Disruption of smooth muscle gap junctions attenuates myogenic vasoconstriction of mesenteric resistance arteries

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Earley, Scott, Thomas C. Resta, and Benjimen R. Walker. Disruption of smooth muscle gap junctions attenuates myogenic vasoconstriction of mesenteric resistance arteries. Am J Physiol Heart Circ Physiol 287: H2677–H2686, 2004. First published August 19, 2004; doi:10.1152/ajpheart.00016.2004—Communication between vascular smooth muscle (VSM) cells via low-resistance gap junctions may facilitate vascular function by synchronizing the contractile state of individual cells within the vessel wall. We hypothesized that inhibition of gap junctional communication would impair constrictor responses of mesenteric resistance arteries. Immunohistochemical experiments revealed positive staining for connexin 37 (Cx37) in both endothelium and smooth muscle of rat mesenteric arterioles, whereas connexin 43 (Cx43) immunoreactivity was not detected in the mesenteric vasculature. Administration of the gap junction inhibitory peptide Gap27, which targets Cx37 and Cx43, significantly diminished myogenic vasoconstriction (8.6 ± 3.8% of passive diameter at 100 Torr) and changes in vessel wall intracellular \([Ca^{2+}]\) of mesenteric resistance arteries compared with vessels treated with either vehicle (physiological saline solution) (33.5 ± 6.1%) or a control peptide (32.1 ± 6.5%). Administration of 18α-glycyrrhetinic acid, structurally distinct from Gap27, also significantly attenuated myogenic constriction compared with its vehicle control (DMSO) (9.6 ± 3.2% vs. 23.8 ± 4.6%). In contrast, phenylephrine-induced vasoconstriction was not altered by gap junction blockers. Attenuated myogenic vasoconstriction resulting from inhibition of gap junctions persisted after disruption of the endothelium. In additional experiments, VSM cell membrane potential was recorded in mesenteric resistance arteries pressurized to 20 or 100 Torr. VSM membrane potential was depolarized at 100 Torr compared with 20 Torr. However, VSM cells in arteries treated with Gap27 were significantly hyperpolarized (−48.6 ± 1.4 mV) at the higher pressure compared with vehicle (−41.4 ± 1.5 mV) and Gap20-treated (−38.4 ± 0.7 mV) vessels. Our findings suggest that inhibition of smooth muscle gap junctions attenuates pressure-induced VSM cell depolarization and myogenic vasoconstriction.

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confirmatory experiments. We found that pressure but not agonist-induced, vasoconstriction was attenuated by gap junction inhibitors and that this effect was independent of the endothelium.

METHODS

Animals

A total of 56 male Sprague-Dawley rats (250–350 g body wt; Harlan Industries) were used for these studies. Animals were provided with fresh bedding, rat chow, and drinking water and maintained under a 12:12-h light-dark cycle. Before experimentation, animals were deeply anesthetized with pentobarbital sodium (50 mg ip) and euthanized by exsanguination after the vessels were harvested according to a protocol approved by the Institutional Animal Care and Use Committee of the University of New Mexico Health Sciences Center.

Immunohistochemistry for Cx Proteins

Tissue preparation. The mesenteric arcade (second- to fifth-order vessels) and segments of the small intestine (~1 cm in length) were isolated from anesthetized rats, rinsed in ice-cold physiological saline solution (PSS; 129.8 mM NaCl, 5.4 mM KCl, 0.83 mM MgSO4, 19 mM NaHCO3, 1.8 mM CaCl2 and 5.5 mM glucose and 10−4 M papaverine), and cryopreserved in OCT compound (Tissue-Tek) cooled by liquid nitrogen. Longitudinal cryosections (10 μm thick, −24°C) were thaw mounted onto Superfrost Plus slides (Fisher Scientific), air dried, and stored at −20°C. Before being immunolabeled, the sections were permeabilized in PBS (0.05 M Na2HPO4, 0.14 M NaCl, pH 7.40) containing 0.1% Triton X-100 (TX). Sections designated for Cx37 and Cx43 immunostaining were then blocked with PBS-TX containing 0.5% bovine serum albumin for 30 min at room temperature. The sections were then incubated for 2 h at 37°C with the following Cx primary antibodies: rabbit anti-mouse Cx 37 polyclonal (Alpha Diagnostic Cx37A11-A; 1:100), mouse monoclonal antibody generated against rat Cx 43 (Chemicon Int. MAB3068; 1:250), and mouse anti-rat Cx 43 monoclonal (BD Transduction Laboratories 610062; 1:100). Some sections were additionally labeled with mouse anti-smooth muscle α-actin monoclonal (Sigma A2547; 1:200) and mouse anti-human von Willebrand factor (vWF) monoclonal antibodies (Serotec MCA1277; 1:200) to selectively label the vascular smooth muscle and endothelium, respectively. The immunogenic peptide sequence for the Cx37 antibody shows no significant homology with other Cx proteins according to the manufacturer (Alpha Diagnostic). For double labeling of Cx37 with homology with other Cx proteins according to the manufacturer genic peptide sequence for the Cx37 antibody shows no significant

