Vanilloid Receptor TRPV1, Sensory C-Fibers, and Vascular Autoregulation

A Novel Mechanism Involved in Myogenic Constriction

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Abstract—Myogenic constriction describes the innate ability of resistance arteries to constrict in response to elevations in intraluminal pressure and is a fundamental determinant of peripheral resistance and, hence, organ perfusion and systemic blood pressure. However, the receptor/cell-type that senses changes in pressure on the blood vessel wall and the pathway that couples this to constriction of vascular smooth muscle remain unclear. In this study, we show that elevation of intraluminal transmural pressure of mesenteric small arteries in vitro results in a myogenic response that is profoundly suppressed following ablation of sensory C-fiber activity (using in vitro capsaicin desensitization resulted in 72.8±10.3% inhibition, n=8; $P<0.05$). Activation of C-fiber nerve endings by pressure was attributable to stimulation of neuronal vanilloid receptor, TRPV1, because blockers of this channel, capsazepine (71.9±11.1% inhibition, n=9; $P<0.001$) and ruthenium red (46.1±11.7% inhibition, n=4; $P<0.05$), suppressed the myogenic constriction. In addition, this C-fiber dependency is likely related to neuropeptide substance P release and activity because blockade of tachykinin NK_1 receptors (66.3±13.7% inhibition, n=6; $P<0.001$), and not NK2 receptors (n=4, NS), almost abolished the myogenic response. Previous studies support a role for 20-hydroxyeicosatetraenoic acid (20-HETE) in myogenic constriction responses; herein, we show that 20-HETE–induced constriction of mesenteric resistance arteries is blocked by capsazepine. Together, these results suggest that elevation of intraluminal pressure is associated with generation of 20-HETE that, in turn, activates TRPV1 on C-fiber nerve endings resulting in depolarization of nerves and consequent vasoactive neuropeptide release. These findings identify a novel mechanism contributing to Bayliss' myogenic constriction and highlights an alternative pathway that may be targeted in the therapeutics of vascular disease, such as hypertension, where enhanced myogenic constriction plays a role in the pathogenesis. (***Circ Res*****. 2004;95:1027-1034.)**

Key Words: mechanotransduction ■ nonselective cation channels ■ cardiovascular physiology

In 1902, Bayliss made the seminal observation that resistance arteries possess an innate ability to constrict in response to elevations in intraluminal pressure.[1] He described this phenomenon as the myogenic response. Today, we understand that myogenic constriction is a major determinant of peripheral resistance and organ perfusion.[2,3] As such, it plays an important role in the maintenance of an appropriate level of perfusion to vascular beds, independent of systemic pressure, providing a mechanism whereby tissues are protected from variations in blood pressure.[2,3] Myogenic responses predominate in small resistance arteries (<500 μm)[4] and are considered to be attributable to an increase in smooth muscle intracellular free calcium concentration ($[Ca^{2+}]_i$) after depolarization of these cells.[5]

Many studies have attempted to elucidate the signaling mechanisms involved in the myogenic response and several possibilities have been proposed including activation of ion channels, ion exchangers/transporters, and enzyme systems/second messengers.[2] In particular, there is compelling evidence supporting a role for stretch activated ion channels and the arachidonate metabolite 20-hydroxyeicosatetraenoic acid (20-HETE).[2] However, the receptor/cell-type that senses changes in pressure on the blood vessel wall and the pathway that couples this to constriction of vascular smooth muscle remain unclear.

Until recently the myogenic response was thought to be derived entirely from a direct effect of intraluminal pressure on the smooth muscle.[6] It is now clear that the endothelium,

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in response to pressure change, influences myogenic constriction[7,8] by providing a tonic inhibitory influence over the myogenic reflex. However, resistance arteries also possess a dense sympathetic, parasympathetic, and/or sensory innervation. Early studies excluded a neuronal sensor for transmural pressure because chemical destruction of sympathetic nerve fibers using 6-hydroxydopamine, or block of neuronal activity and impulse conduction using phentolamine and tetrodotoxin, respectively, had no effect on myogenic responses.[9–11] However, over the past decade, it has become clear that many resistance arteries are also innervated by a subset of unmyelinated and finely myelinated sensory nerves: the C and A∂-fibers.[12] Sensory C-fibers not only function as afferent fibers collecting information about the environment and carrying this information back to the CNS, but they also have an efferent function, independent of conducted electrical impulses, whereby they may be activated locally from within the periphery to release vasoactive sensory neuropeptides including substance P (SP) and calcitonin gene-related peptide (CGRP)[13] from their peripheral nerve endings. The possibility that the efferent, tetrodotoxin-resistant activity of these nerves might be invoked by intraluminal pressure elevation has not been previously addressed.

