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Identification of medium/high threshold extrinsic mechanosensitive afferent nerves to the gastrointestinal tract

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Abstract

Background & Aims—Large distensions reliably evoke sensation from the non-inflamed, non-ischemic bowel, but the specialized afferent axonal structures responsible have not been

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morphologically identified. We investigated whether their transduction sites are located on major blood vessels close to and within the gut wall.

Methods—In vitro extracellular recordings were made from mesenteric nerve trunks in guinea pig ileum, combined with rapid axonal dye filling and immunohistochemical analysis of nerve trunks.

Results—Recordings revealed sensory fibers with focal mechanosensitive sites in the mesenteries that could be activated by von Frey hairs and by stretch. Dye filling revealed varicose branching sensory axons on mesenteric blood vessels but no other anatomically specialized structures in mesenteric membranes or in the serosa. Large amplitude stretch and von Frey hairs also activated sensory endings within the gut wall itself, but only if the submucosa was present; mechanotransduction sites in the serosa or outer muscle layers were sparse. Mechanosensitive sites in the submucosa were exclusively associated with submucosal blood vessels. Submucosal endings had significantly higher thresholds to stretch than specialized low-threshold mechanoreceptors characterized previously in the rectum ($P < 0.05$) and were therefore classified as medium/high threshold mechanoreceptors. Capsaicin (0.3–1 μM) activated most mechanosensitive mesenteric (68%) and submucosal (85%) afferent endings. Similar intramural mechanosensitive afferent endings on blood vessels also exist in the colon and bladder.

Conclusions—Varicose branching axons of sensory neurons on intramural blood vessels, previously shown to mediate sensory vasodilation, are transduction sites for medium/high threshold, stretch-sensitive mechanoreceptors, capable of encoding large distensions in hollow viscera.

Introduction

While cutting, crushing or burning often fail to be detected, distension of hollow organs reliably causes sensations in humans, including pain at higher levels^{1–4} and evokes visceromotor or pseudoaffective responses in animals⁵. Viscera are innervated by spinal mechanosensitive sensory neurons which activate central pain pathways^{1,2,4}. Mechanosensitive afferent nerves, responsive to strong focal compression of arterial branch points have been identified previously, close to several abdominal organs^{6–10}. For the gut, numerous mechanosensitive sites are located near blood vessels in the mesenteric membranes and on the serosal surface of the gut. "Serosal" and "mesenteric" afferents comprise the majority of spinal mechanosensitive afferent nerves to the intestines^{11,12}. High threshold mechanosensitive afferents can be activated by intraluminal distension pressures of 25 mmHg or more⁷. A third, distinct type of spinal mechanoreceptor, characterized by low thresholds and slowly adapting responses to distension, has been studied extensively in the distal large bowel^{9,13,14} and, in the guinea pig, been shown to have transduction sites corresponding to characteristic flattened, rectal intraganglionic laminar endings (rIGLEs) within myenteric ganglia of the distal bowel, but not further orally^{15,16}. These low threshold, specialised rectal afferent fibers have similar mechanical sensitivity to low threshold vagal mechanoreceptors that innervate the upper gut¹⁷.

The mechanotransduction sites of distension-sensitive spinal mechanoreceptors found throughout the entire gut remain to be identified. The presumptive location of sensitive sites in the mesentery and serosa suggest that large distensions of the gut lumen may activate mechanoreceptors which are actually located outside the gut wall proper. Consistent with this, traction applied to the mesenteric membranes evokes pain-like responses in animals¹⁸. However, many studies have implicated mechanosensitive afferent nerves with axons in the muscle layers to encode noxious stimuli^{7,19}. In the present study, mechanosensitive afferents with endings on blood vessels were characterized by responses to von Frey hairs and to distensions. They had significantly higher thresholds to stretch than specialized low threshold rectal afferent nerve endings and were able to encode a wide range of distension amplitudes.

