Neurogastroenterol Motil (2008) **20,** 660–670 doi: 10.1111/j.1365-2982.2008.01082.x

Analysis of innervation of human mesenteric vessels in non-inflamed and inflamed bowel – a confocal and functional study

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Abstract *We investigated the distribution and density of perivascular nerves in human mesenteric arteries and veins and their responses to noradrenaline (NA), ATP and neuropeptide Y (NPY) in control (non-inflamed) and inflamed bowel, using confocal microscopy and in vitro pharmacology. The density of innervation at the adventitial-medial border of arteries and within the medial muscle coat of veins was increased in inflammatory bowel disease (IBD). Expression of markers for both sympathetic nerves and sensory-motor nerves was significantly increased in IBD. Calcitonin gene-related peptide-containing sensory-motor nerves were present in control arteries and IBD, but not in control veins. The density of 5-hydroxytryptamine-containing nerves was variable in controls, but consistently increased (three to four times) in IBD. Vasoactive intestinal peptide (VIP) expression increased (doubled) in arteries and veins. Arteries and veins contracted to NA and ATP, but only veins constricted to NPY. ATP contractions were reduced in arteries and veins in IBD, while contractions to NA were only slightly reduced. Neuropeptide Y induced significantly greater (20%) contractions of IBD veins. In summary, the density of sympathetic and sensory-motor innervation of both mesenteric arteries and veins was increased in IBD. Both 5-hydroxytryptamine and VIP immunoreactivity were also increased. The responses of both arteries and veins to ATP, and to a lesser extent NA, were reduced in IBD while responses to NPY were greater in veins. Decreased responses to ATP indicate changes in purinergic-mediated transmission in the pathological state.*

Keywords *ATP, human mesenteric artery, human mesenteric vein, immunomarker, sympathetic.*

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Received: 21 September 2007
Accepted for publication: 13 December 2007

INTRODUCTION

There is dual local control of vascular tone by perivascular nerves and endothelial cells.[1] Changes in the density of innervation and expression of vasoactive agents coexisting in nerves and endothelial cells have been described in ageing, hypertension and diabetes.[2–5] Studies of vessel innervation were previously assessed by semiquantitative methods open to subjectivity.[6] Newer forms of microscopy, digital image manipulation and image analysis have allowed the development of more accurate, quantitative methods of assessment.

The pathogenesis of inflammatory bowel disease (IBD) remains unclear although vascular abnormalities have been described, affecting the microvasculature and larger vessels.[7,8] Significant alterations in intestinal blood flow have been described in human IBD, which change during disease progression. It was suggested that the decline in blood flow during experimental colitis may result from a diminished capacity of colonic arterioles to respond to endothelium-dependent vasodilators.[9] Changes in perivascular innervation may contribute to these abnormalities.

Differences in the pharmacology of the sympathetic cotransmitters, noradrenaline (NA) and ATP[10] between mesenteric arteries and veins have been reported.[11,12] In this study, quantitative differences in the density and pattern of innervation between arteries and veins were investigated with immunohistochemistry for protein gene product 9.5 (PGP9.5; a general neuronal marker), tyrosine hydroxylase (TH; a marker for NA)

and neuropeptide Y (NPY) for sympathetic nerves, and calcitonin gene-related peptide (CGRP) and substance P (SP) for sensory-motor nerves. The distribution of immunoreactivity for vasoactive intestinal peptide (VIP), nitric oxide synthase (NOS; for nitric oxide), choline acetyltransferase (ChAT; for acetylcholine) and 5-hydroxytryptamine (5-HT) was also studied. A study of human mesenteric vessels using PGP9.5 identified a greater number of nerve fibres using confocal microscopy than conventional epifluorescence microscopy.[13]

There are few studies of neurotransmitter subgroups in human mesenteric vessels; 5-HT-containing nerves[14] and contractions to NA and 5-HT in healthy human mesenteric arteries have been reported.[15] In this study, confocal microscopy and image analysis have been used to assess quantitatively the density of nerves containing neurotransmitters in the human mesenteric vasculature in control vessels and in IBD. Contraction of human mesenteric vessels to NA, ATP and NPY was also investigated, to identify functional changes in vessels from patients with IBD.

METHODS

Mesenteric vessels were obtained from specimens resected from patients undergoing surgery for non-inflammatory (colo-rectal carcinoma) ($n = 6$) and inflammatory ($n = 12$) conditions [ulcerative colitis (UC; colon vessels) and Crohn's disease (CD; ileum or colon vessels)]. Informed consent was obtained. Vessels were dissected from the mesentery close to the resection margin, adjacent to the bowel wall, avoiding interference with mesenteric lymph nodes needed for prognostic information. Patient details are given in Table 1.

Table 1 Patient details – controls

Patient	Age	Sex	Diagnosis	Drug therapy
Controls				
1	80	F	Ca colon	Enalapril
2	59	F	Ca rectum	
3	81	F	Ca colon	Amlodipine, digoxin, senna
4	75	M	Ca rectum	Llisinopril, atenolol, amlodipine
5	60	M	Ca rectum	
6	43	F	Ca rectum	
IBD				
7	58	M	CD colitis	Mesalazine, prednisolone, azathioprine
8	48	M	CD ileitis	
9	46	F	CD ileitis	Prednisolone
10	19	F	CD colitis	Prednisolone, mesalazine
11	35	F	CD ileitis	Prednisolone
12	20	M	CD ileitis	Prednisolone
13	63	F	UC	Prednisolone, lomotil, glicazide
14	29	F	UC	Cortisone, azathioprine
15	28	F	UC	Colifoam
16	18	F	UC	Prednisolone, sulphasalazine, azathioprine, cromoglycate
17	38	M	UC	Prednisolone, sulphasalazine, azathioprine
18	64	M	UC	Prednisolone, colifoam, mesalazine, isosorbide, sulphasalazine

Ca, carcinoma; CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease.