Activation of the peripheral nerve endings of C-fibers can be brought about by a range of diverse stimuli, including acidic pH, heat, and the sensory neurotoxin capsaicin. Capsaicin is known to mediate its effects by binding to the neuronally expressed vanilloid receptor, TRPV1[14,15] (formerly termed VR1[16]). A major recent breakthrough has been the discovery that this 95-kDa receptor, a nonselective cation channel, is also the molecular receptor for acidic pH and heat.[15,17] To date, the TRPV1 has been shown to reside predominantly on sensory C-and A∂-fibers: immunoreactivity is evident in the dorsal root and trigeminal ganglia, along the length of the axons and in the skin and cornea.[18,19] There is no published evidence demonstrating expression associated with the vasculature. Challenging current dogma regarding the mechanisms of myogenic constriction, we have used a range of selective pharmacological tools and biochemical techniques to demonstrate a role for sensory C-fibers and TRPV1, in the myogenic response of resistance arteries.

Materials and Methods

Animals and Tissue Preparation

All experiments were conducted according to the Animals Act 1986, UK. Male Sprague Dawley rats (240 to 280g, bred in-house) were stunned and the neck dislocated. The mesentery was removed and placed in oxygenated cold (4°C) physiological salt solution (PSS) of the following composition (in mmol/L): NaCl 118, KCl 4.69, $CaCl_2$ 2.5, KH_2PO4 1.18, $MgSO_4$ 1.18, $NaHCO_3$ 25, and glucose 11. Fourth order mesenteric arteries were cleaned of surrounding fat and 2- to 3-mm lengths cut. Similarly, mesenteric arteries were isolated from tachykinin NK_1 receptor knockout ($NK_1^{-/-}$) mice that were generated as described by De Felipe and co workers.[20] Experiments were performed using male $NK_1^{-/-}$ mice and compared with responses in the wild-type controls (C57BL/6).

Perfusion Myography

Small arteries were mounted in a perfusion myograph with a 10-mL vessel chamber (Living Systems) and prepared for experimentation as previously described.[7] The artery was continuously superfused with preheated PSS at 37°C, pH 7.4 and gassed with 21% O_2, 5% CO_2 in N_2 at a rate of 10 mL/min. Test drugs were added to the superfusing PSS. Vessels were equilibrated for 45 minutes at an intraluminal flow rate of 10 μL/min and pressure of 10 mm Hg before constructing pressure curves. Changes in diameter were measured in response to 10 mm Hg step rises in intraluminal pressure from 10 to 80 mm Hg. At each pressure step, diameter was measured for 4 minutes or until the response had plateaued. Because the intracellular mechanisms invoked in vascular smooth muscle in response to pressure elevation is thought to vary according to the magnitude of the pressure ramp[21] myogenic constriction generated in response to a dramatic pressure rise from 10 to 80 mm Hg was also tested. At the end of each experiment the passive diameter of the vessel at 80 mm Hg was determined by replacing the superfusing solution with calcium-free PSS containing 2 mmol/L ethyleneglycol-bis-(-aminoethyl-ether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA). For determination of percent constriction in response to pressure the following calculation was conducted: % constriction=($Diameter_{ca\ free}$−$Diameter_{80}$)/$D_{ca\ free}$×100.

To investigate the possible involvement of sensory C-fibers in these responses, arteries were pretreated with a range of different inhibitors and antagonists. In vitro C-fiber desensitization was induced using the selective neurotoxin capsaicin (1 μmol/L for 20 minutes, followed by a 40-minute washout period).[13] Acute exposure to capsaicin will activate the C-fiber; however, prolonged exposure to capsaicin, as in the current protocol, will result in a desensitization of the nerve ending to further activation. A 40-minute washout period ensures removal of any residual neuropeptide that may have been released. In some experiments prior C-fiber destruction was produced by treating rats in vivo with capsaicin (50 mg/kg, IP) or vehicle control and then pressure diameter responses measured. In addition the effects of the sympathetic ganglion blocker guanethidine (5 μmol/L, 30 minutes) or the Na^+ channel blocker tetrodotoxin (1 μmol/L, 15 minutes) were tested. Involvement of the endothelium was determined by testing responses before and after removal of endothelium. The endothelium was removed by perfusion of air as previously described.[7]

Because TRPV1 channels have been identified as a major activation site on C-fiber nerve-endings,[17] we investigated the effect of the selective TRPV1 blocker, capsazepine[23] (3 μmol/L, 30 minutes pretreatment and continuously thereafter) and the nonselective cation channel blocker ruthenium red[24] (30 μmol/L, 30 minutes pretreatment and continuously thereafter) against pressure responses. In addition, there is good evidence to support a dependence of myogenic constriction on activation of stretch activated cation channels. Therefore, effects of gadolinium (10 μmol/L), the mechanogated cation channel blocker, were tested.

To investigate the possibility that neurokinin-1 (NK-1) receptors might be involved in mediating myogenic responses, we tested the effects of selective neurokinin receptor antagonists SR140333 (NK1; 1 μmol/L, 30 minutes pretreatment[25] and MEN 11420 (1 μmol/L, 30 minutes[26]).