characterization of Cx37 Antibody by Western Blot Analysis

Because preliminary experiments revealed immunostaining for Cx37 but not Cx43 within the mesenteric arterial wall, Western blot analyses were performed using a Cx37-transfected rat insulinoma (Rin-mCx37) cell line in which Cx37 expression is inducible by doxycycline (gift from Dr. Janis M. Burt, University of Arizona) to confirm the specificity of the Cx37 antibody used for immunohistochemistry protocols. Rin-mCx37 cells were generated from a Rin-104638 cell line that was stably transfected with a Tet-on vector (Clontech) and with pTre2 containing the coding sequence for mouse Cx37. Cells were grown to ~70% confluency in RPMI-1640 medium supplemented with 10% fetal bovine serum (Hyclone), genetecin (300 μg/ml), and hygromycin (100 μg/ml) in a humidified incubator (6% CO2, balance air) at 37°C. Cx37 expression was induced by treatment with doxycycline (4 μg/ml; 48 h), whereas vehicle-treated cells served as a negative control. Cell lysates were prepared in Laemml buffer (Bio-Rad) and sample protein concentrations determined by the Bradford method (Bio-Rad Protein Assay). A molecular weight standard (Bio-Rad Precision Plus) was added to each gel, and sample proteins (30 μg/lane) were separated by SDS-PAGE (12% Tris-HCl gels, Bio-Rad) and transferred to polyvinylidene difluoride membranes (Bio-Rad). Blots were blocked overnight at 4°C with 5% nonfat milk, 3% bovine serum albumin, and 1% Tween 20 (Bio-Rad) in TBS containing 10 mM Tris-HCL and 50 mM NaCl (pH 7.5). Blots were then incubated for 2 h at room temperature with a rabbit anti-mouse Cx 37 affinity-purified polyclonal antibody (Cx37A11-A; 1:100, Alph Diagnostics). This antibody does not exhibit cross-reactivity with endothelial Cx40 or Cx43 (38, 40). For immunochemical labeling, blots were incubated for 2 h at room temperature with goat anti-rabbit IgG-horseradish peroxidase (1:7,500) (Stressgen). After chemiluminescence labeling (ECL, Amersham), Cx37 bands were detected by exposing the blots to chemiluminescence-sensitive film (Kodak). All reagents were purchased from Sigma unless otherwise noted.

Gap Junction Inhibitors

Two structurally distinct agents were used to disrupt gap junctions. Our initial experiments employed a peptide, Gap27 (amino acid sequence SRPTEKTIFII) (3) that is homologous to an extracellular loop of Cx37 and Cx43. A BLAST search of the GenBank protein sequence database did not detect homology between Gap27 and Cx40 or Cx45. This peptide was chosen as a putative inhibitor of VSM gap junctions based on previous reports (26, 48) showing expression of Cx37 and/or 43 within these cells in mesenteric resistance arteries. Controls for these experiments consisted of the biologically inactive peptide Gap20 (amino acid sequence EIKKKFYGC), homologous to an intercellular loop of Cx43 (4), and the vehicle for both peptides, PSS. Gap peptides were synthesized by the Tufts Medical School Protein Chemistry Facility (Boston, MA) and were dissolved in PSS at a final concentration of 300 μM. 18α-GA was used to block gap junctions in separate experiments. 18α-GA was prepared daily at a concentration of 10 mM in anhydrous DMSO and was diluted with PSS to 20 μM before administration. DMSO (diluted 1/500 in PSS) was used as the solvent control for 18α-GA experiments.

