Endothelium-Dependent Vasodilation in Human Mesenteric Artery Is Primarily Mediated by Myoendothelial Gap Junctions Intermediate Conductance Calcium-Activated K⁺ Channel and Nitric Oxide

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ABSTRACT

Myoendothelial microdomain signaling via localized calcium-activated potassium channel (IKCa) and gap junction connexins (Cx) is critical for endothelium-dependent vasodilation in rat mesenteric artery. The present study determines the relative contribution of NO and gap junction–IKCa mediated microdomain signaling to endothelium-dependent vasodilation in human mesenteric artery. The hypothesis tested was that such activity is due to NO and localized IKCa and Cx activity. In mesenteric arteries from intestinal surgery patients, endothelium-dependent vasodilation was characterized using pressure myography with pharmacological intervention. Vessel morphology was examined using immunohistochemical and ultrastructural techniques. In vessel segments at 80 mm Hg, the intermediate (i)IKCa blocker 1-[(2-chlorophenyl)diphenyl-methyl]-1H-pyrazole (TRAM-34; 1 μM) inhibited bradykinin (0.1 nM–3 μM)-induced vasodilation, whereas the small (S) IKCa blocker apamin (50 and 100 nM) had no effect. Direct IKCa activation with 1-ethyl-2-benzimidazolinone (1-EBIO; 10–300 μM) induced vasodilation, whereas cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimdin-4-yl]-amine (1–30 μM), the SKCa activator, failed to dilate arteries, whereas dilation induced by 1-EBIO (10–100 μM) was blocked by TRAM-34. Bradykinin-mediated vasodilation was attenuated by putative gap junction block with carbenoxylole (100 μM), with remaining dilation blocked by N-nitro-l-arginine methyl ester (100 μM) and [1H-[1,2,4]oxadiazolo-[4,3-α]quinolinaxin-1-one (10 μM), NO synthase and soluble guanylate cyclase blockers, respectively. In human mesenteric artery, myoendothelial gap junction and IKCa activity are consistent with Cx37 and IKCa microdomain expression and distribution. Data suggest that endothelium-dependent vasodilation is primarily mediated by NO, IKCa, and gap junction Cx37 in this vessel. Myoendothelial microdomain signaling sites are present in human mesenteric artery and are likely to contribute to endothelium-dependent vasodilation via a mechanism that is conserved between species.

Introduction

Endothelium-dependent vasodilation plays a critical role in the control of vascular tone and is mediated by NO, prostacyclin, and the non-NO/prostacyclin endothelium-derived hyperpolarization (EDH) mechanism (McGuire et al., 2001; Féleťou and Vanhoutte, 2007; Sandow et al., 2009). The latter mechanism involves an increase in endothelial [Ca²⁺], and activation of localized small and/or intermediate conductance calcium-activated potassium channels (SKCa, as SK3;
K_, Ca2.3/KCa3.1/KCNN4; and IKCa, as IK1; SK4/KCa3.1/KCNN4, respectively. The subsequent endothelial hyperpolarizing current is then transferred to the smooth muscle via myoendothelial gap junctions (MEGJs), and/or endothelial K® is released, which activates smooth muscle Na+/K+-ATPase, closing smooth muscle voltage-dependent calcium channels, thereby hyperpolarizing the smooth muscle and dilating the artery (FéleÁetou, 2009; Sandow et al., 2009).

Specialized myoendothelial signaling sites associated with gap junctions are present in mouse mesenteric artery where EDH-type vasodilator activity is present (Dora et al., 2003; Sandow et al., 2004). In pressurized mouse mesenteric artery, an apparent close spatial association occurs between putative myoendothelial signaling sites as internal elastic lamina (IEL) holes (being prerequisites for MEGJ-related projections) and sites of calcium storage and release (Ledoux et al., 2008). Functional “calcium pulsars” are reported to occur at these sites (Ledoux et al., 2008), an observation consistent with proposed inositol 1,4,5-trisphosphate receptor (IP3R)-mediated signaling at MEGJ sites.