Materials and Methods

Extracellular recording

Adult guinea pigs (180–250g) were killed by stunning and exsanguination, as approved by the Animal Welfare Committee of Flinders University. A specimen of ileum (or distal colon more than 10cm from the anus) was opened near the mesenteric attachment, pinned flat, mucosa uppermost, in Krebs solution (mM: NaCl, 118; KCl, 4.75; NaH₂PO₄, 1.0; NaHCO₃, 25; MgSO₄, 1.2; CaCl₂, 2.5; glucose, 11; bubbled with 95% O₂–5% CO₂). In some preparations the mucosa and/or submucosa was peeled away. In others, the muscularis externa was peeled off the submucosa, using sharp forceps. Several fine mesenteric nerve trunks (2–6 trunks, 20–80µm diameter, 3–5mm long) were dissected free and, with a separate strand of connective tissue, pulled into a small paraffin-oil-filled chamber (1ml volume) under a cover slip, sealed with silicon grease (Ajax Chemicals, Australia). Differential extracellular recordings were made between one nerve trunk and connective tissue via 100µm Pt/Ir electrodes. Signals were amplified (ISO80, WPI, USA) and recorded at 20kHz (MacLab8sp, Chart 5.4 software) and single units were discriminated by amplitude and duration using Spike Histogram software (AD Instruments, Sydney, Australia). Several nerve trunks were recorded until a single unit could be discriminated, determined by a minimum interspike interval >10ms. Preparations of guinea pig bladder were set up similarly, with recordings made from thin nerve trunks close to the bladder trigone.

A 10mm array of hooks connected one edge of the preparation to an isometric force transducer (DSC no. 46-1001-01, Kistler-Morse, Redmond, WA), mounted on a “tissue stretcher”¹⁷. A resting tension of ~1 mN was set and 60 minute equilibration period allowed. Preparations were stretched by the microprocessor-controlled tissue stretcher, while recording intramural tension (5 mm/s, 1–3 mm, held for 10 s, 3 minute intervals). Some preparations were stretched by attaching the hooks to an isotonic transducer and increasing the counterweight thus recording evoked changes in circumference (Harvard Bioscience 52-9511, S. Natick, MA). Mechanotransduction sites (“hotspots”) were identified by probing with a 10mN von Frey hair at multiple points, starting distally and working towards the recording site. Responsive sites were characterised using 0.1–10mN von Frey hairs and marked with fine carbon particles attached to the tip of the hairs¹⁷.

Rapid anterograde and retrograde tracing

Anterograde labelling^{17,20} involved rinsing the recorded nerve trunk with artificial intracellular solution (150mM monopotassium L-glutamic acid, 7mM MgCl₂, 5mM glucose, 1mM ethylene glycol-bis-(β-aminoethyl ether) N, N, N', N'-tetraacetic acid (EGTA), 20 mM hydroxyecosapentanoic acid (HEPES) buffer, 5mM disodium adenosine-5-phosphate (ATP), 0.02% saponin, 1% dimethylsulfoxide (DMSO), 100IU/ml penicillin, 100µg/ml streptomycin, 20µg/ml gentamycin sulphate²⁰). A small drop of 5% biotinamide (N-(2-aminoethyl) biotinamide hydrobromide; Molecular Probes, OR), dissolved in artificial intracellular solution, was then placed under the paraffin oil on the nerve trunk recording site. The preparation was bathed with sterile culture medium (DME/F12, Sigma; 10% foetal bovine serum, 1.8mM CaCl₂, 100IU/ml penicillin, 100µg/ml streptomycin, 2.5µg/ml amphotericin B, 20µg/ml gentamycin, Cytosystems, NSW, Australia). Humidified gas (95% O₂–5% CO₂) was blown to ensure constant mixing for 6–16 hours at 35–36 °C. Positive electrophoretic voltage pulses were applied to the nerve trunk using a Grass SD9 stimulator (2.5Hz, 200ms duration, 1V). Filled preparations were fixed overnight at 4°C in Zamboni's fixative (15% saturated picric acid, 2% formaldehyde in 0.1M phosphate buffer, pH7.0), cleared in dimethylsulfoxide (3×10 minute washes) then rinsed in 0.1M phosphate buffered saline (PBS, 0.15M NaCl, pH7.2). Labelled fibers were visualised with streptavidin-AlexaFluor 488 (1:2000; Molecular

Probes) or -Cy3 (1:400) for 5–24 hours, washed with PBS and mounted in 100% carbonate-buffered glycerol (pH 8.6).