Immunostaining and confocal analysis

Human mesenteric vessels are thick-walled compared to laboratory animals and contain substantial amounts of elastic tissue, which autofluoresces under mercury vapour lamps, making the study of perivascular innervation difficult using conventional techniques, but are overcome by confocal microscopy.[13]

Segments of mesentery were removed in the operating theatre and immediately placed in cold Hanks' balanced salt solution. The marginal vessels or vasa rectae of 1–3 mm diameter were dissected free of excess fat and connective tissue and slit longitudinally. The relaxed dimensions of the vessel segment were measured. The segment was stretched and pinned out on Sylgard and re-measured. The longitudinal and transverse stretch factors were calculated (approximately 10–15% in both longitudinal and transverse directions). The segments were fixed for 2 h in 4% paraformaldehyde. When fixed, the stretched dimensions were retained. The specimens were washed in phosphate-buffered saline (PBS) and stored at 4 °C.

Whole-mount segments of artery and vein were stained using a standard indirect immunofluorescence technique. Briefly, background staining was reduced by incubation in normal donkey serum (1 : 10) for 2 h at room temperature. Rabbit polyclonal antibodies to

Table 2 Details of antisera

Antigen	Source	Dilution
TH	Affiniti (UK)	1 : 500
NPY	Biogenesis (UK)	1 : 2000
VIP	Incstar (USA)	1 : 2000
SP	Genosys (UK)	1 : 1000
CGRP	Affiniti (UK)	1 : 1000
5-HT	Incstar (USA)	1 : 250
PGP9.5	Ultraclone (UK)	1 : 2000
ChAT	Biogenesis (UK)	1 : 250
NOS	Eurodiagnostica (Sweden)	1 : 250

TH, tyrosine hydroxylase; NPY, neuropeptide Y; VIP, vasoactive intestinal peptide; SP, substance P; CGRP, calcitonin gene-related peptide; 5-HT, 5-hydroxytryptamine; PGP9.5, protein gene product 9.5; ChAT, choline acetyltransferase; NOS, nitric oxide synthase.

TH, NPY, VIP, CGRP, 5-HT, NOS, ChAT or PGP9.5 were applied for 36 h in a humid chamber at room temperature, at the concentrations as given in Table 2.

After washing three times in 0.1% Triton in PBS, biotinylated donkey anti-rabbit antibody was applied for 2 h. After further washing, streptavidin-fluorescein was applied for 1 h. The specimens were washed and counterstained with Pontamine sky blue and mounted in Citifluor (Citifluor Ltd., London, UK).

The slides were viewed with a Leica TCS 4D confocal microscope (Leica, Heerbrugg, Switzerland) using an objective magnification of ×25 (or ×40 for very fine nerves). Three representative fields were recorded, having set the depth of tissue scanned ('z-series') to include all visible nerves in each field. The z-series and the projected image were stored digitally.

The digital images were analysed for intercept density (ID) in both transverse and longitudinal orientations, and for total fluorescent area (TFA), using Scion image analysis software (NIH, USA). The ID is proportional to the total length or number of nerve bundles present, whilst TFA is proportional to the total number of nerves within the bundles.

Images were converted to negative black-and-white format. The greyscale level that demarcated the boundary between the background and the nerves was chosen individually for each image and converted to a binary image, which was subjected to the process of 'closing', which has the effect of removing small background speckles up to a predefined size. The TFA of nerves in the visual field was measured and expressed as a percentage of the total image area.

The nerve network was 'skeletonized', reducing the nerves to a network no more than one pixel wide. The ID was then measured using a superimposed array of 15 lines and counting (blind to control and IBD vessels) the number of nerve intersections automatically. The mean transverse ID (TID) for the image was calculated. The previously calculated transverse stretch factor was applied to give the TID.

Confocal analysis statistics

The TFA and TID of nerves containing the immunomarkers were determined by taking that for PGP9.5 as 100% and calculating the TFA and TID of the other immunomarkers as a percent of that of PGP9.5. Control vessels were compared with IBD vessels by an unpaired *t*-test and a one-way analysis of variance (ANOVA) followed by a *post hoc* test. Data are expressed as mean ± SEM ($n = 5$ or more) and $P < 0.05$ was considered significant.

The total number of nerves was assessed with PGP9.5 staining, although it has been shown that PGP9.5 might underestimate the total neuron number, and there is evidence that the pan-neuronal markers Cuprolinic Blue and anti-HuC/D may be more reliable neuronal markers, at least in the gut.[16]

Organ-bath pharmacology

Human mesenteric artery and vein segments (3–5 mm) were suspended in 5-mL organ baths containing oxygenated (95% O_2 and 5% CO_2) Krebs: (mmol L^{-1}) NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, maintained at 37 °C. Ring segments were mounted horizontally by inserting two tungsten wires through the vessel lumen, one attached to a rigid support and the other to a Grass FTO 3C force-displacement transducer. Isometric tension was measured and recorded on a Grass ink writing polygraph (Grass 79D). An initial load of 1–3 g (arteries) and 0.5–2 g (veins) was applied and vessels were equilibrated for 90 min. Corresponding sections of artery and vein were kept together to allow comparison.