Isolated Vessel Preparation

Pressurized mesenteric resistance arteries were studied in isolation. The mesenteric arcade was excised from anesthetized animals and transferred to ice-cold dissecting solution [3 mM MOPS (pH 7.4), 145 mM NaCl, 5 mM KCl, 1 mM MgSO4, 2.5 mM CaCl2, 1 mM KH2PO4, 0.02 mM EDTA, 2 mM pyruvate, 5 mM glucose, and 1% bovine serum albumin]. Fifth-order vessel segments [passive inner diameter (ID) at an intraluminal pressure of 60 Torr = 175.1 ± 4.0 μm, n =...
GAP JUNCTION BLOCKADE ATTENUATES VASOCONSTRICTION

36) were cleaned of adipose tissue, isolated, and transferred to a vessel chamber (Living Systems). The proximal end of the vessel was cannulated with a glass micropipette and secured, blood was gently rinsed from the lumen, and the distal end of the vessel cannulated and secured. Vessels were slowly pressurized to 60 Torr with PSS using a servo-controlled peristaltic pump (Living Systems) and superfused (5 ml/min) with warm (37°C) PSS aerated with a normoxic gas mixture (21% O2-6% CO2-balance N2). After a 30-min equilibration period, intraluminal pressure was slowly increased to 120 Torr, vessels were stretched to remove bends, and pressure was reduced to 60 Torr for an additional 30-min equilibration period. Pressurized resistance arteries were loaded with the cell-permeant ratiometric Ca2+-sensitive fluorescent dye fura-2 AM (Molecular Probes). Immediately before being loaded, fura-2 AM (1 mM in anhydrous DMSO) was mixed with 0.5 volumes of a 20% solution of pluronic acid in DMSO, and this mixture was diluted with dissecting solution to yield a final concentration of 2 μM fura-2 AM and 0.05% pluronic acid. Vessels were incubated in this solution for 45 min at room temperature in the dark. Administration of fura-2 to the abluminal surfaces of pressurized small arteries has been shown to preferentially load VSM cells (30). Under the conditions used for the current study, changes in the fura signal are representative of, but not necessarily identical to, changes in VSM intracellular [Ca2+]. However, ACh mediates vaso dilation and decreases in vessel wall [Ca2+]i, indicative of preferential VSM loading. Vessels were equilibrated for 20 min with warmed, aerated PSS after the loading period to wash out excess dye and to allow for hydrolysis of AM groups by intracellular esterases. Fura-loaded arteries were incubated in the presence of Gap peptides (300 μM), 18α-GA, or appropriate vehicles for 1 h before experimentation. The incubation time and peptide concentration employed for the inhibitory peptide studies were selected based on a prior report (3). A single artery was studied from each rat; thus values of n refer to the number of animals used for a particular experimental group.

Myogenic and Agonist-induced Vasoconstrictor and Ca2+ Responsiveness

The effects of gap junction inhibitors on myogenic and agonist-induced vasoconstrictor and Ca2+ responses were examined. Fura-loaded vessels were subjected to a series of pressure steps between 20 and 120 Torr, and spontaneous myogenic tone was allowed to develop at each step for 3 min. After completion of pressure-response curves, vessels were reequilibrated at 60 Torr for 10 min and then superfused with PSS containing increasing concentrations of the α1-adrenergic agonist phenylephrine (PE) (0.01–100 μM). Completion of myogenic and PE curves required ~45 min. ID was continuously monitored using video microscopy (total magnification ×200, numerical aperture 0.75) and edge-detection software (Ionoptix). In addition, fura-loaded vessels were alternatively excited at 340 and 380 nm at a frequency of 10 Hz and the respective 510 nm emissions were quantified using a photomultiplier tube and recorded with the use of Ionowizard software (Ionoptix, version 4.4). Photometric data were collected from the entire arterial segment under study. Vessel wall [Ca2+]i, representative of VSM cell [Ca2+]i, was expressed as the mean F540/F505 ratio from the background-subtracted 510 nm signal collected over ~3 min. After completion of the pressure-response and PE curves, intraluminal pressure was maintained at 60 Torr and vessels were superfused with Ca2+-free PSS (129.8 mM NaCl, 5.4 mM KCl, 0.83 mM MgSO4, 19 mM NaHCO3, 5.5 mM glucose, and 3 mM EGTA) for 1 h. The pressure-response curve was then repeated under Ca2+-free conditions to obtain passive responses. Myogenic tone was calculated as the percent difference in ID observed for Ca2+-containing versus Ca2+-free PSS at each pressure. PE-induced vasoconstriction was calculated as the percent change in ID relative to baseline. Change in vessel wall [Ca2+]i was calculated as the difference in F540/F380 and was compared with that of vessels pressurized at 20 Torr for myogenic curves or baseline F540/F380 of vessels pressurized at 60 Torr for PE curves. Myogenic tone, PE-induced vasoconstriction, and change in vessel wall [Ca2+]i were determined in the presence of vehicle (PSS) (n = 5), Gap27 (n = 5), or Gap20 (n = 6). In separate experiments, myogenic tone and PE-induced vasoconstriction were recorded in the presence of 18α-GA or its solvent, DMSO (n = 5 for both groups).