Western Blotting

The mesenteric vascular bed was rapidly excised and frozen in liquid nitrogen. Samples were crushed in liquid nitrogen and resuspended in ice-cold lysis buffer (20 mmol/L Tris-HCl [pH 7.4], 50 mmol/L NaCl, 50 mmol/L NaF, 5 mmol/L EDTA, and 20 mmol/L $Na_4P_2O_7.10H_2O$). Supernatants were collected, protein concentration estimated, and samples subjected to SDS-Page gel electrophoresis (10%). Separated proteins were then electrotransferred onto nitrocellulose. Nitrocellulose were then incubated with rat TRPV1 receptor antipeptide antibody (kind gift of Drs Julius and Caterina, University of San Francisco, Calif; dilution 1:30 000[27]) overnight at 4°C followed by peroxidase-coupled donkey anti-rat second antibody (1:2000). Visualization of the antibody-protein complex was achieved using an enhanced chemiluminescence detection reagent (Amersham Pharmacia Biotech). In preadsorption experiments, the anti-peptide antiserum (1:1000) was incubated with its corresponding peptide at a concentration of 5 μg/mL at 4°C for 1 hour before application at a 1:30 000 dilution to the nitrocellulose.

Immunohistochemistry

Immediately after dissection, vessels were placed in Zamboni fixative (2% paraformaldehyde) for 16 hours at 4°C. Whole mounts of vessels were incubated overnight at room temperature in a humid atmosphere with primary antibody of the following dilution, anti-PGP 9.5 (Dako), anti-CGRP 1:200, anti-SP 1:1000, or anti-TRPV1 (1:30 000, kind gift of Drs Julius and Caterina, University of San Francisco, Calif[27]). After incubation with fluorescein isothiocyanate (FITC)–labeled swine anti-rabbit immunoglobulin G (1:40, Dako) or rhodamine-labeled swine anti-rabbit immunoglobulin G (1:40, Dako) second antibodies immunofluorescence imaging was performed with an Improvision imaging system. This consisted of a Zeiss Axiophot 100 mol/L microscope (Zeiss) with either a ×60 or a ×40 oil immersion objective and a xenon polychrome II light source. The images were captured with a Hamamutsa C4742–95 digital camera controlled by OpenLab 2.2.1 software.

TRPV1 Activity Assay

To determine changes in $[Ca^{2+}]_i$ in populations of Chinese hamster ovary (CHO) cells expressing the rat TRPV1 receptor, fluorescence measurements were performed in a Molecular Devices Flexstation.[28] The cells were plated at approximately 25 000 cells/well on Costar black, clear-bottomed plates and grown overnight. The cells were incubated in 2 μmol/L fura-2/AM (Molecular Probes) made up in assay buffer (Hank's Balanced Salt Solution [HBSS, Invitrogen] containing 10 mmol/L HEPES pH 7.4) containing 0.01% pluronic F-127 for 30 minutes at room temperature. After washing twice with assay buffer, 100 μL assay buffer, or antagonists where appropriate, was added to each well and the plate placed in a Molecular Devices Flexstation. The fluorescence was measured over 1 minute at 4-second intervals using excitation wavelengths of 340 and 380 nm and emission of 520 nm. At approximately 17 seconds, 20 μL of the agonists made up at 6-fold the required final concentration were transferred to the cells. For pH experiments, 100 μL HBSS alone pH 7.4 was added to the cells and 20 μL of 60 mmol/L MES in HBSS transferred to the cells. The pH of this solution was adjusted such that it gave the desired pH when diluted 1:6. The ratio of fluorescence intensities after excitation at 340 and 380 nm was calculated for each time point. The agonist-evoked response was calculated as the mean of the ratios in the four time-points after stimulation minus the basal ratio.

Results

Vascular C-Fibers Are Activated by Elevation of Intraluminal Vascular Pressure

Using perfusion myography arteries of 90 to 150 μm diameter showed myogenic constriction in response to graded elevation of transmural pressure (Figure 1B) that was abolished by the absence of extracellular Ca^{2+} (Figure 1B). This myogenic response was markedly suppressed by in vitro C-fiber desensitization, using capsaicin (Figure 2A). This effect was selective on myogenic constriction because constriction to U-46619 was not different in control and capsaicin-treated vessels (pEC50 of −7.2±0.13 and −7.2±0.17 and maximum responses of 106±9.8 and 113±13.5 μm, n=4, in the absence and presence of capsaicin, respectively). Removal of the endothelium had no significant effect on the sensitivity of myogenic constriction to capsaicin (Figure 2E, n=4; $P<0.05$). In contrast, neither guanethidine (5 μmol/L, 30 minutes) or tetrodotoxin (1 μmol/L, 15 minutes) altered the pressure-diameter responses (data not shown, n=6 for each). In vitro capsaicin treatment also significantly attenuated myogenic constriction in response to an acute 80 mm Hg ramp in intraluminal pressure (percent constriction at 80 mm Hg in controls of 40.25±5.3% compared with capsaicin treated of 24.1±3.2%; $P<0.05$, n=4). Similarly arteries taken from animals treated in vivo with capsaicin displayed significantly suppressed myogenic constriction (Figure 2B) in comparison to untreated controls.