Concentration–response curves were constructed for NA, ATP and NPY. The effect of a sub-threshold concentration of NPY (30 nmol L^{-1}) on responses to NA was assessed. Finally, KCl (120 mmol L^{-1}) was applied to provoke maximal contraction of the vessel.

Statistics

Contractions to agonists are expressed as a percentage of the KCl (120 mmol L^{-1}) contraction. Where a maximal agonist response was achieved pD_2 values were

calculated (–log EC_{50}), alternatively, $p[A]_{25}$ values were calculated (–log concentration giving 25% of KCl contraction). The NA contractions in the presence of NPY are expressed as percentage of the maximum control response (taken as 100%).

Comparisons were made between the corresponding arteries and veins, and between non-inflamed and inflamed vessels. All data are expressed as mean ± SEM ($n \geq 5$) and results compared by Student's *t*-tests or one- or two-way ANOVA, as appropriate, followed by a Bonferroni *post hoc* test; $P < 0.05$ was considered significant.

RESULTS

Specimens diagnosed as UC ($n = 6$) and CD ($n = 6$) were examined. The results from the two groups were not significantly different and were therefore grouped together for the purpose of this study under IDB.

Confocal microscopy

Qualitative observations Large paravascular nerve bundles were identified in the outer adventitia in arteries. These bundles were not included in the investigation and were either removed during dissection or excluded during optical sectioning with the confocal microscope. The perivascular nerves in control arteries were orientated largely longitudinally, while in IBD arteries they appeared to have a random orientation.

Nerves immunoreactive to PGP9.5, TH and NPY were identified in all arteries and veins from control and IBD subject (Figs 1A,B and 2A,B), orientated longitudinally in arteries (Fig. 1A,C,E), whereas in the corresponding veins, nerves formed a reticular pattern (Fig. 2A,C,E) and in some instances the nerve fibres appeared to be concertinaed (see Fig. 2D). In IBD arteries, nerves appeared to be present at a higher density than controls, and formed a reticular pattern (Fig. 1B,D,F). The distribution of immunoreactivity to these markers in veins from IBD vessels was similar to controls (Fig. 2B, D,F).

Nerves immunoreactive to VIP were identified in arteries and veins in control (Figs 3A and 4A) and IBD vessels (Figs 3B and 4B), although at a lower density than staining for TH and NPY. The immunoreactivity of VIP was greater in IBD veins than controls. The immunoreactivity of SP was identified in most control arteries (Fig. 3C) and all control veins (Fig. 4C). The nerves were very fine, often with well-defined varicosities, but were usually sparse. Arteries and veins from IBD patients had a similar immunoreactivity pattern to SP (Figs 3D and 4D).

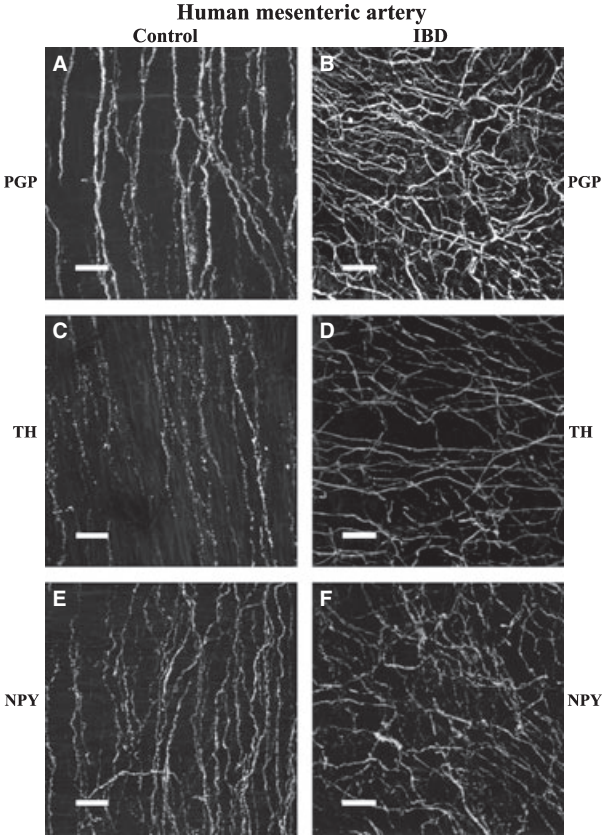

Figure 1 Confocal images of whole-mount preparations of human mesenteric arteries from control subjects (left column) and inflammatory bowel disease (IBD) subjects (right column). The longitudinal axis of the vessels is from top to bottom. Note that the nerve bundles are orientated longitudinally in control, but not so in IBD. Immunostaining is for protein gene product 9.5 (PGP9.5) (A, B), tyrosine hydroxylase (TH) (C, D) and neuropeptide Y (NPY) (E, F). Scale bar = 50μm.

Nerves immunoreactive to CGRP were seen in most control arteries (Fig. 3E) and all IBD arteries (Fig. 3F), with a higher density than SP-immunoreactivity. Control mesenteric veins had no immunoreactivity to CGRP (Fig. 4E), but those from IBD patients did (Fig. 4F).