Disruption of Endothelium

Experiments were performed to determine whether endothelial cell function contributes to attenuated myogenic vasoconstriction associated with gap junction inhibition. The endothelium of small mesenteric arteries was disrupted by perfusing the lumen with ~2 ml of air at an intraluminal pressure of 60 Torr. To demonstrate effective disruption of the endothelium, arteries were preconstricted with PSS (10 μM) and administered the endothelium-dependent vasodilator Ach (1 μM).

VSM Cell Resting Membrane Potential

The effect of gap junction inhibitors on pressure-induced VSM cell depolarization was investigated. Fifth-order mesenteric resistance arteries were isolated and pressurized to 20 or 100 Torr, and VSM cells were impaled through the adventitia with glass intracellular microelectrodes (tip resistance 100–200 MΩ). To allow visual identification of the cell type from which recordings were obtained, the tip of the electrode was filled with a 2% solution of Lucifer Yellow (Sigma) dissolved in 1% LiCl and backfilled with 1 M KCl (10). A Neuroprobe Model 1600 amplifier (A-M Systems) was used for recording membrane potential (Em). Analog output from the amplifier was low-pass filtered at 1 kHz and routed to a Tektronix RM502A oscilloscope and a Gould chart recorder. Criteria for acceptance of Em recordings were the following: 1) an abrupt negative deflection of potential as the microelectrode was advanced into a cell; 2) stable membrane potential for at least 1 min; and 3) an abrupt change in potential to ~0 mV after the electrode was retracted from the cell. After completion of Em recordings, vessels were examined under epifluorescent illumination to visualize dye-loaded cells. For some experiments, recordings from several VSM cells were made for each animal. The mean potential of all VSM cells recorded for an individual rat was considered as a single replicate for statistical purposes. VSM cell Em was recorded in the presence of vehicle, Gap27 or Gap20 at intraluminal pressures of 20 and 100 Torr (n = 5 animals for vehicle and Gap20, n = 7 for Gap27).

Calculations and Statistics

All data are means ± SE. Values of n refer to number of animals employed for each group. Comparisons of vasoconstrictor and vessel wall [Ca2+]i responses between vehicle-, Gap20-, and Gap27-treated vessels were made by one-way ANOVA. Comparisons of VSM cell Em for arteries at different intraluminal pressure were made by two-way ANOVA. If differences were detected by ANOVA, individual groups were compared with the use of the Student-Newman-Keuls post hoc test for all pairwise comparisons. Differences between 18α-GA and vehicle-treated arteries and Gap20 and Gap27 endothelium-disrupted vessels were evaluated using Student’s unpaired t-tests. A level of P ≤ 0.05 was accepted as statistically significant for all comparisons.