Immunohistochemistry and image analysis

Preparations were incubated with primary antibodies (Table 1, supplementary material) for 16–72 hours at room temperature, rinsed with PBS and incubated with secondary antibodies (Table 1) for 2–4 hours, mounted as described above and analysed on an Olympus AX70 epifluorescence microscope fitted with a Hamamatsu Orca digital camera (Model C4742-95, Japan) and IPLab software (Scanalytics Inc, VA). Brightness, contrast, cropping and photomontages were performed using Adobe Photoshop. Analysis of immunoreactivity was carried out using NIH Image (National Institutes of Health, MD, USA) by rapidly switching between a stack of micrographs. An average of 5 axons were analysed in each of 20 stacks, per animal, to minimise double counting.

Data analysis

Results are expressed as means $\pm$ SEM, with n referring to the number of recorded fibers and N to the number of animals. Statistical analysis utilised Student's two-tailed t-test for paired or unpaired data or repeated measures analysis of variance using Prism 4 software (GraphPad Software, Inc., San Diego, CA). Differences were considered significant if $P < 0.05$.

Results

Mesenteric mechanoreceptors

Results were obtained from 155 units from 129 animals. Von Frey hairs applied to the mesenteric membranes evoked firing from single afferent units (Fig 1A), similar to previous reports^{6,8–12}. Most sites required relatively stiff von Frey hairs (3–10mN in the mesentery), and often showed irreversible run-down when probed repetitively. The responsive sites ("hotspots") were associated with vascular bundles, usually within 3mm of the gut. In comparison, low threshold mechanoreceptors in the rectum gave reliable responses to much lighter von Frey hairs (0.1–0.3mN²¹; Fig 1B). Of marked hotspots, 83% were located on mesenteric arteries and 13% were associated with mesenteric veins; fewer than 3% were located away from blood vessels (30 marked hotspots, n=9 preparations, N=7 animals). Of random sites probed on innervated mesenteric blood vessels, 39 $\pm$ 6% had measurable firing to a 10mN von Frey hair (23 of 73 sites, n=5, N=5). Single axons could be activated from several mesenteric hotspots (4 $\pm$ 1 sites, n=8, N=7), confirming previous reports^{6,10}.

Biotinamide fills of recorded nerve trunks labelled mixed sympathetic and sensory axons, which could be distinguished by immunoreactivity for tyrosine hydroxylase and CGRP respectively. Most filled nerve fibers were smooth axons-of-passage, located in paravascular bundles. The only identifiable axonal specialisations were perivascular varicose branching axons on blood vessels. Filling of varicose branching axons often extended into myenteric ganglia, muscularis externa and submucosa (Fig 2A–D). However, unlike the rat²², intraganglionic laminar endings were rare (two filled IGLEs identified in 40 preparations). Thus fine, varicose branching axons running close to the adventitia of blood vessels were the only candidates for mechanosensitive transduction sites in the mesenteries. Measurements confirmed this conclusion; identified "hotspots" were located significantly closer to biotinamide-filled varicose branching axons than random sites ($P < 0.01$, Fig 2C). Previous studies have reported that mesenteric mechanosensitive sites are associated with arterial branch points. In this study, filled varicose branching axons were closer to arterial branch points than randomly generated points on mesenteric arteries (Fig 2D). Thus, varicose branching sensory axons on blood vessels, whose vasodilator effects have been extensively investigated²³, also include mechanosensitive sites.

Mesenteric afferents can also be activated by traction on the mesenteries, a possible cause of pain when the gut is distended or contracts strongly. In 6 of 6 preparations, mesenteric afferents were activated above spontaneous firing rates by stretching with a 100mN load, with an increase in firing (averaged over 20s) of 1.8 ± 0.5 Hz, compared to spontaneous firing of 0.3 ± 0.1 Hz ($n=9$, $N=9$, Figure 3C).