Only one control artery (Fig. 5A) and three control veins (Fig. 5C) showed 5-HT-immunoreactivity, of a low intensity. In IBD arteries there was extensive 5-HT-immunoreactivity (Fig. 5B), although the immunoreactivity to 5-HT in IBD veins did not differ from that of controls (Fig. 5D).

No nerves immunoreactive to NOS or ChAT were identified in either control or IBD artery or vein (data not shown).

Quantitative observations: Total fluorescent area For arteries, the plane of focus was confined to the plexus

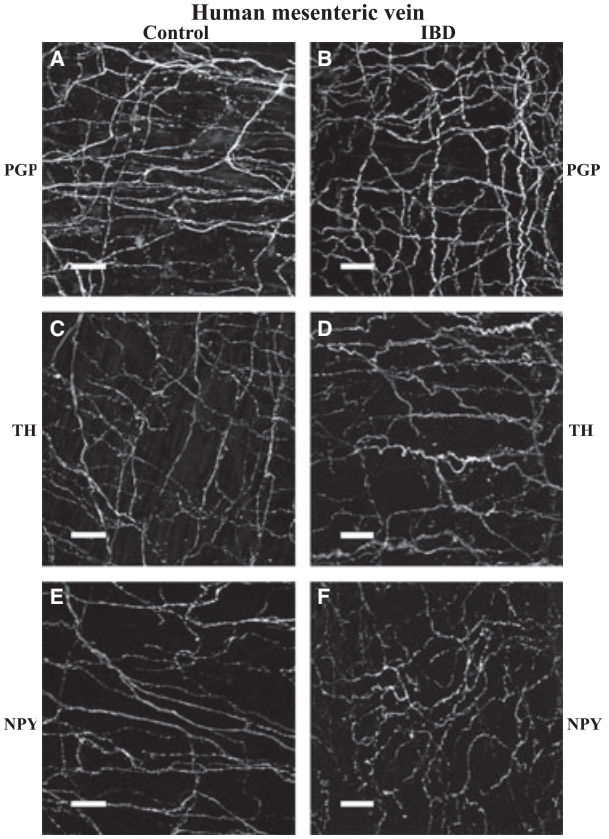

Figure 2 Confocal images of whole-mount preparations of human mesenteric veins from control subjects (left column) and inflammatory bowel disease (IBD) subjects (right column). The longitudinal axis of the vessels is from top to bottom. Note that the nerve bundles appear to be orientated predominately circularly in controls and more randomly in IBD, with some fibres appearing concertinaed. Immunostaining is for protein gene product 9.5 (PGP9.5) (A, B), tyrosine hydroxylase (TH) (C, D) and neuropeptide Y (NPY) (E, F). Scale bar = 50μm.

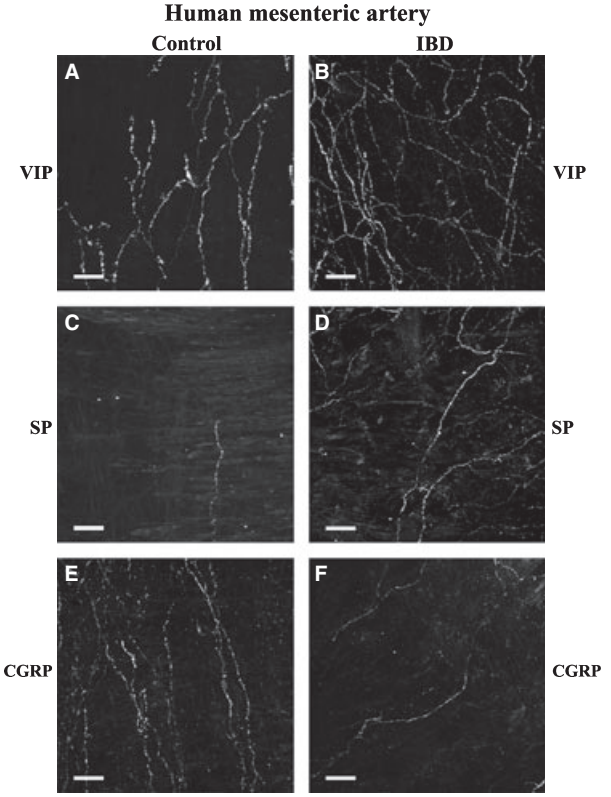

Figure 3 Confocal images of whole-mount preparations of human mesenteric arteries from control subjects (left column) and inflammatory bowel disease (IBD) subjects (right column). Note increase in density of vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP)-immunostained nerves in IBD. Immunostaining is for VIP (A, B), SP (C, D) and CGRP (E, F). Scale bar = 50μm.

at the adventitial/medial border (about 20 μm thick), while in veins the confocal analysis allowed nerves from the entire medial coat to be visualised (about 100 μm thick). The TFA for the immunomarkers was calculated using conventional light microscopy.

The TFAs for PGP9.5, TH, NPY and 5-HT were significantly greater (at least two times) in inflamed arteries and veins compared with controls ($P \leq 0.01$) and for VIP in inflammation compared with control arteries. There was no significant difference in TFA from arteries and veins from non-inflamed and inflamed vessels for SP or from inflamed veins *vs* control for VIP (Fig. 6A).

The IBD arteries had a significantly reduced (by ½) TFA for CGRP, whereas the veins had a greater TFA than controls (absent).