In rat mesenteric artery, EDH-mediated vasodilation is dependent on myoendothelial microdomain signaling (Dora et al., 2008; FéleÁetou, 2009; Sandow et al., 2009). The sites for such signaling occur at endothelial to smooth muscle cell projections via IEL holes, a proportion of which are associated with localized myoendothelial expression of IP3Rs (Sandow et al., 2009) and gap junction connexins (Cx) 37 and 40 and IKCa (Mather et al., 2005; Sandow et al., 2006, 2009; Dora et al., 2008). In a manner similar to IP3R-mediated events at contact sites in mouse aortic endothelial and smooth muscle cell coculture (Isakson and Duling, 2006; Isakson, 2008), localized IP3R are potentially associated with the inositol 1,4,5-trisphosphate dependence of EDH-type vasodilation in rat mesenteric artery (Fukao et al., 1997; Cao et al., 2005).

The relative contribution of EDH to vasodilation differs within and between vascular beds, within species, and in ageing, development, and disease (McGuire et al., 2001; Sandow, 2004; FéleÁetou, 2009). This finding suggests that the potential relationship between myoendothelial microdomain components may vary in a similar manner. However, the contribution of microdomain-dependent KCa and gap junction Cx to endothelium-dependent vasodilation in human arteries is unknown.

The aim of the present study was to investigate the mechanisms involved in endothelium-dependent vasodilation in human mesenteric artery and specifically to determine the relative contribution of NO and KCa/gap junction-mediated microdomain signaling to this activity. The hypothesis tested was that microdomain signaling associated with EDH-type vasodilation is characterized by KCa and MEGJ activity in human mesenteric artery via a mechanism, which is conserved between species.

Materials and Methods

Patients. Artery segments were obtained from male and female patients; male patients were heavier than female patients (51 patients total; Table 1). Two were smokers, whereas two had smoked previously but had stopped for ≥25 years. Two patients had type 2 diabetes, and none had undergone radiochemotherapy. All but one patient were not on drug therapy [the exemption to this was taking antihypertensive medication telmisartan (Micardis)]. Thirty-five patients were treated for colon cancer, and the remaining 15 were treated for diverticulitis, bowel disorder, polyps, and adhesions; one was unknown.

Vessel Isolation. Immediately after lower intestinal surgery, mesenteric arcades were excised from resected tissue and washed several times overnight in ice-cold Krebs’ solution containing 112 mM NaCl, 25 mM NaHCO3, 4.7 mM KCl, 1.2 mM MgSO4, 7H2O, 0.7 mM KH2PO4, 10 mM HEPES, 11.6 mM glucose, and 2.5 mM CaCl2·2H2O, pH 7.3. Successive washing was carried out to remove anesthetic and excess blood. In preliminary studies, inadequate washing of tissue resulted in a lack of vascular responses. Mesenteric arteries with an inner lumen diameter of 451 ± 11 μm (at 80 mm Hg in zero calcium, as maximal dilution; n = 51) were isolated and carefully cleaned of connective and adipose tissue. Procedures were performed in accordance with the ethics guidelines of the National Health and Medical Research Council of Australia.

Pressure Myography. Segments of artery were cannulated in a pressure myograph (Living Systems Instrumentation, St. Albans, Victoria, Australia), with continuous superfusion of Krebs’ solution (37°C, 5% CO2) at a rate of 2 ml/min, and diameter was monitored using Diamtrak software (Adelaide, SA, Australia). Arteries were pressurized to 80 mm Hg, and vessel viability was assessed in response to 80 mM KCl. After several washes, arteries were submaximally preconstricted with vasopressin (0.03–3 μM), and subsequent endothelium-dependent vasodilation was measured in response to increasing concentrations of bradykinin (BK; 0.1 nM-3 μM).

Vasopressin was chosen as the constrictor agonist (Gillham et al., 2007; noting that these authors also used preconstriction to the thromboxane agonist 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F20, U46619), as preliminary experiments (n = 3) showed inconsistent and predominantly no constrictor action to phenylephrine (1–30 μM). Bradykinin was chosen as the vasodilator agonist, as preliminary experiments (n = 3) showed inconsistent and predominantly no vasodilator action to acetylcholine (0.01–30 μM). The use of BK is consistent with previous endothelium-dependent vasodilation studies on human mesenteric artery (Matoba et al., 2002).