RESULTS

Cx Proteins Expressed by Small Mesenteric Arteries

Immunohistochemistry for Cx37 in second- through fifth-order mesenteric arteries outside the gut wall revealed fine punctate staining in the medial layer (Fig. 1, A and B), which
exhibited frequent colocalization with immunoreactive smooth muscle α-actin (Fig. 1A). Larger Cx37-immunoreactive plaques were identified in the endothelial layer (Fig. 1, A and B) as determined by costaining with vWF (Fig. 1B). Similar patterns of Cx37 staining were observed in mesenteric arteries from two animals. Cx43 was present only in the inner circular layer of gastrointestinal (GI) smooth muscle and was not detected in the outer longitudinal GI smooth muscle layer or vascular tissue (Fig. 1C). An identical pattern of staining for Cx43 was observed for both Cx43 monoclonal antibodies. A similar lack of Cx43 staining was observed in mesenteric arteries outside the gut wall in two animals and in submucosal arteries of the gut wall in four rats. Staining for all three Cx antibodies was eliminated when primary antibodies were preadsorbed with appropriate blocking peptides. Furthermore, no specific staining was observed in sections treated only with secondary antibodies or in samples treated with mouse IgG or rabbit serum instead of primary antibodies. These results validated the use of Gap27 as an inhibitor in subsequent functional studies, as this peptide is homologous to extracellular loops of Cx37 and Cx43.

**Characterization of Cx37 antibody.** The Cx37 antibody detected a single band at 37 kDa in doxycycline-induced

![Fig. 1. A: dual labeling for connexin 37 (Cx37; red) and smooth muscle α-actin (green) in a transverse section of a mesenteric arteriole (a). Fine punctate staining for Cx37 (arrows) was observed in vascular smooth muscle (vsm) cells, with larger Cx37-immunoreactive plaques present in the endothelial (e) layer. B: dual labeling for Cx37 (red) and endothelial von Willebrand factor (vWF; green) in a transverse section of a mesenteric arteriole. Cx37 staining (arrows) exhibited colocalization with vWF. C: Cx43 staining in a longitudinal section of small intestine. Cx43 immunoreactivity was detected in the inner circular layer of gastrointestinal (GI) smooth muscle (arrow) but not in mesenteric arterioles. Scale bars = 20 μm.](http://ajpheart.physiology.org/)

![Fig. 2. Representative Western blot for Cx37 in doxycycline-induced rat insulinoma (Rin)-mCx37 cells (Cx37+) and noninduced cells (Cx37−). The positions of molecular mass standards are indicated at the left side of the blot. Cx37 was identified as a single band at 37 kDa in induced cells.](http://ajpheart.physiology.org/)
Rin-mCx37 cell lysates but not in lysates from noninduced cells (Fig. 2).

**Effects of Gap Junction Inhibitors on Myogenic Vasoconstrictor and Ca2+ Responses**

Myogenic vasoconstriction of arteries treated with the inhibitory peptide Gap27 was greatly attenuated compared with both vehicle and Gap20-treated vessels (Fig. 3, A–C). Consistent with this finding, increases in vessel wall [Ca2+]i associated with elevation of intraluminal pressure were diminished for small mesenteric arteries treated with Gap27 compared with Gap20- and vehicle-treated vessels (Fig. 3, A, B, and D). Pressure-induced constrictor and vessel wall [Ca2+]i responses did not differ between vessels treated with Gap20 or vehicle (Fig. 3, C and D). Administration of 18α-GA also decreased myogenic vasoconstriction of mesenteric resistance arteries compared with vehicle (Fig. 4).

Effects of Gap Junction Inhibitors on PE-Induced Vasoconstrictor and Ca2+ Responses

In contrast to the effects gap junction inhibitors on myogenic constriction, mean PE-induced vasoconstriction (Fig. 5C) and increases in vessel wall [Ca2+]i (Fig. 5D) did not differ between arteries treated with Gap27, Gap20, or vehicle. Interestingly, we found that oscillations in ID and vessel wall [Ca2+]i observed for control (Gap20 treated) vessel after administration of high concentrations of PE (Fig. 5A) were attenuated for vessels treated with Gap27 (Fig. 5B). We also found that PE-induced vasoconstriction did not differ between 18α-GA and vehicle-treated vessels (Fig. 6).