Transverse intercept density The TID for each immunomarker followed a similar pattern to that of the TFA (Fig. 6B), and was significantly greater ($P \leq 0.01$) in IBD arteries compared to controls for PGP9.5 (two times), TH (three times), NPY (two times), VIP (two times) and 5-HT (three to four times). There was no significant difference in the TID from pathological veins for these immunomarkers, with the exception of 5-HT. There was no significant difference in the TID from non-inflamed and inflamed arteries and veins for SP and in non-inflamed and inflamed veins for VIP. The TID for CGRP from inflamed arteries was significantly ($P < 0.01$) less (two times) than that of controls, whereas that for veins was greater than controls (absent).

Functional studies

Contractions to KCl, NA and ATP Contractions to KCl were greater from control arteries than control veins

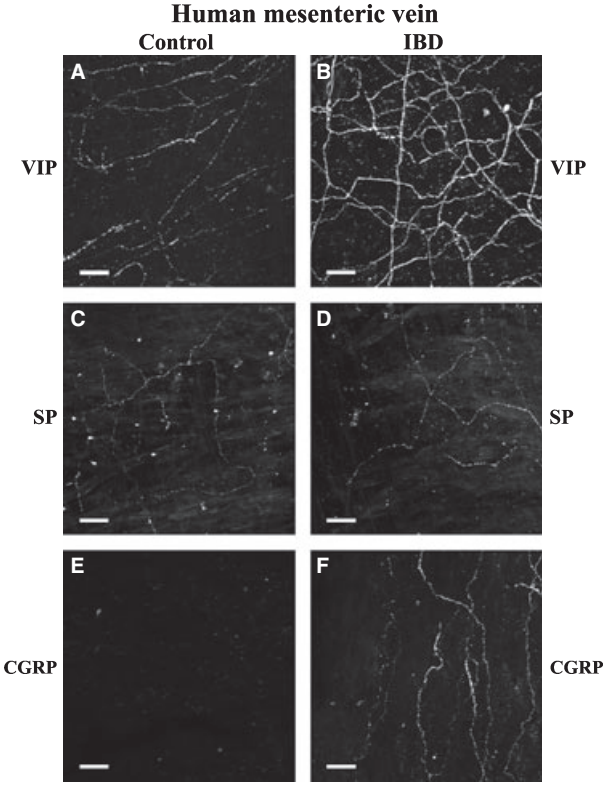

Figure 4 Confocal images of whole-mount preparations of human mesenteric veins from control subjects (left column) and inflammatory bowel disease (IBD) subjects (right column). Immunostaining is for vasoactive intestinal peptide (VIP) (A, B), substance P (SP) (C, D) and calcitonin gene-related peptide (CGRP) (E, F). Note increase in density of VIP and CGRP-immunostained nerves in IBD. Scale bar = 50μm.

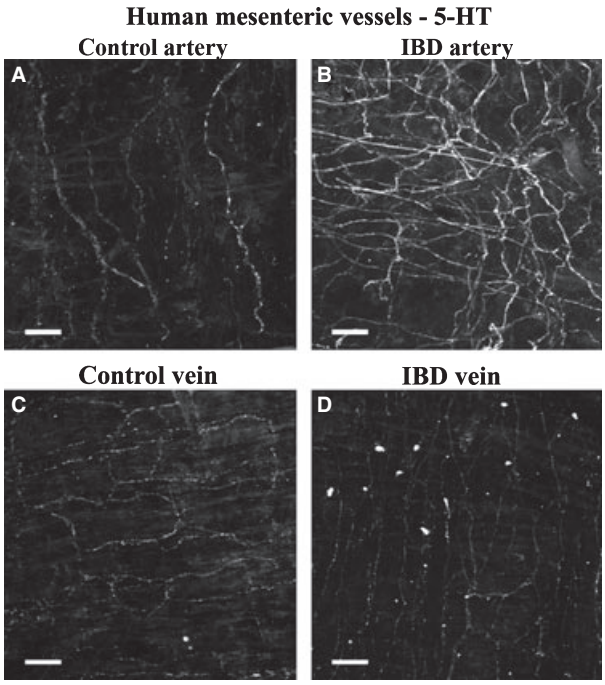

Figure 5 Confocal images of immunoreactivity to 5-hydroxytryptamine (5-HT) in whole-mount preparations of control human mesenteric artery (A) and vein (C) and inflammatory bowel disease (IBD) artery (B) and vein (D). Note the striking increase in 5-HT-immunoreactive nerves in IBD artery and changed appearance in IBD vein. Scale bar = 50μm.

($P < 0.05$); there was a tendency for greater KCl contractions in diseased tissue compared to non-diseased tissue, but not significantly so (see Table 3).

Noradrenaline induced concentration-dependent contractions in arteries and veins from control and IBD subjects (Fig. 7A), those from arteries were greater than veins. There was no significant difference in the concentration–response curves from controls and IBD subjects in either arteries or veins. The pD_2 values for NA are shown in Table 3.

Exogenous ATP induced concentration-dependent contractions in both arteries and veins from control and IBD subjects (Fig. 7B), those from veins being larger than arteries. $p[A]_{25}$ values were calculated and are shown in Table 3. Contractions to ATP were significantly ($P < 0.01$) smaller in arteries and veins from IBD subjects than controls.