Experiments were conducted in the presence and absence of selective blockers of endothelium-mediated vasodilator pathways. l-NAME (100 μM) and ODQ (10 μM) were used to examine NO synthase and soluble guanylyl cyclase activity, respectively (McGuire et al., 2002; Mauban and Weir, 2004; Hilgers et al., 2010). Indomethacin (10 μM) was added to clarify the potential role of cyclooxygenase (Laneuville et al., 1994). Apamin (50 and 100 nM) and 1-[2-chlorophenyl]diphenyl-methyl-1H-pyrazole (TRAM-34) (1 μM; Eicher et al., 2003) were used to selectively block SKCa and IKCa, respectively (Gillham et al., 2007; Dora et al., 2008; Weston et al., 2010). The putative gap junction uncoupler carbobenzoxolone (100 μM; Coleman et al., 2001; Dora et al., 2008) and the H2O2-decomposing enzyme catalase (from Bovine Liver; Sigma-Aldrich, St. Louis, MO) were used to assess the respective contribution of gap junctions and H2O2. Based on previous studies of human vessels (Larsen et al., 2008; Luksha et al., 2008), the 2000 units/ml used in the present study is apparently sufficient to block potential H2O2-dependent vasodilator activity. However, given that 6250 units/ml catalase was previously shown to attenuate human mesenteric artery vasodilator activity (Matoba et al., 2002), its effect at this concentration was also examined.

### Human mesenteric artery general patient characteristics

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Weight (kg)</th>
</tr>
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<tbody>
<tr>
<td>Male (24)</td>
<td>Female (27)</td>
</tr>
<tr>
<td>59 ± 2 (51)</td>
<td>79 ± 2 (48)</td>
</tr>
<tr>
<td>58 ± 2 (24)</td>
<td>86 ± 2 (23)</td>
</tr>
<tr>
<td>60 ± 2 (27)</td>
<td>73 ± 2 (25)</td>
</tr>
</tbody>
</table>

* P < 0.05, significant (from male weight).

**TABLE 1** Human mesenteric artery general patient characteristics. The number of patients in each group is indicated within parentheses.
Blockers were incubated for at least 20 min before repeated agonist responses were examined. In addition, the SKCa and IKCa agonists cyclo-hexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine (CyPPA) (1–30 μM; NeuroSearch A/S, Ballerup, Denmark; Weston et al., 2010) and 1-ethyl-2-benzimidazolinone (1-EBIO) (10–300 μM; Tocris Bioscience, Bristol, UK; Kusama et al., 2005), respectively, were added to the bath after preconditioning, with the latter in the presence and absence of TRAM-34 to examine direct channel activation to further verify blocker data. Artery diameter changes are expressed as a percentage of the maximal vasodilation achieved by the control Krebs solution with [Ca2+]o-free Krebs’ solution.

**Immunohistochemistry.** The potential distribution of myoendothelial SKCa, IKCa, and Cx37, Cx40, and Cx43 was examined in human mesenteric artery using conventional confocal immunohistochemistry (Rummery et al., 2005; Sandow et al., 2006). Fresh tubular vessel segments were pinned to a Sylgard dish containing Krebs’ solution, with 0.1% sodium nitrite added to induce maximal vessel dilation. Segments were cut along the lateral plane and pinned out as a flat sheet with the intima uppermost and fixed in fresh 2% paraformaldehyde in PBS, pH 7.4, or ice-cold (4°C) acetone for 5 to 10 min.

