Hemodynamic and proinflammatory actions of endothelin-1 in guinea pig small intestine submucosal microcirculation

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Hemodynamic and proinflammatory actions of endothelin-1 in guinea pig small intestine submucosal microcirculation. Am J Physiol Gastrointest Liver Physiol 284: G940–G948, 2003. First published February 5, 2003; 10.1152/ajpgi.00373.2001.—The hemodynamic and proinflammatory effects of endothelin-1 (ET-1) in proximal (1st/2nd order) and terminal (3rd/4th order) arterioles and venules were examined in small intestine submucosa of anesthetized guinea pigs. Vessel diameter (D), red blood cell velocity, and blood flow (Q) were determined in eight proximal and eight terminal microvessels before and at 20 min of ET-1 suffusion (10−10, 10−9, and 10−8 M) and then with endothelin-A (ETα) receptor blockade with BQ-123 (10−5 M). This protocol was repeated with platelet-activating factor (PAF) inhibition (WEB-2086, 1.0 mg/kg iv; n = 6). The ET-1-mediated microvascular responses were also examined with endothelin-B (ETβ) receptor blockade using BQ-788 (10−5 M; n = 11) alone or with ETα, ETβ receptor blockade with BQ-123 + BQ-788 (n = 10). Microvascular permeability was assessed by FITC-albumin (25 mg/kg iv) extravasation in seven series: 1) buffered modified Krebs solution suffusion (n = 6), 2) histamine suffusion (HIS; 10−3 M, n = 5), 3) ET-1 suffusion (10−8 M, n = 5), 4) BQ-123 (10−5 M) plus ET-1 suffusion (n = 5), 5) PAF inhibition before ET-1 suffusion (n = 5), 6) histamine-1 (H1) receptor blockade (diphenhydramine, 20 mg/kg iv) before ET-1 suffusion (n = 5), and 7) ETα-receptor blockade before (BQ-788 10−5 M; n = 3) or with ET-1 suffusion (n = 3). D and Q decreased at 10−8 M ET-1 and returned to control values with BQ-123 and BQ-123+BQ788 but not with BQ-788 in proximal microvessels. D did not change in terminal microvessels with ET-1 (10−8 M) but decreased with BQ-788 and increased with BQ-123. PAF inhibition did not affect the D and Q responses of proximal microvessels to ET-1 but prevented the fall in Q in terminal microvessels with ET-1. ET-1 increased vascular permeability to ~1/3 of that with HIS; this response was prevented with BQ-123 and WEB-2086 but not with H1 receptor blockade. This is the first evidence that submucosal terminal microvessel flow is reduced with ET-1 independent of vessel diameter changes and that this response is associated with increased microvascular permeability mediated via ETα-receptor stimulation and PAF activation.

blood flow

IN PREVIOUS EXPERIMENTS, we found that intra-arterial infusion of endothelin-1 (ET-1; 0.1 μg·kg−1·min−1) caused a significant reduction in blood flow in canine skeletal muscle (15) and small intestine (14). In response to this reduction in blood flow, O2 extraction increased to maintain muscle O2 uptake. No compensatory increase in O2 extraction occurred in the canine small intestine, and as a result, O2 uptake fell. The failure of gut to increase O2 extraction following ET-1 might be the result of either a reduction in gut metabolic demand or a change in microcirculatory flow, which limited O2 extraction. We discarded the former because no evidence is available to support a negative metabolic action of ET-1 in gut tissue. The likely mechanism was an alteration in microcirculatory flow distribution that increased O2 diffusion distances and limited O2 extraction.

ET-1 could influence the distribution of flow in the microcirculation through vasoconstrictor and/or proinflammatory actions. Other vasoconstrictor agents do result in the increased heterogeneity of blood flow in the microcirculation seen with ET-1 (1). Furthermore, in preliminary experiments, we observed that vascular stasis occurred with only a small change in vessel diameter (10−9 M ET-1) in rat mesenteric microvessels (~30 μm), suggesting that ET-1-mediated vasoconstriction was not totally responsible for the cessation of flow in these small vessels (14). Others (10) have shown that intravenous administration of ET-1 (1 nmol/kg) in conscious rats increased albumin extravasation in the bronchi, spleen, kidney, stomach, and duodenum. This effect was abolished by endothelin-A (ETα) receptor blockade or platelet-activating factor (PAF) inhibition, indicating that ET-1 can induce an inflammatory response leading to the formation of tissue edema. With the use of another indicator of inflammation, Boros et al. (4) demonstrated that ET-1 (3 nmol/kg iv) increased leukocyte rolling and adherence in postcapillary venules of the ileal submucosa in the anesthetized rat; a response that was attenuated by ETα-receptor blockade. The increased vascular leakage that occurs with ET-1-mediated inflammation in the small intestine may result in hyperviscosity of the blood, which would promote stasis in the microcirculation (22, 23). This could explain our findings in the rat mesentery. The functional consequence of such a re-
response would be the formation of areas of stasis within the microcirculation resulting in a heterogeneous distribution of O\textsubscript{2} within the microcirculation and subsequent decrease in O\textsubscript{2} extraction.