NPY and its effect on contractions to NA Exogenous NPY induced concentration-dependent contractions of control and IBD veins (Fig. 7C), but failed to contract arteries. Contractions to NPY from IBD subjects were significantly ($P < 0.01$) greater from inflamed veins than controls, although pD_2 values were not significantly different (Table 3).

The effect of a sub-threshold concentration (30 nmol L^{-1}) of NPY was examined against NA contractions. The NPY (30 nmol L^{-1}) had no significant effect on contractions to NA in control arteries and veins (see Table 3). Concentration–response curves from inflamed veins were significantly ($P < 0.05$) reduced in the presence of NPY, while in inflamed veins responses were significantly increased ($P < 0.01$), although pD_2 values were not significantly different.

DISCUSSION

The mesenteric circulation is highly specialized, supplying the metabolic needs of the gut and other organs; blood flow is altered in response to mechanical stimulation of food in the lumen[17] and as part of inflammatory responses.[18] Mesenteric veins form the largest capacitance bed in man and control of venous tone are important in the effective maintenance of blood pressure,[19] particularly when posture changes. The

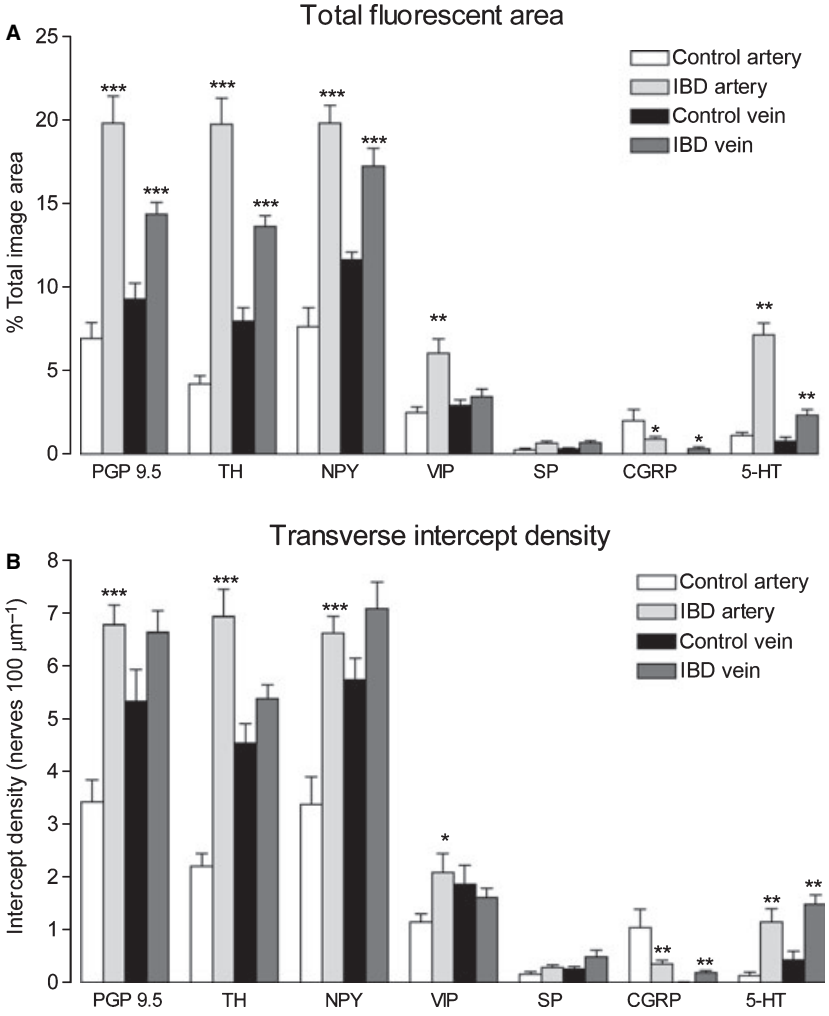

Figure 6 Bar graphs showing total fluorescent area (A) and transverse intercept density (B) of the individual immunomarkers for human mesenteric arteries and veins from control and inflammatory bowel disease (IBD) subjects. All bars are mean ± SEM (n = 5 or more). Statistical significance was tested by an unpaired t-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 3 Contractions to KCl (120 mmol L^{-1}) and agonist pD_2 or $p[A]_{25}$ values

Agonist	Control artery	IBD artery	Control vein	IBD vein
KCl (g)	5.42 ± 1.1 (6)	7.90 ± 0.80 (12)	3.10 ± 0.51(6)	5.09 ± 0.74(12)
NA pD_2	5.23 ± 0.08 (6)	5.38 ± 0.10 (12)	6.08 ± 0.05 (6)	5.94 ± 0.14 (12)
NA pD_2 (+NPY 30 nmol L^{-1})	5.29 ± 0.14 (6)	5.34 ± 0.07 (12)	6.02 ± 0.06 (6)	6.00 ± 0.18 (8)
ATP $p[A]_{25}$	3.22 ± 0.08 (6)	2.72 ± 0.16*** (12)	4.28 ± 0.28 (6)	3.59 ± 0.25 (12)
NPY pD_2	–	–	7.37 ± 0.10 (5)	7.59 ± 0.13 (6)

NA, noradrenaline; NPY, neuropeptide Y.
All data are expressed as mean ± SEM (n). Statistical analyses were by unpaired t-tests, comparing control with inflamed vessels. The NA pD_2 values refer to NA in the presence of a subthreshold concentration of NPY *vs* the same vessel in the absence of NPY.
$^{***}P < 0.001$.

greater nerve density in human mesenteric veins compared with arteries shown in this study may reflect this role.