The method described above maximizes the size of the flat area of vessel visible in the narrow focal region of the IEL holes and associated potential channel localization at the IEL-endothelial/smooth muscle cell interface, as per previous studies (Sandow et al., 2006; 2009) and is thus optimal for intact vessel methods or sections, where the “flat” area is limited. Whole-mount tissues were incubated in blocking buffer as PBS containing 1% bovine serum albumin, 0.2% Tween 20 for 2 h at room temperature, rinsed in PBS (3 × 5 min), and incubated in primary antibody as SK3 (SKCa; 1:100; M75; Glaxo-SmithKline, Stevenage, Hertforshire, UK), IK1 (IKCa; 1:100, M20; GlaxoSmithKline), Cx37/40 (1:100; Australian National University, Canberra, ACT, Australia), and Cx43 (1:100; Invitrogen, Carlsbad, CA) in blocking buffer as PBS containing 1% bovine serum albumin, 0.2% Tween 20 for 2 h at 4°C. Tissue was then rinsed in PBS (three times for 5 min each) and incubated in secondary antibody (AlexaFluor 633; Invitrogen), diluted in 0.01% Tween 20 for 2 h at room temperature, rinsed in PBS (3 × 5 min), and incubated in primary antibody as SK3 (SKCa; 1:100; M75; Glaxo-SmithKline, Stevenage, Hertforshire, UK), IK1 (IKCa; 1:100, M20; GlaxoSmithKline), Cx37/40 (1:100; Australian National University, Canberra, ACT, Australia), and Cx43 (1:100; Invitrogen, Carlsbad, CA) in blocking buffer as PBS containing 1% bovine serum albumin, 0.2% Tween 20 for 2 h at 4°C. Tissue was then rinsed in PBS (three times for 5 min each) and mounted intima uppermost in antifade glycerol and examined with a confocal microscope (Olympus FV1000; Olympus, Center Valley, PA) using consistent settings for each preparation. CellR software (Olympus) was used for quantitative measurements. Localizations of KCa and Cx were counted as bright spots of fluorescence at the IEL-smooth muscle cell and IEL-smooth muscle cell interface (Sandow et al., 2006, 2009; Dora et al., 2008; Chadha et al., 2010). Multiple IK1/Cx37 localizations at single sites (<2 µm apart) were counted as single localizations.

Extensive controls for antibody specificity were conducted previously and involved use of transfected cells, as well as with positive and negative controls, Western blotting, and immunoelectron microscopy (Boettger et al., 2002; Chen et al., 2004; Rummery et al., 2005; Haddock et al., 2006; Sandow et al., 2006).

**Transmission Electron Microscopy.** Tissue preparation for serial section electron microscopy is as described previously (Sandow et al., 2004). Serial transverse sections (~100 nm thick) totaling approximately 5 µm of vessel length were cut, and MEGJs and their surrounding endothelial and smooth muscle cell regions were counted and imaged at 10,000 to 40,000× on a Phillips 7100 transmission electron microscope at 16-megapixel resolution (camera from Scientific Instruments and Applications, Inc., Duluth, MN). Quantitative wall properties were made from measurements of vessel cross-sections cut 90° perpendicular to the longitudinal vessel axis from ultrastructural montages taken at magnifications ranging from 1500 to 2500× at 16-megapixel resolution. CellR software (Olympus) was used for gross quantitative measurements. The number of smooth muscle cell layers was counted as the mean of smooth muscle cell profiles ≥5 µm in length, from four areas per vessel, 90° apart. Adventitial thickness was measured as the mean of four areas, 90° apart.

**Statistical Analysis.** Agonist concentrations causing half-maximal responses (pEC50 value) were calculated using nonlinear regression analysis (Prism; GraphPad Software, Inc., San Diego, CA) and expressed as the negative logarithm of the molar concentration (pEC50 values). Percentage relaxation evoked by agonist was taken to refer to the same extent after exposure to TRAM-34 alone or to vehicle-treated controls and the pEC50 of apamin (50 nM) and TRAM-34 (1 µM) respectively, compared with vehicle-treated controls (approximately 1.3-log unit rightward shift and approximately 30% reduction in Emax). (P < 0.05; Fig. 1A; Table 2). Vasodilation to BK was attenuated to the same extent after exposure to TRAM-34 alone (P < 0.05; Fig. 1A; Table 2), although apamin alone (50 and 100 nM) did not alter dilation compared with vehicle control (Fig. 1A, 50 nM shown; with 100 nM alone showing the same lack of effect; Table 2).

In preconstricted arteries, the IKCa activator 1-EBIO (10–300 µM) caused concentration-dependent dilation (18–86%.