We developed a guinea pig submucosal microcirculation preparation to measure vascular hemodynamic and permeability changes at all levels of the microcirculation. We chose to use the submucosal rather than mesenteric microcirculation to obtain findings more relevant to the effects of ET-1 on nutritive blood flow to tissue. Furthermore, we wished to assess vessel diameter and red blood cell (RBC) velocity at all branch levels of the microcirculation to determine whether ET-1 had a predominant effect at a specific level of the microcirculation to alter microvascular flow distribution. We believe that if ET-1 induces vasoconstriction equally across all levels of the microcirculation, then a homogeneous reduction in O\textsubscript{2} delivery will occur. However, if the vasoconstriction is applied to a much greater extent at lower-order vessels leading into the capillary bed, this action will limit the cross-sectional area of capillary bed available for exchange of O\textsubscript{2} and metabolites. With this in mind, we arbitrarily designated the first- and second-order microvessels as "proximal" microvessels and viewed these primarily as conduit vessels. Third- and fourth-order microvessels were designated as "terminal" microvessels because we viewed these vessels as "distribution" vessels that were close to or leading into the capillary bed of the villi. Given these suppositions, the intent of the current study was to determine the effect of ET-1 suffusion at various levels of the submucosal microcirculation in the anesthetized guinea pig. We hypothesized that ET-1 would cause a greater disruption in blood flow in terminal compared with proximal microvessels and that this response would involve both the vasoconstrictor and proinflammatory actions of ET-1.

**METHODS**

**Animal and Tissue Preparation**

All animals were cared for according to the Canadian Council of Animal Care guidelines and all experimental protocols being approved by the Queen’s University Animal Ethics review committee. Male guinea pigs (200–225 g; n = 72) were anesthetized with pentobarbital sodium (45 mg/kg ip); additional intravenous anesthetic was given as required. The trachea was cannulated, and the animals were placed on a rodent ventilator (Ugo Basile) and ventilated at a rate of 60 breaths/min with a tidal volume of 2 ml. The right external jugular vein and the right carotid artery were cannulated for injection of anesthetic and substances and for measurement of mean arterial pressure, respectively. The guinea pig was then placed on a Plexiglas platform warmed to 37°C. A laparotomy was performed along the abdominal midline, and an ileal segment of the small bowel was exteriorized and placed on the viewing pedestal of the Plexiglas platform. A 2-cm length of bowel was selected for study. An open catheter was inserted into the lumen of the intestine 5–6 cm proximal to the selected area to divert intestinal debris. With the use of a fine electrocautery tip, a 2-cm incision was made along the antimesenteric border of the segment. An adjustable double arterial microvascular clamp was used to spread and hold 1.5 cm of the selected gut tissue. The clamped tissue (mucosal side down) was placed on the viewing pedestal, which was designed to hold the tissue flat, and a 37°C buffered modified Krebs solution was continuously suffused over the tissue.

**Intravital and Fluorescence Video Microscopy**

The animals were placed on the stage of a trinocular ELR-Intravital Microscope (Wild-Leitz). The microscope was fitted with both transmitted (50 W halogen) and fluorescence (50 W mercury incidence with an I 2/3 beam splitter) light accessories. Images were focused with a Leitz-Wetzlar ×25 NPL Floutar long-distance working lens and Periplan ×10 eyepieces with final magnification being approximately ×1,500. In each animal, a suitable area of submucosal vessels was selected for study. The images were viewed through the photodiode array of an optical Doppler velocimeter (Texas A&M) to measure RBC velocity, passed through a Nikon ×0.35 TV lens, and captured with a SONY DSC-390m 3CCD color video camera. The images were sent to a Hitachi SVHS video recorder and to a Matrox Meteor II multichannel (RGB) image capture card for online video-image processing and analysis with Image-Pro Plus 4.0 software (Media Cybernetics).