Sympathetic perivascular nerves were previously thought to contain only NA, but are now recognized as utilizing cotransmitters, notably ATP and NPY.[10] The neuronal markers for this study were selected on the basis of neurotransmitters commonly identified in the four classes of perivascular nerves,[20] TH and NPY for sympathetic nerves, SP and CGRP for sensory-motor nerves, and NO and VIP for intrinsic nerves projecting from the enteric nervous system (ENS).

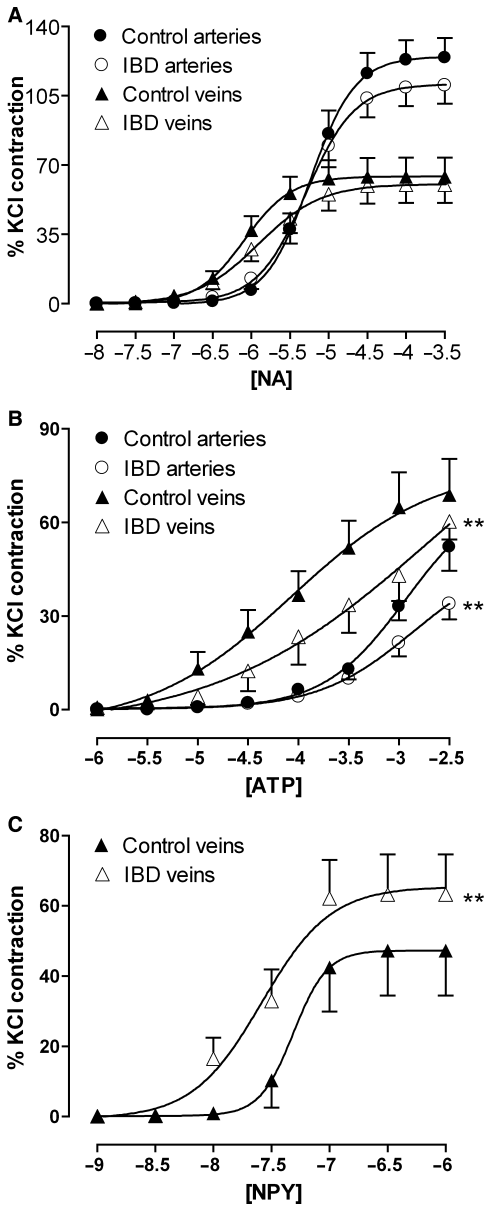

Figure 7 Concentration response curves of human mesenteric arteries and veins from control and inflammatory bowel disease (IBD) subjects. (A) noradrenaline (NA), (B) ATP and (C) neuropeptide Y (NPY). Note NPY failed to induce contractions in mesenteric arteries. All symbols show mean ± SEM (n = 5 or more). Statistical significance was tested by 2-way ANOVA followed by a Bonferroni's *post hoc* test. ***P < 0.001.

5-HT has previously been identified in human mesenteric perivascular nerves, probably after being taken up by sympathetic nerves.[14] ATP is a cotransmitter in most, if not all of these nerve types, but there is no immunohistochemical method for its localization, and detecting its presence relies on functional studies. Noradrenaline and ATP are the major sympathetic vasoconstrictors, acting via postjunctional α_1-adrenoceptors and $P2X_1$ receptors.[10] Although in some blood vessels NPY acts as a vasoconstrictor, in many vessels NPY acts as a neuromodulator, postjunctionally enhancing the contraction produced by other transmitters and/or acting prejunctionally to reduce transmitter release.[10] Perivascular nerves, in concert with signals from the endothelium, regulate local blood flow.[1]

Expression of immunomarkers

In arteries and veins from IBD patients, there was an increase in overall nerve density measured as the TFA for PGP9.5, compared with controls. While increased immunoreactivity implies an increased number of fibres, it may also be indicative of increased branching or increased immunoreactivity within fibres. The majority of human mesenteric perivascular nerves were of sympathetic origin. All vessels had a plexus of TH- and NPY-containing nerves. Neuropeptide Y is also found in subpopulations of neurons in the ENS[21] and in some parasympathetic nerves.[22] There was a greater nerve density for NPY and NA in arteries and veins from patients with IBD.

A moderately dense plexus of nerves containing VIP was identified in arteries and veins. The VIP is a cotransmitter with acetylcholine and other neuropeptides in some parasympathetic nerves.[22] However, the role of parasympathetic innervation of the human mesenteric circulation has been questioned[23] and, in mesenteric vessels of the pig, VIP-immunoreactive nerves are derived from the ENS.[24] In IBD vessels, VIP-immunoreactive nerves accounted for 30 and 25% of the total innervation (as shown by PGP9.5 staining) of arteries and veins, respectively, although this was not significantly different from control vessels. It has been suggested that VIP is involved in suppression of chronic inflammatory responses of the gut.[25] Studies on changes in VIP innervation in the bowel wall have been contradictory[26,27] and they may only occur close to the site of mucosal inflammation.[28]

Fewer than 5% of nerve fibres in control vessels were SP- and CGRP-immunoreactive probably indicating extrinsic sensory-motor nerves.[2,29] Both are potent vasodilators and also interact with mast cells and may have a role in immune functions in inflamed bowel[25,30] and in the protection of the mucosa.[31] However, some of these nerves may represent projections of intrinsic neurons of the ENS, which have been shown to contain CGRP and SP in humans.[32] There were no significant differences in the SP-immunoreactive perivascular nerve density between IBD and

control arteries. In control veins, CGRP was absent, but present in all veins from IBD patients, suggesting a change in the role of the sensory motor innervation in IBD.