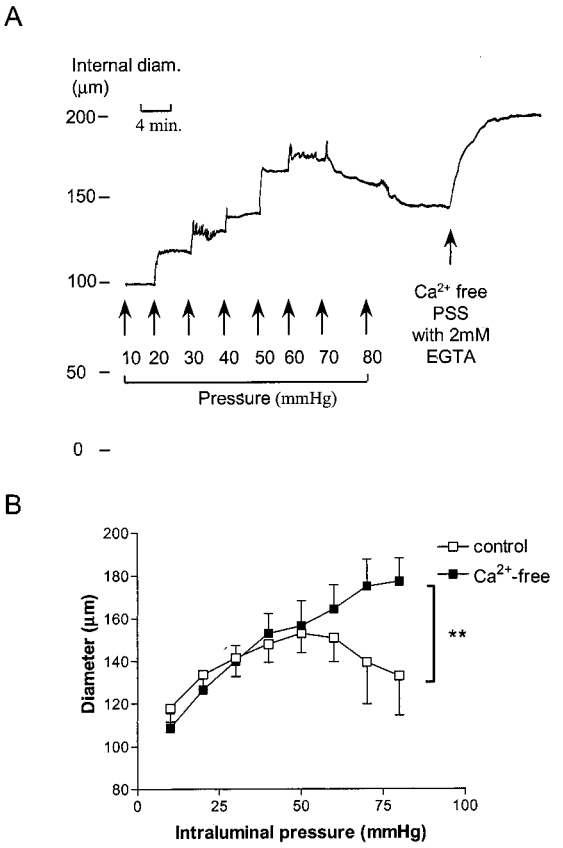

Figure 1. Myogenic constriction of rat mesenteric resistance arteries. A, Representative trace of a control pressure-diameter curve. B, Pressure-diameter curve in normal PSS (□) and Ca^{2+}-free PSS (■). Data are mean±SEM. of n=8 experiments. Statistical significance $^{**}P<0.01$ using two-way ANOVA

TRPV1 Are Involved in Mediating Myogenic Constriction

Both capsazepine (n=7, Figure 2C) and ruthenium red (n=4, Figure 2D) caused significant (>50%) depression of myogenic constriction. At the concentrations used, neither of these drugs affected constriction to U-46619 (10 nmol/L) (percent constriction to U-46619 of 37.7±3.5% and 44.1±11.5% in the absence and presence of capsazepine, respectively, n=7, and 42.4±4.3% and 30.0±5.1% in the absence and presence of ruthenium red, respectively, n=4). The effects of both capsazepine and ruthenium red were reversible because removal of the drug from the perfusate resulted in an increase in the myogenic response to control levels.

TRPV1 Are Expressed on Neurones Innervating Mesenteric Resistance Arteries

Immunohistochemical analyses of these arteries demonstrate positive immunostaining for the general neurone marker protein gene product (PGP) 9.5 (Figure 3A) and CGRP (Figure 3B) and SP (Figure 3C), supporting the concept that these resistance arteries are innervated by C-fibers. In addition,

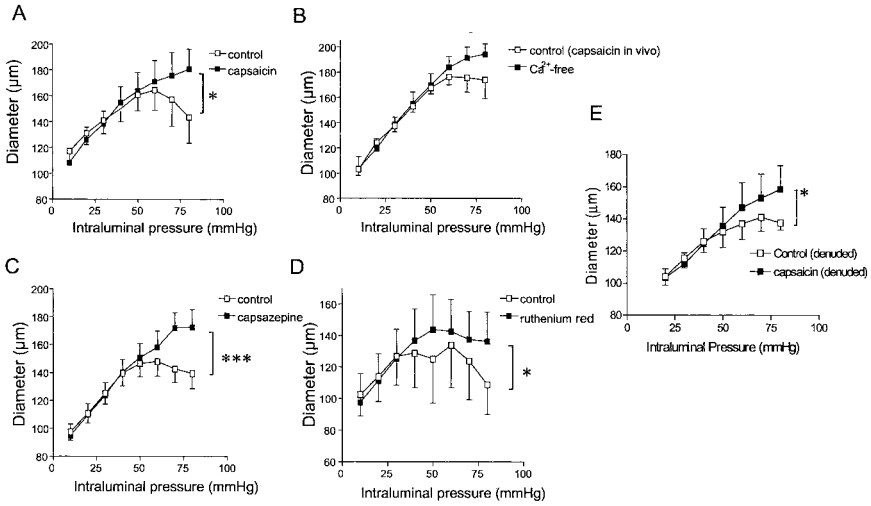

Figure 2. Involvement of sensory C-fibers in the myogenic response of rat mesenteric resistance arteries. Pressure diameter curves in the absence and (A) presence of in vitro C-fiber desensitization achieved using a 20-minute capsaicin (1 μmol/L, n=7) pretreatment (B) following in vivo desentization of C-fibers (capsaicin 50 mg/kg SC, n=4), (C) presence of capsazepine (3 μmol/L, n=9) and (D) in the presence ruthenium red (30 μmol/L, n=4), (E) following endothelium removal (n=4). Data are mean±SEM. Statistical significance $^{*}P<0.05$ and $^{***}P<0.001$ using two-way ANOVA.

