pathway stimulated by mucosal capsaicin has been shown in the gastric mucosa (16). These studies with capsaicin demonstrate a functional link between extrinsic afferents and the intestinal vasculature and support a role for luminal stimuli in modulating intestinal blood flow. Such neurally mediated events could help orchestrate optimal conditions for nutrient absorption and digestion in addition to serving a protective or “emergency” role, mediating gastrointestinal hyperemia in response to noxious stimuli (see Refs. 1, 15, 16, 36).

Effects of Intra-Arterial Capsaicin (Gut-Off)

Vasodilatation may also occur after release of a vasodilator directly from the nerve terminal without propagation of axon reflexes. This mechanism could explain the capsaicin-induced vasodilatation observed in the gut-off preparations in our study, where dilation induced by intra-arterial capsaicin was unaffected by TTX. A similar mechanism appears to mediate capsaicin-induced vasodilatation in the perfused mesenteric vascular bed (26). Because the gut body was removed in these and in our own gut-off preparations, local submucosal reflexes (see Ref. 40) cannot be involved. We only used capsaicin as an experimental tool to support a link between afferent activation and changes in blood flow (vascular tone) and therefore, refer the reader to more detailed studies of the mechanism of action (5, 14, 23, 39).

Effects of Changing Vascular Perfusion

Having established that afferent activation does influence vascular tone in our models, we then examined whether hemodynamic changes themselves could be “sensed.” The effects of increasing perfusion rate step-wise on AD was examined in both intact and gut-off
preparations. In intact gut-on studies (where segments weighed ~1 g), basal vascular perfusion rate was set at 0.6 ml/min, slightly less than basal flow rate measured in the rat in vivo [1 ml·min⁻¹·g⁻¹ (2)]. Perfusion rate was increased in 0.4 ml/min steps from 0.6 to 1.0, 1.4, and 1.8 ml/min, such that maximum perfusion amounted to three times basal and vascular pressures ranged from 76 ± 9 (basal) to 132 ± 19 mmHg (maximum flow). Hence, perfusion rates and pressures lie within the physiological range. However, because it is difficult to mimic physiological parameters such as blood pressure, which is also under considerable sympathetic nervous control in vivo, perfusion pressure acts only as an index of blood flow in our model.

In gut-on preparations, increases in vascular perfusion rate caused concomitant falls in AD. In addition, discharge returned to baseline levels as perfusion rate was returned to basal. Because these falls in AD were not secondary to falls in gut luminal pressure, AD appears to be influenced by vascular perfusion itself. What then is the stimulus? Because it is difficult to separate flow from pressure in our model, we focused on three potential stimuli, namely the vascular delivery of oxygen, “factors” associated with the gut body, and finally involvement of the vascular endothelium.

Oxygen Delivery?

It is well known that mesenteric afferents are sensitive to ischemia in vivo (10, 19). Because oxygenation would improve with increased vascular perfusion rate, this may in turn have altered AD. However, similar falls in AD still occurred when O₂-CO₂ was replaced with N₂-CO₂ gassing the vascular perfusion. Hence, vascular delivery of O₂ is not the stimulus for flow-associated falls in AD. In addition, the afferent response to terminating perfusion does not represent an effect of hypoxia, because responses still occurred in preparations receiving vascular N₂-CO₂. It is also important to remember that in these preparations, oxygen is still present in the “serosal” bathing solution. Clearly, afferent responses to stopping perfusion in our model represent more direct afferent sensitivity to mechanical forces associated with stopping flow. In addition, influences from blood-borne cells/factors can be excluded in our in vitro models. Indeed, in our studies, the afferent response profile to terminating perfusion was rapid and sustained, unlike the more complex pattern of activity resulting from localized mesenteric ischemia in vivo (19). Although similar ischemic-sensitive afferents probably still exist in our preparations, they are not activated under our in vitro conditions where oxygen can still be obtained from the bathing media. Our simplified in vitro approach allows us to investigate stimuli that are sensed more directly by extrinsic afferents. It would seem unlikely that vascular O₂ would be sensed directly by afferents located within the adventitia. Indeed, a recent study visualizing SP-containing nerves in small mesenteric arteries from the rat indicated that most primary afferent varicosities lay between 0.4 and 2 μm from the vascular smooth muscle, slightly farther away compared with sympathetic terminals (22).

Involvement of Gut Body?

Similar perfusion-associated changes in AD were also observed when the gut body was removed. This suggests that the sensory machinery is not confined to the mucosal and/or submucosal circulation or involve other gut cells, including enteric neurons. The sensing machinery could therefore, reside in larger vessels within the mesenteric fan. Although generally assumed that smaller submucosal arterioles comprise the main resistance vessels in the mesenteric circulation, more recent studies have shown that 40–55% of the total network resistance can indeed be found in larger arterioles and small arteries (31). This is supported by our observations with intra-arterial capsaicin in gut-off preparations where a considerable vasodilatation could still be induced. Thus control of these larger “feed” vessels by extrinsic afferents could still have an important physiological role in the overall regulation of gut blood flow. Furthermore, control mechanisms located upstream could serve a protective role, possibly regulating blood flow to prevent damage caused by inadequate perfusion. Thus, in this highly simplified preparation, the sensing system could be localized to the vessels themselves, an observation that would support the novel concept that mesenteric blood vessels and extrinsic afferent nerves may communicate as a functional unit sensing hemodynamic factors.

Role of the Endothelium?