**Effects of Disruption of the Endothelium**

Before disruption of the endothelium, ACh (1 μM) administration reversed PE-induced tone by 96.7 ± 0.7% for Gap20-treated vessels and 94.7 ± 2.6% for Gap27-treated vessels. ACh-induced vasodilation was nearly abolished (1.48 ± 0.5% for Gap20, 4.8 ± 1.6% for Gap27) after the lumen had been perfused with air, demonstrating that this procedure effectively disrupts endothelial cell function (Table 1). Gap junction inhibitors had similar effects on vasoconstrictor and Ca2+ responses of endothelium-disrupted vessels compared with endothelium-intact vessels. Pressure-induced constriction and increases in vessel wall [Ca2+]i were diminished for arteries treated with Gap27 compared with Gap20-treated vessels (Fig. 7, A and B), whereas PE-induced responses did not differ between these groups (Fig. 7, C and D).

Comparisons of myogenic and PE-induced vasoconstriction of endothelium-intact and endothelium-disrupted arteries are shown in Table 2. Myogenic vasoconstriction was not different between endothelium-intact or endothelium-disrupted vessels treated with Gap20 or Gap27. In contrast, agonist-induced vasoconstriction of endothelium-disrupted arteries was greater
than that of endothelium-intact vessels at [PE] of 1, 10, and 100 μM for both Gap20- and Gap27-treated arteries. In addition, oscillations in ID and vessel wall [Ca^{2+}]_i after administration high concentrations of PE in endothelium-intact control (Gap20 and vehicle treated) vessels (Fig. 5A) were not present for endothelium-disrupted arteries.

Effects of Gap Junction Inhibitors on VSM Cell E\textsubscript{m}

Lucifer yellow loading allowed visual identification of the cell type within the vascular wall from which E\textsubscript{m} recordings were obtained. Dye-labeled VSM cells were easily identified by their characteristic spindle-shaped morphology and perpendicular orientation relative to the long axis of the artery (Fig. 8A). In agreement with an earlier report (16), lucifer yellow loading was confined to a single VSM cell, suggesting that gap junctions formed by these cells are unable to transport the dye. VSM cell E\textsubscript{m} was significantly (P < 0.05) depolarized when vessels were pressurized at 100 Torr compared with 20 Torr within all treatment groups (Fig. 8E). However, VSM cells in vessels treated with Gap27 and pressurized to 100 Torr were hyperpolarized compared with arteries treated with Gap20 or vehicle at this pressure (Fig. 8, B–E). Interestingly, some (2 of 7) VSM cells in mesenteric resistance arteries treated with Gap27 and pressurized to 100 Torr exhibited an unusual E\textsubscript{m} with steady oscillation between -34 and -43 mV at a frequency of ~0.5 Hz (Fig. 8F). Because these recordings apparently constituted a second population of cells, they were excluded from the mean data shown in Fig. 8E. In addition, vessels treated with Gap20 were slightly depolarized compared with Gap27- and vehicle-treated arteries at intraluminal pressures of 20 Torr (Fig. 8E). This may be a nonspecific effect related to the relatively high (300 μM) peptide concentration employed for these studies.

Table 1. Effects of endothelial disruption on agonist-induced vasoconstriction and vasodilation of mesenteric arteries

<table>
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<tr>
<th></th>
<th>Endothelium-Intact Diameter</th>
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<td>27.4 ± 1.2*</td>
<td>32.1 ± 2.4†</td>
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Values are means ± SE; n = 10 for all groups. PE, phenylephrine. *Different from endothelium-intact vessels administered PE; †different from endothelium-intact vessels administered PE and ACh.
Effects of endothelial disruption on myogenic and PE-induced vasoconstriction of Gap20- and Gap27-treated arteries

Table 2.

<table>
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<tr>
<th>Intraluminal pressure, Torr</th>
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<th>Gap20 Denuded</th>
<th>Gap27 Intact</th>
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Values are means ± SE; n = 5 for all groups. [PE], PE concentration. Data are from Figs. 3, 5, and 7 and are shown to facilitate comparisons between endothelium-intact and endothelium-denuded vessels. *Diffs from Gap20-treated arteries with intact endothelium; †differences from Gap27-treated arteries with intact endothelium.