immunohistochemistry of whole mount rat mesenteric resistance arteries, using a selective rat TRPV1 antibody, showed TRPV1-positive immunostaining (Figure 3D) that was absent when the antibody was preadsorbed with the TRPV1 peptide against which the antibody was raised[27](Figure 3E). Western blotting of homogenized mesentery for TRPV1 demonstrated the presence of ≈95-kDa band, the predicted size of this protein[27] (Figure 3F).

TRPV1 Are Not Mechanoreceptors

The stretch-activated nonselective cation channel blocker gadolinium[29] significantly attenuated myogenic constriction (percent constriction at 80 mm Hg being 32±8.1% in the absence and 12±8.1% in the presence of gadolinium, respectively; 10μmol/L, n=3, $P<0.05$).[29] Because TRPV1 is a nonselective cation channel, we investigated the possibility that the TRPV1 may be the vascular mechanoreceptor sensitive to gadolinium using a TRPV1 expression system. Up to 100 μmol/L gadolinium had no effect on pH 5.0– or capsaicin (30 nmol/L)-induced TRPV1 activation measured as Ca^{2+} flux in CHO cells, transfected with rat TRPV1 loaded with the Ca^{2+}-sensitive dye Fura-2. Only higher nonselective concentrations of gadolinium (up to 1 mmol/L) displayed partial suppression of TRPV1 activation in response to capsaicin and complete inhibition of the pH 5.0–induced response (Figure 4A).

20-HETE Activates TRPV1 in Resistance Arteries

Recent evidence suggests that certain arachidonate products display agonist activity at TRPV1, in particular 12-HETE.[30] Because there is good evidence for the role of the arachidonate metabolite 20-HETE in the myogenic constriction response and 20-HETE is structurally related to 12-HETE, we tested the possibility that 20-HETE–induced constriction of mesenteric resistance arteries involved activation of TRPV1. 20-HETE (3 nmol/L) constricted pressurized (60 mm Hg) resistance arteries (n=5). This response was significantly suppressed by capsazepine (3 μmol/L, n=5; Figure 4B).

NK_1 Receptor Knockouts Display Altered Myogenic Responses

The postjunctional effects of sensory C-fiber activation are mediated by neuropeptide neurotransmitters released from peripheral nerve terminals, in particular SP, which acts at tachykinin receptors, of which the NK_1 receptor is predominantly expressed in the vasculature. Our studies show that the NK_1 receptor antagonist SR140333 (n=7; Figure 5A) significantly suppressed myogenic constriction of rat mesenteric arteries, whereas the NK2 receptor antagonist MEN11420 (1 μmol/L) did not (n=5; Figure 5B). Additionally, whereas myogenic constriction in small mesenteric arteries of NK1 receptor wild-type mice was suppressed by capsaicin treat-

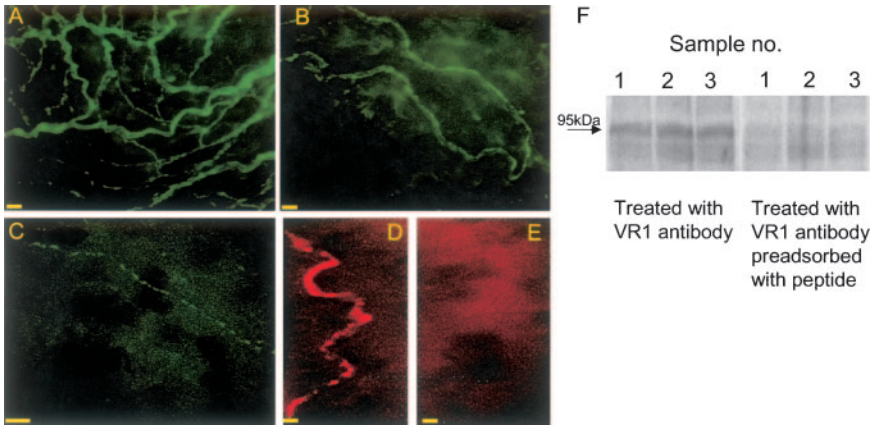

Figure 3. TRPV1 expression in rat mesenteric resistance arteries. Immunohistochemistry of whole mount rat mesenteric artery showing neuron immunoreactivity to (A) PGP 9.5, (B) CGRP, (C) Substance P, (D) TRPV1, and (E) preadsorbed TRPV1 (scale bars=1 μm). F, Western blot of 3 individual rat mesentery supernatant.