Luminal distension of the gut activates pain pathways *in vivo*^{5,24}; while this could theoretically be mediated by traction on mesenteric afferent axons, a contribution by more directly coupled intramural afferent axons has also been suggested^{8–10}. We tested whether intramural mechanosensitive afferent fibers exist and, specifically, whether they respond like “serosal” mechanoreceptors identified previously^{11,12,19}.

Intramural mechanoreceptors

Preparations consisting of the outer muscle layers of ileum or colon including the myenteric ganglia, with intact serosa, but with mucosa and submucosa removed¹⁷, were stretched circumferentially with a 100mN load. This usually evoked no afferent activation or small phasic responses at the onset of stretch, (Figure 3B,C “myent”). In responding fibers, an increase in firing of 0.9 ± 0.2 Hz, compared to spontaneous firing of 0.4 ± 0.2 Hz, (averaged over 20s), was seen ($n=9$, $N=9$, Fig 3C). Few mechanically-sensitive hotspots were detected by stiff von Frey hairs (10mN); consistent with the paucity of intraganglionic laminar endings seen in biotinamide fills. Of randomly probed sites in such preparations, $6 \pm 3\%$ (21 of 434 sites, $N=5$) showed firing to a 10mN von Frey hair; a significantly lower proportion than mesenteric blood vessels ($p < 0.05$). The serosa, external muscle layers and myenteric plexus were all intact in these preparations, suggesting that high threshold mechanosensitive transduction sites are sparse within the outer layers of the intestine.

In contrast, when the submucosa was present, von Frey hairs activated numerous mechanosensitive “hotspots”, suggesting that intramural mechanotransduction sites in guinea pig small intestine are concentrated in the submucosa. To test this, preparations were dissected to remove the mucosa. They were then turned over and the muscularis externa and serosa removed from most of the preparation, apart from a 1mm strip along the mesenteric border (where blood vessels and extrinsic nerves penetrate). Thus most of the preparation consisted only of submucosa and adhering muscularis mucosa. Distension of such preparations reliably evoked strong afferent firing (100mN load evoked an increase in firing of 2.8 ± 0.8 Hz compared to basal firing of 0.8 ± 0.3 Hz ($n=10$, $N=10$, Fig 3A). Quantitative analysis revealed that stretch-activated responses were more frequent in the submucosa than mesenteric or muscularis externa/serosal preparations (Fig 3B). Submucosal afferents showed stronger responses to 100mN distension than axons in muscularis externa (Fig 3C).

The numerous von Frey hair-sensitive sites in the submucosa were all visibly associated with submucosal blood vessels (fig 4), often where there was no underlying muscularis externa or serosa. Probing between blood vessels never evoked firing, but $51 \pm 8\%$ of sites tested on innervated submucosal vessels (up to the third level of branching), evoked firing (Fig 3B, 63 of 124 sites, $N=5$), more responding sites than in muscularis externa or serosa. Submucosal hotspots were significantly more sensitive to von Frey hair probing than mesenteric hotspots (Fig 2B), with half maximal firing frequency at 1.9 ± 0.3 mN (11 hotspots, $N=4$) compared to 3.6 ± 0.6 mN for mesenteric afferents (12 hotspots, $N=4$). More mesenteric and submucosal hotspots had thresholds above 0.5 mN than low threshold rectal afferents ($\chi^2=14.3$, $df=1$, $P < 0.001$); the latter had half maximal firing at 0.4 ± 0.1 mN (data from²¹).

Using loads of 50–200mN, submucosal afferents were more strongly activated than mesenteric afferents or fibres in the muscularis externa (Fig 5A). In comparison, in rectal preparations of similar size, subjected to the same loads, rectal afferents were more sensitive still, with

significantly lower distension thresholds (1.47 ± 1.26 vs 3.3 ± 1.48 mN, $n = 8, 10$ $P = 0.012$ $t = 2.768$). We tested whether the same nerve fiber could have mechanotransduction sites on both mesenteric and submucosal vessels. This was confirmed experimentally with von Frey hairs (Fig 5B) for a few fibers, but was not quantified, due to the frequent damage to nerve pathways entering the gut wall during the complex dissection.