DISCUSSION

The primary goal of this study was to examine the contribution of intercellular communication to myogenic and agonist-induced vasoconstriction of small arteries. Our major findings include the following: 1) both VSM and endothelial cells of small mesenteric arteries appear to express Cx37, although Cx43 immunoreactivity was not detected in these arteries; 2) gap junction inhibitors greatly attenuate myogenic vasoconstriction and pressure-induced increases in vessel wall [Ca2+]i; 3) mean PE-induced constrictor and Ca2+ responses are not altered by gap junction inhibitors; 4) blunted myogenic vasoconstriction associated with gap junction inhibitory peptides persists after disruption of the endothelium; and 5) depolarization of VSM cells associated with increases in intraluminal pressure is attenuated for arteries treated with a gap junction inhibitory peptide. These findings suggest that VSM intercellular communication via Cx37-dependent gap junctions contributes to myogenic, but not agonist-induced, vasoconstriction of small mesenteric arteries. Furthermore, attenuated myogenic vasoconstriction associated with gap junction inhibition is independent of endothelial cell function.

Studies of Cx37 and Cx43 expression within the rat peripheral circulation have revealed substantial heterogeneity in localization depending on the vascular bed and vessel size examined. Consistent with our present findings, Hill and colleagues (24, 34) identified strong expression of Cx37 in the endothelium of rat caudal and basilar arteries but only fine punctate Cx37 immunoreactivity in the medial layer of these vessels using the same Cx37 antibody employed in the current study. However, these investigators detected Cx43 within the smooth muscle layer of hepatic arteries. Discrepancies have also been revealed with respect to Cx37 and Cx43 localization in rat mesenteric arteries. For example, Gustafsson et al. (22) detected both Cx37 and Cx43 plaques in the endothelium of mesenteric resistance arteries but no Cx staining in the media. In contrast, a separate study found relatively low levels of Cx37 and Cx43 in the medial layer of
elastic but not muscular rat mesenteric arteries, with strong expression of Cx37 in the endothelium (29). The reason for these apparent discrepancies in the literature is not clear but may reflect differences in methodology and heterogeneity in Cx expression between vessel types and sizes.

Our finding that administration of either gap junction inhibitory peptides or pharmacological agents that block intercellular communication greatly attenuates pressure-induced vasoconstriction of mesenteric resistance arteries is in agreement with those of Lagaud et al. (32), showing loss of myogenic tone after administration of heptanol or 18α-GA to pressurized cerebral arteries. Myogenic behavior results primarily from stretch-induced VSM cell depolarization associated with elevations in intraluminal pressure (23). In addition, Ca\(^{2+}\) sensitization also contributes to this response in cerebral (31) and mesenteric (42) arteries. Smooth muscle depolarization elicits activation of voltage-dependent Ca\(^{2+}\) channels, Ca\(^{2+}\) influx, and vasoconstriction (30). Our findings clearly demonstrate that administration of either Gap27 or 18α-GA nearly abolishes myogenic vasoconstriction (Fig. 3C) and that this response is associated with blunted VSM cell depolarization (Fig. 8E) and diminished increases in pressure-induced vessel wall [Ca\(^{2+}\)] (Fig. 3D). Given that earlier studies have shown that pressure-induced vasoconstriction is blocked by inhibitors of voltage-dependent Ca\(^{2+}\) channels (30), these findings suggest that attenuated myogenic vasoconstriction after gap junction inhibition may result from diminished voltage-dependent Ca\(^{2+}\) influx. To examine the possibility that blunted myogenic vasoconstriction during blockade of intercellular gap junctional communication was due to increased production endothelial cell-derived hyperpolarizing or vasodilatory factors, additional experiments were performed using vessels with dysfunctional endothelium. However, attenuated myogenic constriction associated with gap junction inhibitors persisted after disruption of the endothelium (Fig. 7, A and B), demonstrating that interrupted smooth muscle communication is central to this response. Furthermore, because the Gap27 inhibitory peptide is selective for Cxs37 and 43, and that Cx37 but not 43 was detected by immunostaining in VSM cells of mesenteric arteries, we conclude that smooth muscle gap junctions containing Cx37 appear to be required for myogenic vasoconstriction of mesenteric resistance arteries.