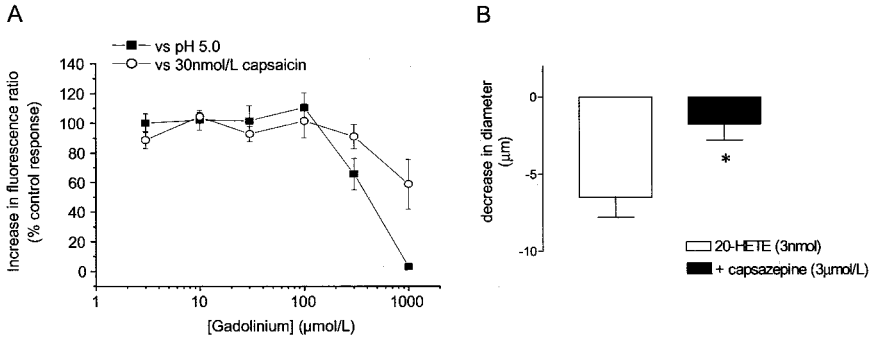

Figure 4. Mechanism of vascular TRPV1 activation. A, Effect of mechanoreceptor blocker gadolinium (3 to 1000 μmol/L) on activation of rat TRPV1, expressed in CHO cells, by pH 5.0 or capsaicin (30 nmol/L). B, Constriction of pressurized (50 mm Hg) rat mesenteric arteries by 20-HETE. Data are mean±SEM of n=4 to 5 experiments. Statistical significance *$P<0.05$ using paired *t* test.

ment (Figure 5C), capsaicin had no effect on arteries of $NK_1^{-/-}$ mice (Figure 5D). This effect did not appear to be attributable to some altered capacity to constrict because the response to U-46619 (10nmol/L) in the arteries of each species was no different (percent constriction of 48.2±9.6%, n=5, and 43.5±4.3%, n=4, in the wild-type and knockout, respectively).

Discussion

Bayliss originally described the myogenic response in 1902[1]; since that time, the significance of this phenomenon with respect to maintenance of normal physiological hemodynamics has been recognized and demonstrated many times over.[2,3] However, the exact mechanisms involved in the processing and translation of an elevation in intraluminal pressure into vasoconstriction of the blood vessel has remained unclear. We now demonstrate that in mesenteric resistance arteries myogenic constriction is in part attributable to the activation of sensory C-fibers. Moreover, that it is the pressure-induced generation of the arachidonate metabolite, 20-HETE, that activates TRPV1 on C-fiber nerve endings to result in depolarization of these nerve fibers. After activation, SP is released from these neurones and causes contraction of vascular smooth muscle by binding to tachykinin NK_1 receptors. We believe that these studies identify a novel pathway involved in determining resistance artery tone and highlights novel targets in the treatment of conditions such as hypertension associated with exaggerated myogenic responses and vascular autoregulation.[31–33]

The rat mesenteric arteries used in this study demonstrated typical myogenic constriction in response to physiological transmural pressures that these vessels would normally experience in vivo. At low transmural pressure (10 to 40 mm Hg) diameter increased linearly with pressure. The threshold pressure for spontaneous myogenic constriction occurred between 50 and 60 mm Hg, which is typical for arteries of this size.[2] Indeed normal in vivo intraluminal pressure in arteries of the size used in the present study are thought to be 60% to 80% of mean arterial pressure (see review[34]). Therefore, with a mean arterial pressure of 95 to 100 mm Hg, intraluminal pressure of these arteries in vivo should range between 60 to 80 mm Hg (ie, within the range tested). Moreover, the dependency of this response on Ca^{2+} influx demonstrates that the myogenic response studied in these vessels is of a similar nature to that reported in resistance arteries of other vascular beds and species.[2,6]

As expected, neither sympathetic ganglion blockade or block of impulse conduction affected myogenic constriction confirming no role for conventional neuronal activity. However, in vitro capsaicin treatment, resulting in "desensitiza-