**Protocol: Hemodynamic Studies**

Control ET-1 dose response. In the first series of experiments (control group; n = 16), resting measurements of arteriolar and paired venular blood vessel diameter and centerline RBC velocity were made. Measurements were repeated following suffusion of ET-1 at concentrations of $10^{-10}$, $10^{-9}$, and $10^{-8}$ M and following blockade of ET\textsubscript{A} receptors with the specific ET\textsubscript{A}-receptor antagonist BQ-123 (10 $^{-5}$ M) (3) added to the suffusate containing $10^{-8}$ M ET-1. Measurements were obtained after 20 min suffusion of each concentration ET-1 and after 30 min of suffusion with BQ-123 + ET-1 in eight proximal (1st/2nd order) and eight terminal (3rd/4th order) paired arteriole/venule submucosal microvessels. Diameter was reported as internal diameter in micrometers. Mean RBC velocity was calculated as centerline velocity divided by 1.6 (16). Blood flow was calculated as mean RBC velocity times the vessel cross-sectional area (16).

Endothelin receptor antagonist studies. Studies were performed to determine the contribution of endothelin-B (ET\textsubscript{B}) receptors and ET\textsubscript{A} + ET\textsubscript{B} receptors to the gut microvascular responses to ET-1 ($10^{-8}$ M). A protocol similar to that described above was followed. Control measures of vessel diameter and RBC velocity were obtained, and blood flow was calculated. Then, ET-1 ($10^{-8}$ M) was suffused for 20 min followed by either suffusion for 30 min with the specific ET\textsubscript{B}-receptor antagonist [N-cis-2,6-dimethylpiperidinocarbonyl-L-$\gamma$-methylleucyl-L-1-methoxy-carbonyl-tryptophanyl-$\alpha$-norleucine (BQ-788); 10 $^{-5}$ M] (12) or with both ET\textsubscript{A} + ET\textsubscript{B}-receptor antagonists (BQ123 + BQ788; $10^{-5}$ M).

**PAF inhibition studies.** In the second series of experiments, control measurements were obtained, and then PAF was inhibited using WEB-2086 (1.0 mg/kg iv; Boehringer-Ingelheim) (5). Measurements were obtained 15 min after WEB-2086 administration and then the same protocol as in the control series was repeated (WEB, n = 16).

**Protocol: Microvascular Permeability Studies**

Microvascular permeability was assessed by the quantification of extravasated FITC-labeled albumin (Sigma) in the intestinal wall of the guinea pig ileum. With the use of the mercury lamp, fluorescent images were captured at 1, 5, 10,
and 20 min after FITC-albumin administration (25 mg/kg iv) in six experimental series: control (n = 6); 20-min suffusion of buffered modified Krebs solution; histamine (n = 5); 20-min suffusion of histamine (10⁻⁸ M); ET-1 (10⁻⁸ M); ET-1 + BQ-123 (n = 5); BQ-123 (10⁻⁵ M) was suffused for 30 min followed by a 20-min suffusion with BQ-123 (10⁻⁵ M) and ET-1 (10⁻⁸ M); PAF inhibition (n = 5): WEB-2086 (1 mg/kg iv) was administered 15 min before commencing suffusion with ET-1 (10⁻⁸ M) for 20 min; histamine-receptor blockade (n = 5): the histamine-1 (H1) receptor antagonist diphenhydramine (20 mg/kg iv) was administered 15 min before commencing suffusion with ET-1 (10⁻⁸ M) for 20 min. Additional studies were performed with ETₐ-receptor blockade using BQ-788 (10⁻⁵ M), which was suffused for 30 min followed by a 20-min suffusion with ET-1 (10⁻⁸ M). In three animals, FITC was given concurrently with the ET-1, and in three animals, FITC was administered at 20 min of ET-1 suffusion. Captured images were converted to eight-bit grayscale (0–255 pixel assignment), and mean optical intensity was measured in a 220 × 270-μm “area of interest” using the software program Image-Pro Plus (Media Cybernetics). As greater quantities of FITC-albumin accumulated in the interstitial space due to increases in vascular permeability, an increase in mean optical intensity was observed (17).

Statistical Analysis

Data are presented as means ± SE. In the control ET-1 dose response and PAF inhibition experiments, differences within groups were determined using repeated-measures ANOVA with an α-level of 0.10 (23) followed by a post hoc Bonferroni analysis with significance accepted at P < 0.02 (α/ nº of comparisons). Differences between groups at each measurement point were determined using unpaired t-test analysis, and significance was accepted at P < 0.05. In the vascular permeability studies, differences between specific group comparisons (histamine and ET-1 vs. control; ET-1 + BQ-123, ET-1 + WEB-2086, and ET-1 + H1 block vs. ET-1) were determined using unpaired t-test analysis, and significance was accepted at P < 0.05.

RESULTS

Effect of ET-1 on Submucosal Vessel Diameter and Blood Flow

The values for mean arterial pressure at the beginning and at the end of the experimental protocol in both the control and WEB groups are listed in Table 1. Local suffusion of increasing concentrations of ET-1 over the small bowel intestinal segment and the additional suffusion of BQ-123 did not affect mean arterial pressure.