5-Hydroxytryptamine is often described as a 'false neurotransmitter' in sympathetic nerves.[33] It is not synthesized in the nerve varicosities, but is taken up from extracellular sources after release from platelets and stored in vesicles for subsequent release, although a subpopulation of enteric neurons contain 5-HT as a principal transmitter.[34] Vasoconstrictor nerves containing 5-HT have been identified in human mesenteric vessels.[14] In spite of the low density of innervation in healthy vessels, there was a marked increase (three to four times) in the number of 5-HT-immunoreactive nerves in IBD, indicating greater release of 5-HT from platelets and perhaps enterochromaffin cells in this pathological condition. The number of 5-HT-containing mucosal enterochromaffin and mast cells was increased in inflamed bowel.[35] Release of 5-HT could contribute to vasospasm and disturbances in blood flow, characteristic of the inflammatory response. This may indicate an underlying abnormality in 5-HT release or metabolism in the gut of IBD patients.[36] Nitric oxide synthase-positive nerves were not detected in this study. This is consistent with a previous study on human infant mesenteric vessels.[21]

These neurochemical changes that we have identified in mesenteric vessels in IBD are similar to those identified in the myenteric plexus of ileum from patients with CD, where an increase in the number of nerves with TH-, NPY- and 5-HT-immunoreactivity was observed.[37] Similarly, in a study comparing biopsies of normal and UC bowel, the number of sympathetic nerves, the mean diameter and the number of varicosities were seen to be increased in inflamed bowel.[38] However, a loss of sympathetic nerve fibres from the mucosa and submucosa has been reported in IBD patients[39] and in a mouse model of colitis.[40] It was suggested that loss of sympathetic fibres might be more apparent in 'hot' inflammatory areas in the mucosa and submucosa. It has also been speculated that loss of sympathetic fibres is due to a repulsion of the fibres from the inflammatory area,[41] which could lead to the concertinaed appearance of nerve fibres seen in this study. Ultrastructural studies in CD identified axonal proliferation accompanied by necrosis, both in the inflamed region of the bowel and the resection margins.[42] In our study of mesenteric vessels in IBD, nerve proliferation was identified but there was no evidence of axonal degeneration. Nerve proliferation appears to be a widespread feature in chronic gut inflammation.[43] The increase in sympathetic innervation would be likely to contribute to the vasospasm, non-synchronized bowel contraction and oedema observed in IBD. Whether nerve proliferation is the result of the inflammatory processes, perhaps by initiating increase in sympathetic nerve activity, or to an unknown environmental factor, has yet to be determined.

It should be noted that the IBD patients in this study were receiving corticosteroids. This may have an effect on the innervation, as intracolonic application of the corticosteroid, budesonide, to rats in which colitis had been induced, resulted in a dose-dependent prevention of nerve loss.[44]

Functional responses

Contractions to NA in IBD artery and vein were reduced, although not significantly, compared to controls. However, contractions to ATP were significantly decreased in both IBD artery and vein, suggesting that the purinergic component of sympathetic cotransmission may be selectively altered in the inflamed state. In a recent study of a mouse model of colitis, ATP did not induce vasoconstriction of submucosal arerioles,[45] further supporting this view. In contrast to veins, arteries from control and IBD subjects did not respond to NPY. The contractile effects of NPY on healthy human mesenteric veins have been described previously.[46] Significant decreases in the contractile responses of human mesenteric arteries to phenylephrine in CD have been described.[47]

The alteration in contractility of veins is likely to be of clinical importance. Localized venous contraction would contribute to engorgement and oedema of the gut, which is a prominent feature of CD. Furthermore, non-invasive tests of autonomic nervous system function have identified systemic abnormalities in UC and CD. Changes in contractility of splanchnic veins would be reflected in tests such as these.[48] Abnormalities in blood flow in mesenteric arteries have been identified using Doppler sonography.[49] Differential blood flow in the layers of the bowel wall may divert blood away from the mucosa, leading to paradoxical ischaemia. Increased flow in the face of contraction of the postcapillary resistance vessels may also contribute to vascular engorgement and oedema. It has been suggested that ischaemia plays a part in the pathogenesis of IBD.[7] Experimental evidence in mice indicates that microcirculatory disturbances precede histological abnormalities.[8]

A further factor that may be of importance is ATP, which is released from both nerves and non-neuronal cells, as it has many actions in the inflammatory

process, including mast cell degranulation, leucocyte adhesion to the endothelium, production of prostaglandins and inflammatory cytokines and potentiation of the oxidative burst. The response to ATP is determined by the receptor subtypes present in the tissues, which have been found to be altered in chronically inflamed tissues[45,50], as well as alterations in ATP degradation.[45]

Abnormalities in the perivascular innervation of human mesenteric vessels have been demonstrated in the present study. The perivascular nerves are one arm of the dual control system for controlling blood flow,[1] and changes in this system in patients with IBD may be important in its pathogenesis.

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