In contrast to the dramatic effects of gap junction inhibitors on myogenic responsiveness, mean PE-induced vasoconstrictor and vessel wall [Ca\(^{2+}\)] responses were not altered by inhibitors of intercellular communication (Fig. 5). This finding is in disagreement with earlier reports suggesting that the agonist-induced vasoconstriction of aortic rings is attenuated by inhibition of gap junctions. For example, Christ et al. (8) demonstrated that administration of heptanol relaxed aortic rings that had been precontracted with PE, whereas rings constricted with 60 mM extracellular KCl were not affected by heptanol. A similar study by this group also reported heptanol-induced relaxation of aortic ring precontracted with endothelin-1,5-hydroxytryptamine and prostaglandin F\(_{2\alpha}\) (9). The discrepancy between the current findings and prior reports may be due to heterogeneity between conduit and resistance arteries in the relative significance of gap junctions in these responses. In addition, diffusional distances and barriers are much greater in large arteries containing more concentric layers of VSM compared with resistance vessels. Therefore, a greater proportion of VSM cells in large arteries may not be directly activated by vasoconstrictor agents and therefore must be stimulated indirectly via gap junctions. In contrast, in smaller vessels, it appears that most cells are directly influenced by vasoconstric-

![Figure 8](http://ajpheart.physiology.org/)
tion of mesenteric resistance arteries, suggesting that these signaling events are not influenced by interrupted intercellular communication. Furthermore, these findings imply that most if not all VSM cells with the vessel wall are equally sensitive to stimulation by this agonist.

Interestingly, we found that although administration of gap junction blockers did not alter mean PE-induced vasoconstriction, administration of an inhibitory peptide reduced oscillations in artery diameter and vessel wall [Ca2+]i, associated with high concentrations of PE (Fig. 5, A and B). These findings are consistent with a report by Chaytor and co-workers (3) demonstrating a similar loss of PE-induced oscillations of mesenteric artery rings treated with the Gap27 peptide. In addition, similar to prior studies (14, 49) we found that disruption of the endothelium increased PE-induced vasoconstriction of mesenteric arteries. Furthermore, in agreement with an earlier report (28), we found that disruption of the endothelium also decreased diameter and Ca2+ oscillations associated with PE administration (not shown). These findings are consistent with the hypothesis that in mesenteric resistance arteries, myoendothelial communication involving gap junctions may contribute to oscillations in diameter and vessel wall [Ca2+]i, associated with PE administration.

Depolarization of VSM cells associated with elevated intraluminal pressure may result from activation of mechanosensitive ion channels (12, 43, 44). Recent studies have shown that VSM cells express stretch-activated nonselective cation channels (43) and that downregulation of these channels by an antisense oligonucleotide approach attenuates myogenic vasoconstriction (44). These data support the hypothesis that these channels play a central role in pressure-induced vasoconstriction. Given that mechanosensitivity appears to be inherent to VSM cells (12), if all cells within the vascular wall responded equally to mechanical stimuli, it would be predicted that inhibition of gap junction communication would not alter stretch-induced changes in VSM cell Em and myogenic vasoconstriction. However, the current study shows that inhibition of VSM intracellular communication greatly attenuated pressure-induced VSM cell depolarization, Ca2+ influx and vasoconstriction. Thus our findings are consistent with the possibility that heterogeneity in stretch sensitivity exists among VSM cells. Differential VSM cell depolarization may result from heterogeneity in inherent mechano sensitivity or from differences in mechanical stretch sensed by various smooth muscle layers within the vascular wall. Consistent with this hypothesis, heterogeneity in the pressure-induced deformation of smooth muscle cell layers has been reported (20). Layers that are subjected to relatively higher levels of deformation may depolarize to greater extent than cells experiencing lower levels of mechanical stress. This hypothesis predicts that some VSM cells strongly depolarize after vessel wall stretch and transmit this response to adjacent, less mechanosensitive cells through gap junctions, thereby eliciting coordinated vasoconstriction. Recordings obtained from vessels pressurized to 100 Torr and treated with a gap junction inhibitory peptide that exhibit an unusual oscillating membrane potential (Fig. 8F) may represent these strongly depolarizing cells. Although additional work is needed to fully develop and verify this hypothesis, the proposed differences in response to mechanical stress among VSM cells could have significant implications for the understanding of vascular disease.

In conclusion, this study demonstrates that inhibition of gap junctions attenuates myogenic vasoconstriction of rat mesenteric resistance arteries by blunting stretch-induced VSM cell depolarization and increases in vessel wall [Ca2+]i. This effect was independent of endothelial cell function, suggesting that VSM intercellular communication via gap junctions is required for normal myogenic behavior.

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