Anterograde filling of mesenteric nerves labelled varicose branching axons on both mesenteric and submucosal blood vessels, some of which were immunoreactive for the neuropeptide, CGRP (Figure 6A). A dense plexus of CGRP-immunoreactive sensory fibers ran along and over 1st–3rd order submucosal vessels, but rarely extended to finer, more distal branches, similar to submucosal hotspots detected with von Frey hairs. In the mesentery, 96.5% of CGRP axons were also immunoreactive for TRPV1; in the submucosa 95.1% of CGRP axons contained TRPV1 immunoreactivity (Figure 6B,C). Application of 10^{-12} moles of capsaicin topically (in 1–3 μ l volume), to marked 20 marked hotspots activated 85% (17) of submucosal fibers ($n = 4$, $N = 4$), evoking a burst of firing with short latency. Similarly, the majority of mesenteric afferent hotspots (68%) were also activated by 1 μ M capsaicin (Fig. 6D,E). In mesenteric receptors, capsaicin evoked an increase in firing of 7.6 ± 8.5 Hz in normal Krebs ($n = 15$, $N = 5$), reduced by 1 μ M capsazepine to 0.8 ± 2.2 Hz ($n = 19$, $N = 5$, $P < 0.05$, $t = 3.86$, $df = 32$) confirming that effects were mediated via TRPV1.

Similar studies were carried out in distal colon, more than 10 cm proximal to the anal border, in the region lacking low threshold, rectal mechanoreceptors^{15,16}. Mechanosensitive sites on submucosal blood vessels could be activated by von Frey hairs, by stretching with large loads (100 mN, Fig 7A, $N = 4$) and by capsaicin (3 μ M, Fig 7B). However, few mechanosensitive units could be detected when the submucosa and mucosa were removed¹⁵. Thus, similar intramural mechanoreceptors, responsive to high amplitude distension and to stiff von Frey hairs, exist in submucosal blood vessels of large intestine (fig 7C). Biotinamide filling revealed bundles of smooth axons-of-passage and varicose branching axons on blood vessels; no other axonal specialisations were seen. Similar observations were also made in the rat small intestine (not shown).

Intramural medium/high threshold mechanosensitive axons in the bladder

Similar *in vitro* recording techniques were used in small preparations of guinea pig bladder with regions of mucosa removed to reveal subepithelial blood vessels. After excluding 3 low threshold mechanosensitive units²⁵, 13 units were recorded ($N = 9$) which responded to calibrated von Frey hair (0.1–10 mN, Fig 8C). The average distance from marked hotspots to subepithelial blood vessels was significantly closer than randomly generated sites (68 ± 27 μ M, $n = 13$, $N = 7$; 537 ± 56 μ M, $n = 70$, $P < 0.01$, Fig 9B). None of these afferent fibers responded to small stretches (2 mm), which activated low threshold mechanoreceptors, but 8 of 9 units responded to high amplitude stretch (4–6 mm distension, Fig 9A). Of these afferent nerves 8 of 12, ($N = 8$) were activated by 3 μ M capsaicin. Anterograde tracing showed that the only axonal specialisations close to marked hotspots were perivascular varicose branching axons ($N = 4$, Fig 9E), of which 2 of 3 were immunoreactive for CGRP.

Discussion

The present study localized the specialised axons of medium/high threshold afferents to the gastrointestinal tract and demonstrated that their mechanotransduction sites correspond to varicose branching axons on blood vessels, both in the mesenteries and after they penetrate the gut wall, particularly in the submucosa.