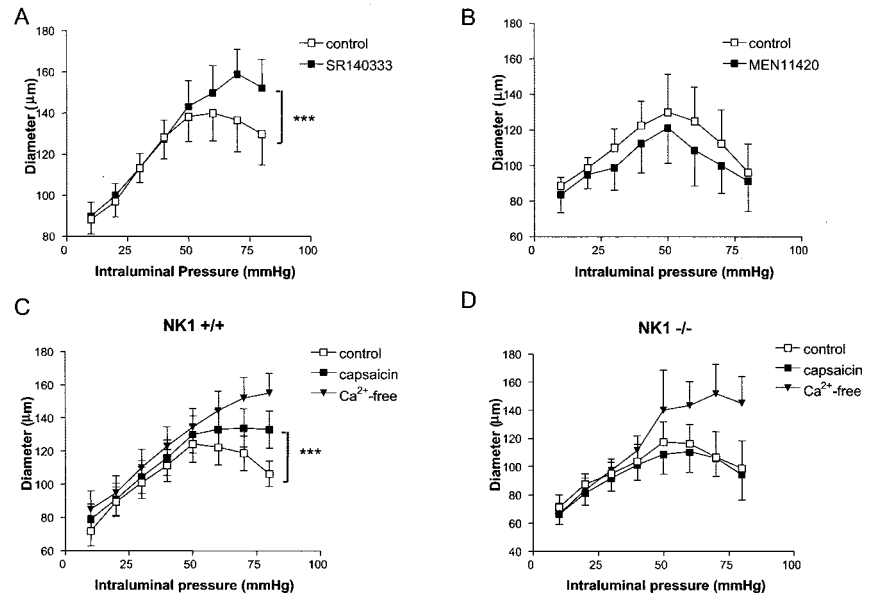

Figure 5. Pressure diameter curves in the absence and presence of (A) NK_1 receptor blocker SR140333 (n=6), (B) NK2 receptor blocker MEN 11420 (n=4) in rat mesenteric resistance arteries, (C) following capsaicin desensitization in NK_1 receptor wild type murine arteries (n=5), or (D) in $NK_1^{-/-}$ murine arteries (n=4). Data are mean±SEM. Statistical significance ***$P<0.001$ using two-way ANOVA.

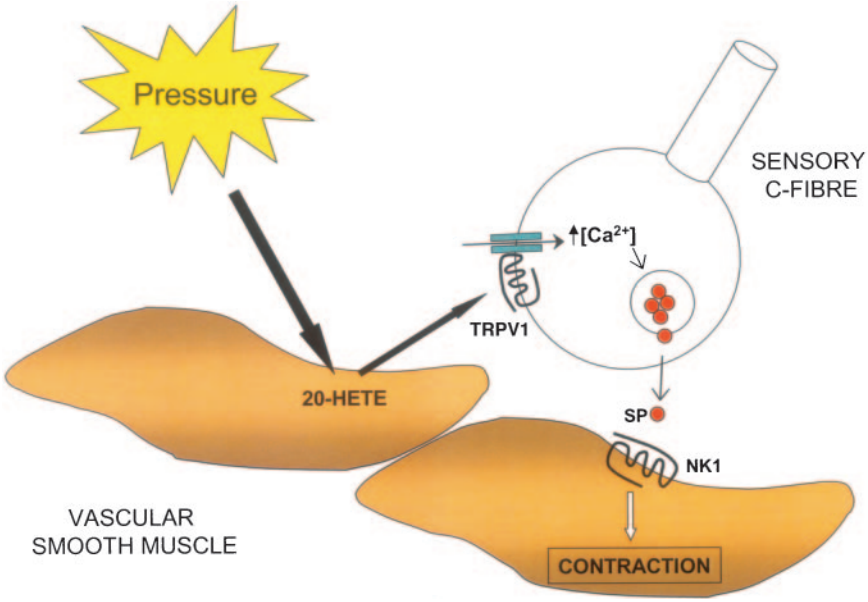

Figure 6. Schematic representation of the mechanisms involved in the C-fiber dependent component of the myogenic response. Transmural pressure results in the elevation of vascular smooth muscle cell-derived 20-hydroxyeicosatetraenoic acid (20-HETE) that in turn binds to vanilloid receptor TRPV1 and activates C-fibers causing depolarization and consequent substance P (SP) release that binds to tachykinin NK_1 receptors on vascular smooth muscle to cause contraction.

tion" of C-fibers, profoundly suppressed the myogenic response. Similarly, arteries removed from animals previously subjected to an in vivo capsaicin treatment, producing selective depletion of C and A∂-fiber sensory neuropeptides (>90%),[35,36] displayed profoundly suppressed myogenic reactivity. Furthermore, as with myogenic responses to stepwise increase in pressure, c-fiber desensitization suppressed the response to a large ramp increase in pressure. Thus, it appears that effective "silencing" of vascular sensory C-fibers prevents the blood vessel from responding normally to increases in intraluminal pressure, whether this increase be a slow graded elevation or a large rapid increase in pressure. Since the first demonstration of the functional vasoactive implications of vascular C-fiber activation by Kawasaki in 1988,[37] many other groups have demonstrated the vascular effects of C-fiber activation, produced artificially either by electrical stimulation or the application of capsaicin,[13] including in the rat mesenteric vascular bed. Indeed, of those vascular beds that display acute myogenic responsiveness, almost all of them are densely sensory C-fiber innervated, eg, coronary, cerebral, renal, and mesenteric.[12,38] However, although it is clear that activated fibers release vasoactive mediators, the endogenous-activator of sensory C-fibers, and the role these nerves play in the physiological regulation of vascular tone have remained uncertain. Our findings identify a, hitherto unknown, physiological role for C-fibers in the vasculature as mediators of the myogenic response.

TRPV1 has recently come to the fore as a key site for activation of C-fibers from within the periphery,[15] and our Western and immunohistochemical studies clearly demonstrate the presence of TRPV1 on nerves penetrating the walls of mesenteric resistance arteries. Using agents shown to selectively interfere with TRPV1 activity, capsazepine and ruthenium red,[23,24] we demonstrated a dependence of myogenic responses on TRPV1 activation. These agents were selective in their action because they had no effect on constriction induced by the thromboxane A_2 mimetic U-46619. In light of these findings, we then went on to determine exactly how and what might activate TRPV1 after elevation of intravascular pressure.