**Table 2.** Human mesenteric artery bradykinin-induced vasodilation drug intervention characteristics

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<tr>
<th></th>
<th>pEC50</th>
<th>Emax</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>8.0 ± 0.1</td>
<td>92.9 ± 1.7</td>
<td>29</td>
</tr>
<tr>
<td>Indomethacin (10 µM)</td>
<td>8.0 ± 0.2</td>
<td>93.1 ± 3.3</td>
<td>4</td>
</tr>
<tr>
<td>Apamin + TRAM-34 (1 µM)</td>
<td>6.5 ± 0.2*</td>
<td>64.6 ± 13.2*</td>
<td>5</td>
</tr>
<tr>
<td>Apamin</td>
<td>7.4 ± 0.1*</td>
<td>66.8 ± 7.4*</td>
<td>5</td>
</tr>
<tr>
<td>Apamin (100 nM)</td>
<td>8.1 ± 0.1*</td>
<td>95.0 ± 2.4*</td>
<td>3</td>
</tr>
<tr>
<td>1-NNAME (100 µM) + ODQ (10 µM)</td>
<td>8.0 ± 0.2*</td>
<td>89.3 ± 4.6*</td>
<td>3</td>
</tr>
<tr>
<td>1-NNAME + ODQ + apamin + TRAM-34</td>
<td>6.7 ± 0.2*</td>
<td>77.8 ± 6.4*</td>
<td>4</td>
</tr>
<tr>
<td>1-NNAME + ODQ + TRAM-34 + indomethacin</td>
<td>5.1 ± 1.6*</td>
<td>7.2 ± 8.1*</td>
<td>4</td>
</tr>
<tr>
<td>1-NNAME + ODQ + apamin + indomethacin</td>
<td>6.9 ± 0.2**</td>
<td>57.6 ± 4.8**</td>
<td>3</td>
</tr>
<tr>
<td>Carbenoxolone (100 µM)</td>
<td>7.3 ± 0.3*</td>
<td>76.7 ± 6.1*</td>
<td>4</td>
</tr>
<tr>
<td>L-NNAME + ODQ + carbenoxolone</td>
<td>7.0 ± 0.4*</td>
<td>60.6 ± 9.0*</td>
<td>4</td>
</tr>
<tr>
<td>L-NNAME + ODQ + carbenoxolone + TRAM-34</td>
<td>18.7 ± 11.1*</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05, significant (from vehicle).
† P > 0.05, not different from vehicle or indomethacin alone.
†† P > 0.05, not different from 1-NNAME + ODQ alone, 50 nM apamin, unless otherwise stated.
Inhibition of NO synthase and guanylyl cyclase with L-NAME (100 μM) and ODQ (10 μM) respectively, caused a rightward shift in the BK concentration response curve (approximately 1.4-log unit shift and approximately 17% inhibition in E_max; P < 0.05; Fig. 2A). Moreover, BK-induced vasodilation was abolished by L-NAME and ODQ in addition to combined apamin (50 nM) and TRAM-34 (P < 0.05; Fig. 2A; Table 2).

Bradykinin-induced vasodilation in human mesenteric arteries was unaffected by indomethacin (10 μM; n = 4), with pEC_{50} and E_max being the same as vehicle control (Table 2; Fig. 2A). In addition, inclusion of indomethacin with L-NAME, ODQ and apamin showed no difference in the response compared with L-NAME and ODQ (Table 2; Fig. 2, compare B with A), whereas the addition of indomethacin with L-NAME, ODQ and TRAM-34 reduced the response in a manner similar to L-NAME and ODQ with apamin and TRAM-34 (Table 2; Fig. 2, compare B with A), although an approximate 20% maximal dilation persisted with 1 μM BK (Fig. 2B).

**Gap Junction-Mediated Endothelium-Dependent Vasodilation.** The putative gap junction uncoupler carbenoxolone (100 μM) caused a rightward shift in the BK concentration-response curve (0.8-log unit shift and an approximate 18% inhibition in E_max; P < 0.05; Fig. 2C; Table 2). Carbenoxolone plus L-NAME (100 μM) and ODQ (10 μM) further attenuated maximal (E_max) BK-induced vasodilation by approximately 17%, with the further addition of TRAM-34 (1 μM) blocking the response to BK (P < 0.05; Fig. 2C).