Mesenteric medium/high threshold mechanoreceptors

Mechanosensitive varicose branching axons on mesenteric blood vessels are probably a subset of the perivascular sensory vasodilator axons²³. It is not clear whether all perivascular sensory axons are mechanosensitive, or only a subset. Focal mechanosensitive sites in mesenteric arteries were reported to be close to arterial branch points^{6,10,12}; the present study confirmed this. We speculate that branch points are exposed to distortion during strong contractions or distension of the intestine. Branching nociceptor axons with regular swellings in extensive terminal fields are found on vascular structures in testis²⁶, where ultrastructural specialisations have been detailed, comparable to nociceptor terminals in the knee joint, pleura and dura mater²⁷. Whether the mesenteric mechanosensitive axonal structures identified here actually constitute “nerve endings” and share similar ultrastructural characteristics remains to be determined.

Submucosal medium/high threshold mechanoreceptors

The outer layers of the intestine consist of the serosal membrane, longitudinal smooth muscle, the myenteric plexus and circular smooth muscle. Von Frey hairs applied to the outside of the gut wall activate mechanoreceptors, suggested to lie in the serosa^{10–12,28}. The present study did not detect mechanotransduction sites in the serosa, *per se*, but rather on blood vessels both outside or within the gut wall, particularly in the submucosa. Intramural hotspots (in the submucosa) could be activated by von Frey hairs applied to either the mucosal surface of the gut, or to the serosal surface (data not shown). Intramural mechanotransduction sites were localised to primary or secondary submucous arteries; they were rarely present in finer branches and were usually restricted to about one third to one half of the circumference from where blood vessels entered the layer. Removal of the muscularis externa and serosa certainly did not block mechanosensitivity to distension; whether it sensitized submucosal afferent neurons could not be determined. Biotinamide tracing consistently failed to detect specialised axonal structures in the serosa. We have made similar observations in rat small intestine (unpublished observations). It is likely that there are significant species differences in the distribution of blood vessels within the gut wall: certainly in human tissue, large blood vessels in the plane of the myenteric plexus receive a sensory innervation (unpublished observations); some of these may be mechanosensitive. Our results suggest that “serosal” mechanoreceptors largely correspond to afferent nerve fibers associated with intramural blood vessels.

Afferent axonal branches in myenteric ganglia

Biotinamide applied to mesenteric nerve trunks filled many varicose axons in myenteric ganglia and muscle layers; most belong to post-ganglionic sympathetic neurons²⁰, but a small proportion were CGRP-immunoreactive spinal sensory neurons. Sensory axons in the myenteric plexus are immunoreactive for CGRP and/or substance P²⁹ and TRPV1³⁰, with lower density in the muscle layers. The present study suggested that few of these axons were activated by stiff von Frey hairs. Circumferential stretch was similarly ineffective. In the few cases where distension responses were seen, these were usually phasic and could be localised to mechanosensitive sites on remnant stumps of blood vessels; extrinsic sensory axons in myenteric ganglia and external muscle layers are probably not mechanosensitive but may be the sites of contact with enteric neurons³¹. In rats, there are numerous vagal IGLEs in myenteric ganglia of the small intestine²² which are transduction sites of low threshold vagal mechanoreceptors¹⁷ and can be recorded electrophysiologically³². Few IGLEs were encountered in the guinea pig distal ileum during the present study, suggesting an interspecies difference. “Viscerofugal” enteric neurons, with axons in mesenteric nerves are also activated by distension³³. Many were filled during this study, but did not appear to contribute to the distension or von Frey hair-evoked electrophysiological responses.

Classes of submucosal and mesenteric receptors

Different types of extrinsic spinal afferent neurons to the viscera have been distinguished by various criteria including the neurochemistry of cell bodies after retrograde tracing. In small or large intestine of rat, mouse and sheep, many spinal afferents are immunoreactive for CGRP, but consistently a proportion lack this neuropeptide^{34–36}. In the present study, many sensory fibers anterogradely labelled were CGRP immunoreactive. The methodology to identify the morphology of mechanosensory transduction sites allows afferent axons to be characterised structurally, providing an additional criterion to distinguish classes^{15,17,25,37} and can be used in other species including rat, mouse and human (not shown). Additionally it facilitates investigation of mechanisms of activation^{21,38,39}.