There is considerable support for the concept that activation of stretch-activated cation channels is an integral step involved in the sensing and reactive constrictor response to pressure in arteries of many vascular beds that display myogenic autoregulation.[2,6] This hypothesis is based in part on studies using gadolinium, an inhibitor of mechanogated cation channels with no specificity for single or nonselective channels.[29] In the present study, gadolinium also significantly attenuated myogenic constriction. However, the possibility that TRPV1 itself might be the nonselective cation channel sensitive to gadolinium was excluded because gadolinium did not alter TRPV1 activation using a CHO cell TRPV1 expression assay. Extremely high concentrations of gadolinium (up to 1 mmol/L) displayed apparent partial suppression of TRPV1 activation; however, this is most likely attributable to the nonselective nature of gadolinium in such excess. Thus, although high concentrations appeared to show activity, it is unlikely that this relates to TRPV1 activation and intimates that it is unlikely that the TRPV1 itself is a mechanoreceptor.

Several reports support a role for the vascular smooth muscle–derived vasoconstrictor 20-HETE as a mediator of the myogenic response[2,6] in a number of different resistance artery types,[39] including rat mesenteric arteries.[40,41] 20-HETE is produced from ω-hydroxylation of arachidonic acid via cytochrome P450. Interestingly, recent studies suggest that certain arachidonic acid–derived products activate, and are potential endogenous ligands for, TRPV1.[30,42] In particular, there is compelling evidence for agonist activity of 12-HETE (a 12-lipoxygenase product) at TRPV1.[30] Similarly, we now show that the structurally related 20-HETE also constricts pressurized arteries, a response attenuated by capsazepine. These results demonstrate that 20-HETE activates vascular TRPV1, and because there is considerable support for the generation of 20-HETE in the myogenic response after pressure elevation, this may provide the mechanism by which

TRPV1 are activated after an elevation of intraluminal pressure.

Finally, the peripheral effects of sensory C-fiber activation are mediated by neuropeptides released from the nerve terminals. Our studies using the selective NK_1 receptor antagonist, SR140333, indicate that NK_1 receptors are involved. SP activity in most arteries is associated with vasodilator activity after interaction with endothelial NK_1 receptors; however, there are some reports demonstrating a vasoconstrictor capacity of this peptide and expression of NK_1 receptors on vascular smooth muscle.[43,44] A direct action of SP at the smooth muscle is supported by the data demonstrating that removal of the endothelium had no influence over the sensitivity of the myogenic response to capsaicin. In addition, studies with the selective antagonist were supported by the finding that in vitro capsaicin desensitization of C-fibers had no effect on myogenic responses of arteries of $NK_1^{-/-}$ mice. However, contrary to our expectations, the absolute magnitude of myogenic tone was not attenuated in these arteries compared with wild-type arteries. This absence of suppression suggests developmental adaptation in the NK_1 receptor knockouts compensating for the dysfunction of this pathway and thereby, maintaining the capacity of resistance arteries to respond to pressure change. Activation of compensatory mechanisms following knockout of specific proteins essential to blood pressure control is not uncommon and has been identified in various systems including in nitric oxide synthase knockout animals.[4,7] This finding perhaps highlights the importance of myogenic constriction in normal physiology and might explain why there appear to be several mechanistic components to this phenomenon, ie, in the situation that one particular pathway might be disturbed or disrupted other pathways are upregulated to maintain the physiological integrity of the blood vessel response to intraluminal pressure. Interestingly, in contrast, absolute myogenic constriction was altered after disruption of this pathway by desensitization of C-fibers in normal animals (in vivo capsaicin treatment). These findings suggest that the compensatory mechanisms activated to maintain myogenic tone in $NK1^{-/-}$ mice are likely to occur in the early stages of development.

Our findings indicate that vascular sensory C-fibers are involved in mediating Bayliss' myogenic constriction of arteries, ie, the acute vasoconstriction response to a rise in intraluminal pressure. Moreover, it appears that the molecular receptor for this mechanosensitivity of vascular peripheral C-fibers is the TRPV1. After elevation of pressure, we propose that TRPV1 located on C-fibers penetrating the blood vessel wall are activated after the generation of 20-HETE and that one mechanism involved in 20-HETE–induced contraction, apart from its other reported effects,[2] is that this lipid acts at TRPV1 located on peripheral nerve endings resulting in neuron depolarization and the release of SP from these nerve-endings. This SP, in turn, binds to postjunctional NK_1 receptors, located on the smooth muscle to bring about vascular smooth muscle contraction and hence myogenic constriction (see Figure 6). These results highlight a novel pathway for therapeutic targeting in those cardiovascular diseases where an altered myogenic responsiveness is thought to play a role, such as hypertension[31,32] and diabetes.[45]

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