Due to their anatomic location, it is unlikely that extrinsic afferents sense flow within a vessel directly. However, pressure could distort tissues around the vessel, including afferents within the medio-adventitial border. In contrast, afferent nerves could detect changes in flow indirectly by responding to factors released locally by another sense cell, either vascular smooth muscle and/or the endothelium. Evidence is growing to support functional interactions between vascular endothelium and sensory perivascular nerves in the dual control of vessel tone and blood flow (6, 29). Because the sensing mechanism appears to reside in the mesenteric fan, we examined involvement of the endothelium as a primary “sensor” for perfusion in the gut-off preparation. The enzyme neuraminidase (0.2 U/ml ia, 20 min) was used to disrupt the endothelial glycocalyx. This proved a more subtle approach that did not affect afferent integrity, unlike preliminary studies with vascular saponin, hypotonic shock, and air (not published). Neuraminidase treatment prevents flow-dependent vasodilatation in rabbit mesenteric vessels (30) and perfusion with higher concentrations (2 U/ml, 40 min) inhibited shear stress-dependent nitric oxide release from rabbit femoral arteries (13). In both these studies, enzyme treatment did not affect endothelial responses to ACh, suggesting that the endothelium remained functionally viable. Similarly, our findings support functional viability of both vascular
smooth muscle and endothelium after enzyme treatment applied intra-arterially. Furthermore, afferent terminals were also functionally unaffected by the enzyme, because vasodilatation induced by capsaicin remained similar after intra-arterial neuraminidase.

Despite the lack of effect on endothelial, afferent, and muscle functional viability, perfusion-associated falls in AD were abolished by neuraminidase. Although it is tempting to conclude that neuraminidase disrupts the glyocalyx and, in turn, prevents the endothelium responding to flow, other effects of neuraminidase cannot be excluded. Indeed, because serosal neuraminidase had a similar inhibitory effect (4 of 7 experiments), it is unlikely that the enzyme acts selectively, at least on the endothelial glyocalyx. Although the endothelium is a prime candidate for a “flow-sensor” (8, 9, 18, 20), our findings, at present, cannot support a role for the endothelium. Possible signal transduction pathways are summarized in Fig. 10.

The action of neuraminidase therefore, remains unresolved. Although our control experiments indicate clear extravascular effects of the enzyme, these actions are not necessarily unspecific. Neuraminidase could not only cleave sialic acid residues in the endothelial glyocalyx but also remove residues from neuronal membranes. Although the effects of removing sialic acid-containing substrates from nerves is not fully understood, some functional changes have been documented, including effects on transmitter release (4).

**Effects of Stopping Vascular Flow**

Despite the clear inhibitory effects of neuraminidase on perfusion-associated falls in AD, this enzyme treatment (either intra-arterial or serosal) had no effect on the afferent response to stopping vascular perfusion. Thus afferent responses, which can be up- or down-regulated by rates of vascular perfusion, do not appear to be mediated by a single common mechanism. A separate, neuraminidase-insensitive mechanism underlies the afferent response to stopping vascular perfusion. What then could be the stimulus? Although other workers have demonstrated that gastrointestinal afferents can be activated by global ischemia in vivo (10), this must represent a more complex response involving multiple cell types, including blood-borne mediators. Observations from our in vitro experimental model support the concept that afferents may respond to more physical stimuli associated with terminating perfusion, because hypoxia cannot account for increased AD in our model. Mechanical deformation caused by vessel collapse could provide the major stimulus. Deformation of the endothelium or vascular smooth muscle could release factors that, in turn, alter AD. Alternatively, perivascular afferent nerve terminals may themselves possess mechanosensitivity. Interestingly, the protein stomatin, thought to play an important role in linking mechanically gated ion channels to the cytoskeleton, is expressed by all sensory nerves in the mouse dorsal root ganglia (25). In addition, a mechanically gated whole cell current has also been demonstrated in sensory neurons from rat dorsal root ganglia (27). These currents were sensitive to gadolinium and benzamil, suggesting involvement of mechanosensitive ion channels belonging to the degenerin/epithelial sodium channel family. This raises the possibility that such channels could also mediate mechanosensitivity at the terminals of perivascular afferents.

In conclusion, our data support a role for extrinsic afferents in regulating intestinal vascular tone. In addition to a motor action, these nerves appear to have a sensory function at the level of blood vessels, responding to changes in vascular perfusion. These flow-associated changes in afferent firing appear to reflect a response to physical forces associated with flow/pressure rather than O₂ delivery or local gut factors. More direct sensing mechanisms must exist even at the level of the mesenteric fan. Separate mechanisms, however, must underlie the afferent response to terminating perfusion.

The physiological relevance of flow-associated changes in AD is now under question. Flow is known to modulate vessel tone in the microcirculation and increases in flow are associated with vasodilation (see Refs. 18, 28, 35). However, falls in afferent firing (after increased flow) would tend to reduce the release of afferent vasodilators, thus reducing vasodilation. This apparent discrepancy needs to be addressed. One possible explanation is that afferents are not the primary sensors of flow but merely passive responders to

![Fig. 10. Schematic summary of how perivascular afferents may respond to changes in vascular flow rate. Forces associated with changes in flow (shear or pressure) must influence afferents located “outside” the vessel within the adventitia. Endothelial signals must either 1) diffuse across the vascular muscle layer to directly influence afferent sensitivity or 2) interact with the underlying muscle and in turn influence afferents. Alternatively, 3) afferents may detect these changes by more direct sensing mechanisms, possibly responding to stretch in the vessel wall.](http://ajpgi.physiology.org/)
other mechanisms. In contrast, the endothelium and afferent nerves may serve as a functional unit with dual regulation of vessel tone. One could speculate that endothelium-induced vasodilatation would be simultaneously opposed (or modulated) by a reduction in afferent-mediated vasodilator “tone.” Clearly, further studies are required to determine whether these afferent responses serve a role in the moment-moment regulation of intestinal blood flow or reflect a pathophysiological function of these nerves.

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