Many mechanosensitive afferents have transduction sites associated with blood vessels supplying the gut^{6,8,10} and other viscera^{10,40}. These afferents have sometimes been traced onto the walls of viscera and have been described as forming mesenteric, serosal and intramuscular populations^{11,12,19}. While mesenteric and submucosal afferent axons are both closely associated with blood vessels, they had different sensitivities to distension and to von Frey hairs. It is not clear whether this is a function of the axons themselves or their mechanical coupling to surrounding tissues.

Spinal afferent axons to viscera have previously been classified as low or high threshold, or “wide-dynamic range” fibers which encode across the innocuous to noxious range^{1,14,32,41–43}. In the rat, most afferents at S1 had low thresholds, but a distinct population of high threshold afferents was also reported^{14,44}. In the upper gut of cat, low and high threshold spinal afferents were reported, with differential sensitivity to ischemia⁴³ and in the colon, 4 classes of mechanoreceptors could be distinguished by their adaptation rates⁷. We found that submucosal vascular afferent units are slightly more responsive to both distension and von Frey hairs than mesenteric vascular afferent fibers and are readily activated by luminal distension. Specialised rectal afferents, which have rectal IGLEs in myenteric ganglia¹⁵ were significantly more sensitive to distension than submucosal afferents. On the basis of such responses to stretch, vascular afferents can be considered as “medium/high threshold” compared to the “low threshold rectal mechanoreceptors”. We cannot rule out the possibility that subtypes of mesenteric and submucosal vascular afferents may exist within these populations.

Medium/high threshold mechanoreceptors and their adequate stimuli

While medium/high threshold vascular mechanoreceptors can be activated by stiff von Frey hairs, this intensity of stimulation is unlikely to be encountered under physiological or even pathophysiological conditions. The same receptors can however be activated by strong mechanical distension applied to either the gut wall or to the mesenteric membranes. Submucosal intramural vascular afferents may mediate the slowly-adapting, high threshold (>25mmHg) response to distension reported in the cat colon⁷. Our findings do not, however, exclude the possibility that other types of stimuli may excite intestinal medium/high threshold mechanoreceptors, including reduced perfusion of mesenteric blood vessels⁴⁵ or transient ischemia⁴³ during strong contraction or distension. The situation may be further complicated if activation of vascular afferent fibers causes vasodilator axon reflexes⁴⁶.

Mechanoreceptors and inflammatory sensitization

Colonic inflammation can induce hypersensitivity to intestinal distension⁴⁷. Bradykinin, 5-hydroxytryptamine, prostaglandins, TRPV1 activators and hypoxia/ischemia, all sensitize afferent axons^{9,28,40,48} and increased release could contribute to the sensitization associated with inflammation⁴¹. However, it has been difficult to understand the sensitizing effects of such mediators if high threshold mechanoreceptors are exclusively located outside the gut

proper, in the serosal and mesenteric membranes. The present demonstration that extrinsic sensory neurons frequently have transduction sites in the submucosa within the gut wall, provides a ready explanation for how intramural inflammation may cause sensitization of distension-sensitive reflex pathways. We speculate that these intramural vascular afferent axons have close mechanical coupling with gut distension and appropriate properties to play a role in distension-induced sensation, including pain. We cannot exclude the possibility that lower threshold mechanoreceptors elsewhere in the gut also contribute to pain pathways.

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Figure 1.

Responses to von Frey hairs. A. Axons of mesenteric afferent nerves give repeatable responses to localised probing with a von Frey hair (7mN) on mesenteric blood vessels, with little adaptation. B. Averaged responses to von Frey hairs from axons on mesenteric blood vessels (14 hotspots, N=4), submucosal blood vessels (13 hotspots, N=4) and, for comparison, to low threshold rectal mechanoreceptors in the myenteric ganglia of guinea pig rectum (8 hotspots, n=4, data from Lynn et al 2005¹⁵). C. Varicose branching axons filled from the recording site were significantly closer to marked “hotspots” than to random sites ($P<0.01$) on mesenteric blood vessels C. Varicose branching axons filled from the recording site were significantly closer to arterial branch points than randomly generated sites on mesenteric arteries ($P<0.02$)

Figure 2.