**Absence of Catalase Effect on Endothelium-Dependent Vasodilation.** Dilation to BK in arteries exposed to catalase (2000 units/ml: pEC_{50}, 8.1 ± 0.1; E_max, 95.6 ± 1.6; 6250 units/ml: pEC_{50}, 7.8 ± 0.1; E_max, 98.4 ± 0.9) for 40 min was no different compared with vehicle-treated arteries (pEC_{50}, 8.1 ± 0.1; E_max, 95.1 ± 1.1; Fig. 3).

**Myoendothelial Microdomain Anatomy.** Conventional confocal immunohistochemistry using characterized antibod-
Human mesenteric arteries (Fig. 4). Intense punctate expression of IKCa was identified at the IEL/EC-SMC interface (Fig. 4, A–C). Expression was localized near holes in the IEL, as sites for potential contact between endothelial and smooth muscle cells and typical of MEGJ projection sites (Fig. 4, A–C; Table 3). In a similar manner, confocal imaging demonstrated punctate myoendothelial expression of Cx37 aligned to holes in the IEL as potential MEGJ projection sites (Fig. 4, D–F; Table 3). It is noteworthy that expression of SK3, Cx40, and Cx43 was not detected using immunohistochemistry at the myoendothelial interface, although Cx40 and Cx43 were detected at nearby and adjacent endothelial cell associations (data not shown).

Serial section electron microscopy was used to examine MEGJ incidence and general vessel characteristics (Fig. 5, A–C; Table 3). Approximately two MEGJs were present in every 10³-μm² region. It is noteworthy that the origin of MEGJ projections in human mesenteric artery is approximately 60:40 on endothelial cell compared with smooth muscle-derived stalks, whereas in rat mesenteric arteries, all MEGJs are on endothelial derived “stalks” (Sandow et al., 2002). In addition, vessels had approximately seven layers of smooth muscle and an adventitial thickness of approximately 11 μm (Table 3). Preliminary studies attempted to measure smooth muscle cell membrane potential, as a primary indicator of EDH-type dilator activity. However, because sharp microelectrode penetration of the adventitia was very difficult, it is unfortunate that this was not possible. It is noteworthy that human mesenteric artery adventitia is >2.5-fold thicker (Table 3) than in similar order vessels of the adult male Sprague-Dawley rat where it is 4.0 ± 0.2 μm thick (n = 8; S. L. Sandow, R. E. Haddock, and P. S. Chadha, unpublished results).

Of interest, the IEL in human mesenteric artery has a highly variable morphology, in that it is often intermittent, “crispy,” and/or stringy in appearance with deposits of electron dense (possibly calcified) material. Of eight human mesenteric arteries examined as flat preparations with confocal microscopy, only one preparation had IEL holes typical of rodent arteries (Sandow et al., 2006), and it was from a 43-year-old patient.

**Discussion**

Myoendothelial microdomain signaling sites are present in human mesenteric artery. In this vessel, correlation of functional IKCa and Cx activity and IKCa and MEGJ Cx37 distribution is consistent with the suggestion that these two observations are related causally. With previous observations from mouse and rat mesenteric artery (Dora et al., 2003; Mather et al., 2005; Sandow et al., 2006, 2009), the present data suggest that such sites contribute to endothelium-dependent vasodilation via a mechanism whose essential elements are conserved between species in specific vascular beds.

In human mesenteric artery, the present data are consistent with endothelium-dependent vasodilation mediated by NO, IKCa, and gap junction Cx, with the latter non-NO mech-

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**Figure 3.** Catalase effect on endothelium-dependent vasodilation. Arteries were exposed to 2000 and 6250 units/ml catalase for 40 min before experimentation and remained present in the bath during BK exposure (n = 3–4).

**Figure 4.** IK1 and Cx37 endothelial immunohistochemical expression at the IEL-endothelial-smooth muscle interface in human mesenteric artery. Confocal images from the same vessel region (A–C and D–F, respectively), with IEL autofluorescence (A and D) demonstrating IEL holes (dark spots; examples shown with arrows in A and D) as potential sites of contact between endothelial and smooth muscle cells, typical of myoendothelial gap junction sites. Characterized IK1 antibody (Chen et al., 2004; Neylon et al., 2004), conjugated to AlexaFluor 633, representative of IKCa (A–C) and Cx37 (D–F) labeling, shows intense punctate localization at discrete points (examples are shown with arrows). Arrows (in A–C and D–F) correspond to the same region in each panel. Longitudinal vessel axis runs left to right. Controls show absence of IK label with peptide block, as did incubation in secondary only (data not shown). No labeling for SKCa and Cx40/43 was present at the IEL-endothelial-smooth muscle interface. Scale bar, 20 μm.