Anterograde dye fills of axons in mesenteric nerve trunks. The only specialised axonal structures in the mesentery, revealed by biotinamide fills (apart from bundles of smooth axons of passage), were varicose branching axons on blood vessels. A: map of biotinamide fill of guinea pig ileum shows location of all axonal specialisations filled from the asterisk. All were varicose branching axons (black dots) usually located close to mesenteric arteries or, in the gut to myenteric ganglia. B: Fluorescent, biotinamide-labelled axons in the mesentery consisted of bundles of smooth axons-of-passage and occasional varicose branching axons on arteries (arrows). C: Varicose branching axons were also abundant in myenteric ganglia. D: in the submucosa, filled axons were primarily associated with blood vessels, with the only specialisation being varicose branching axons, but some axons also ran in the submucous plexus

Figure 3.

Responses to von Frey hair and distension. A. Afferent axon on a submucosal blood vessel was strongly activated by a 3mN von Frey hair and by circumferential distension by a 10mN load. B. Afferent axons in the submucosa (N=5) were more likely to show a response to a 100mN load than axons in either the external muscle layers including myenteric ganglia (“myent”, N=5) or axons in the mesentery (“mesent”, N=5). C. Mean firing rate evoked by a 100mN load, averaged over 20s (Hz), showed that afferent axons in the submucosa (N=10) and mesentery (“mesent” N=11) had significantly larger responses than axons in external muscle layers (“myent” N=7)

Figure 4.

Responses to von Frey hairs. A. 3mN von Frey hair evoked consistent bursts of firing from a submucous artery. B. micrograph of preparation with mucosa and most underlying external muscle layers removed, but submucosa intact. Responsive sites ("hotspots") are marked with asterisks. Note 7 "hotspots" (responsive sites) located on blood vessels in the mesentery and 9 hotspots in the submucosa, all of which are located on submucous blood vessels

Figure 5.

Comparison of sensitivity of different types of extrinsic afferent nerve endings to stretch. A. Rectal mechanoreceptors (N=10) had significantly lower thresholds ($P<0.02$ see text) and were more responsive than submucosal receptors (N=8). Mesenteric endings (N=6) and endings in muscularis externa ("myenteric" N=5) were less sensitive still. B. The same afferent unit was activated by a 6mN von Frey hair applied to both a mesenteric blood vessel and to a submucosal blood vessel (8 superimposed spikes (right), suggesting that some vascular afferents innervate both tissues).

Figure 6.

Sensory markers in mesenteric and submucosal afferent axons. **A.** Biotinamide fills revealed varicose branching axons, some of which are likely to be sensory, with CGRP but not tyrosine hydroxylase (TH) immunoreactivity. **B.** There was a high degree of coexistence of the sensory markers, CGRP and TRPV1 in perivascular nerve fibers (mesenteric, N=5, submucosal N=6). **D,E:** Most mesenteric (68%) and submucosal (85%) mechanosensitive vascular afferent fibers (responsive to 3mN von Frey hair), were activated by topical 1 μ M capsaicin (asterisks)

Figure 7.

Mechanosensitive innervation of the colon associated with submucosal blood vessels. A. Von Frey hairs (0.5mN) activated two afferent axons (Units 1,2), both located on submucosal blood vessels; which also responded to 6mm circumferential distension. B. Unit 1 was activated by 3 μ M capsaicin applied in the bath, but unit 2 was not. D. Both units had mechanosensitive sites associated with submucosal blood vessels (hs1, hs2)

Figure 8.

Intramural bladder receptors, located on blood vessels, respond to mechanical stimuli. A. 0.5mN von Frey hair evoked firing in an intramural bladder afferent. Stretch (6mm distension) also activated the same unit. B. Marked “hotspots” on tissue were significantly closer to blood vessels than randomly generated sites. C. Afferent unit shows graded response to von Frey hairs. D; Micrograph shows carbon marker and hotspots (arrows). E. At higher magnification, biotinamide-filled axons had varicose branching axons on intramural blood vessel, close to the hotspot.