**Table 3.** Human mesenteric artery, MEGJ, punctate IKCa, and Cx37 characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Values</th>
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<tbody>
<tr>
<td>Number of SMC Layers</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>Adventitial Thickness</td>
<td>10.8 ± 1.1</td>
</tr>
<tr>
<td>MEGJs/10³ μm²</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>IK1 at IEL-EC/SMC Interface/10³ μm²</td>
<td>4.1 ± 0.6*</td>
</tr>
<tr>
<td>Cx37 at IEL-EC/SMC Interface/10³ μm²</td>
<td>2.2 ± 0.5</td>
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*P < 0.05, significant (from MEGJ density).
anism consistent with the pharmacological profile of EDH-mediated vasodilation in this vessel. Such a myoendothelial microdomain-dependent mechanism is well characterized in rat mesenteric artery. In the rat mesenteric artery, SKCa and IKCa activation and a role for MEGJs are key components of the EDH mechanism (Féle'tou, 2009; Sandow et al., 2009). Moreover, noting potential species differences, in the mouse mesenteric artery, localized myoendothelial IKCa is apparently associated with microdomain signaling sites and modulation of localized IP3R-mediated [Ca2+]i storage and release (Ledoux et al., 2008).

The present study suggests a key role for IKCa and MEGJs in human mesenteric artery endothelium-dependent vasodilation. Vasodilator responses to BK are sensitive to selective IKCa block with TRAM-34 and putative MEGJ uncoupling with carbenoxolone. Furthermore, IKCa-mediated vasodilation was induced using the concentration-selective IKCa activator 1-EBIO at concentrations ≤100 μM. It is noteworthy that in human mesenteric artery, 1-EBIO shows apparent nonspecificity at >100 μM (approximately 28% dilation remaining after addition of TRAM-34, post-1-EBIO application at this concentration; Fig. 1B).

The lack effect of the SKCa agonist CyPPA in eliciting vasodilation or of the selective SKCa blocker apamin on BK-induced vasodilation, as well as the absence of myoendothelial SKCa (SK3) protein expression, suggests an absence of SKCa in vasodilation in the human mesenteric artery. However, the small residual TRAM-34-insensitive vasodilatory component (Fig. 2B) suggests the potential for a minimal contribution of SKCa in endothelium-dependent vasodilation of human mesenteric artery. Thus, perhaps SKCa is expressed at a low level, albeit below the detection limit of conventional confocal immunohistochemistry.

Expression of endothelial IKCa and Cx37 near holes in the IEL is consistent with their role in microdomain-mediated signaling events, SKCa, Cx40, and Cx43 being absent at such sites. Furthermore, MEGJ incidence in these arteries suggests potential for significant gap junction-dependent electrical coupling between the endothelium and smooth muscle. It is noteworthy that myoendothelial IKCa density is twice that of MEGJ and Cx37-microdomain incidence at IEL holes sites (Table 3). This suggests that either there are more IKCa-only associated myoendothelial microdomain sites than MEGJ-related ones or that IKCa is recruited to IEL hole sites before the formation of gap junctions at such sites.

Data in the present study support a role for Cx37 in myoendothelial signaling in human mesenteric artery, which is in contrast to observations in human subcutaneous vessels where Cx43 is implicated in such activity (Lang et al., 2007). Indeed, in rat mesenteric artery, Cx37 and Cx40 underlie MEGJ-mediated coupling (Mather et al., 2005; Sandow et al., 2006), whereas in human mesenteric artery, Cx37 is likely to be associated with this response. Because different Cx exhibit distinct biophysical properties, such as conductance and permeability (Söhl and Willecke, 2004), these data provide further support for the presence of significant heterogeneity in myoendothelial microdomain signaling characteristics in different vascular beds of the same species, as well as in the same vascular beds of different species (Sandow, 2004; de Wit et al., 2008; Johnstone et al., 2009).

In a manner similar to Cx-mediated vascular signaling mechanisms, the present data also support a differential contribution of SKCa and IKCa between species, as well as in different vascular beds of the same species. In rat and mouse mesenteric artery, both SKCa and IKCa underlie significant components of EDH-type relaxation (Hill et al., 2000; Sandow et al., 2002; Dora et al., 2003), whereas in human mesenteric artery, an SKCa component is minimal. Indeed, similar to the rat mesenteric artery (Shimokawa et al., 1996; Hill et al., 2000; reviewed in Sandow, 2004), it is possible that the specific contribution of SKCa, IKCa, and Cx-mediated signaling to EDH differs in different segments of the human mesenteric vascular bed, further studies on different segments of the human bed being required to clarify this issue. Furthermore, in contrast to the primary IKCa dependence of EDH-type activity in human mesenteric artery, both SKCa and IKCa underlie significant components of such activity in human omental and myometrial arteries (Gillham et al., 2007). These data further collectively support the presence of significant heterogeneity in endothelial vascular signaling.
mechanisms between species and within and between vascular beds (Hill et al., 2001; Aird, 2007).

Of interest, previous studies have suggested a role for gap junctional coupling and endothelial-derived H$_2$O$_2$ in EDH-mediated vasodilation of human mesenteric artery (Matoba et al., 2002). Although the present data support a role for gap junctions in EDH-mediated vasodilation in this vessel, the present data are not consistent with a role for H$_2$O$_2$ in such activity. In contrast to Matoba et al. (2002), the present study found that exposure of human mesenteric arteries to catalase, as a defining feature of the putative role of H$_2$O$_2$ (Matoba et al., 2002), had no effect on endothelium-dependent vasodilation. The discrepancy between the present results and those of Matoba et al. (2002) is unknown. However, a lack of apparent catalase/H$_2$O$_2$ discrepancy between the present results and those of Matoba et al. (2002) had no effect on endothelium-dependent vasodilation. The disconnection-dependent vasodilator signaling mechanism may be due in part to the combination of blockers apparently required to be present with catalase before seeing an effect, as suggested by Matoba and Shimokawa (2003) in mouse mesenteric artery. In addition, the type of preconstrictor agonist may influence the apparent relative involvement of the different facets of endothelium-dependent vasodilator activity, as found in human myometrial and omental arteries (Gillham et al., 2007).

Nevertheless, in several other vascular beds, H$_2$O$_2$ does not seem to contribute to EDH-mediated activity (Ellis et al., 2003; Gluais et al., 2005). Furthermore, it is possible that the potential effects of catalase on EDH-type activity may be related to altered gap junction coupling (Chaytor et al., 2003) or, at a minimum, an interaction with such coupling. Indeed, among other effects, H$_2$O$_2$ has been shown to increase and decrease gap junction coupling, cause vasoconstriction, modulate the sensitivity of the contractile apparatus to calcium, and activate smooth muscle Na$^+$/K$^+$-ATPase, large KCa, and ATP-sensitive potassium channels (reviewed in Ellis and Triggle, 2003; Sandow, 2004), observations that are not directly consistent with its proposed role in EDH activity.

In conclusion, the present study provides evidence for microdomain-dependent signaling in EDH-type vasodilation in human mesenteric artery, IK$_{Ca}$ and MEGJ Cx expression and activity being critical for this phenomenon. In combination with previous mouse and rat mesenteric artery data, the present data support the proposal that myoendothelial microdomain-dependent signaling and associated EDH-type activity is conserved across species. Differences in specific aspects of the microdomain-dependent vasodilator signaling mechanism may represent vascular bed-specific targets for therapeutic intervention.

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Authorship Contributions
Participated in research design: Chadha, Murphy, and Sandow. Conducted experiments: Chadha, Senadheera, Murphuy, and Sandow. Contributed new reagents or analytic tools: Chadha, Liu, Rikard-Bell, Senadheera, Howitt, Bertrand, Grayson, Murphy, and Sandow. Performed data analysis: Chadha, Senadheera, Murphuy, and Sandow.

Wrote or contributed to the writing of the manuscript: Chadha, Liu, Rikard-Bell, Senadheera, Howitt, Bertrand, Grayson, Murphy, and Sandow.


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