The responses of proximal and terminal submucosal arterioles to increasing concentrations of ET-1 are shown in Fig. 1, A and B, respectively. The resting diameter of proximal arterioles averaged 72 ± 3 μm compared with 29 ± 3 μm in terminal arterioles. The control values for blood flow in proximal and terminal arterioles were 5.3 ± 1.2 and 0.40 ± 0.03 μl/s, respectively. Suffusion of ET-1 at concentrations of 10⁻¹⁰ and 10⁻⁸ M had no significant effect on either the diameter or blood flow of proximal and terminal arterioles. At an ET-1 concentration of 10⁻⁸ M, proximal arteriole diameter decreased 54%, with a resultant 81% reduction in blood flow. This concentration of ET-1 (10⁻⁸ M) had no significant effect on terminal arteriolar diameter; however, blood flow in these terminal vessels was reduced 70% (P < 0.02). In six of the eight terminal arterioles studied, complete stasis of flow (zero flow) occurred with suffusion of ET-1 (10⁻⁸ M). The ETA-receptor antagonist BQ-123 completely reversed the proximal arteriolar vasorelaxation with ET-1 and partially reversed the reduction in blood flow. In terminal arterioles, BQ-123 in the presence of ET-1 (10⁻⁸ M) resulted in a significant increase in vessel diameter, and blood flow was greater than control values (P < 0.02).

The effects of ET-1 and BQ-123 on proximal and terminal venular diameter and blood flow (Fig. 2, A and B, respectively) were similar to those observed in proximal and terminal arterioles. Control proximal and terminal venular diameters averaged 119 ± 12 and 56 ± 8 μm, respectively. Venular blood flow averaged 3.7 ± 0.9 and 0.5 ± 0.1 μl/s in the proximal and terminal vessels, respectively. Suffusion of ET-1 at concentrations of 10⁻¹⁰ and 10⁻⁹ M had no effect on proximal and terminal venular diameter and blood flow. Suffusion of 10⁻⁸ M ET-1 decreased proximal venular diameter and blood flow 31 and 38%, respectively. Terminal venular diameter was not significantly affected by suffusion with ET-1 (10⁻⁸ M); however, terminal venular blood flow was reduced 85% (P < 0.05), with stasis occurring in many of the studied terminal venules. BQ-123 reversed the ET-1 (10⁻⁸ M)-induced vasoconstriction in proximal venules. The reduction in blood flow that occurred in response to ET-1 (10⁻⁸ M) was completely reversed by BQ-123, and blood flow returned to control levels in proximal and terminal venules, respectively.

The relative proximal and terminal arteriolar diameter and blood flow responses with ET-1 (10⁻⁸ M) and subsequent changes with ETA, ETB, and ETₐ+ETB-receptor blockade compared with control (1.00) are shown in Fig. 3. In proximal arterioles, vessel diameter decreased with ET-1 and returned to control values with ETA and ETₐ + ETB-receptor blockade. Proximal arteriole blood flow was significantly reduced with ET-1. ETA + ETB-receptor blockade returned flow to near-control values, but ETₐ or ETB-receptor blockade alone did not. In contrast, little change in terminal

Table 1. Mean arterial pressure (mmHg) at the beginning and conclusion of the protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>Start of Protocol</th>
<th>End of Protocol</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>Proximal vessel group</td>
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<td>50 ± 4</td>
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<tr>
<td>Terminal vessel group</td>
<td>48 ± 2</td>
<td>48 ± 3</td>
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<tr>
<td>WEB-2086</td>
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<tr>
<td>Proximal vessel group</td>
<td>48 ± 4</td>
<td>42 ± 3</td>
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<tr>
<td>Terminal vessel group</td>
<td>57 ± 2</td>
<td>53 ± 2*</td>
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Values are means ± SE. *Significant difference between the values at the start and end of protocol at P < 0.05.
arteriole diameter was observed with ET-1 and did not change with ETA + ETB-receptor blockade. ETB-receptor blockade resulted in a decrease in terminal arteriole diameter, whereas ETA-receptor blockade significantly elevated vessel diameter above control levels. The decrease in terminal arteriole blood flow with ET-1 was enhanced with ETB-receptor blockade, not significantly affected by ETA + ETB-receptor blockade, whereas ETA-receptor blockade resulted in a hyperemic flow response (1.67 of control).

**Fig. 1.** Values are means ± SE for vessel diameter (top) and blood flow (bottom) in submucosal proximal (1st/2nd order (A)) and terminal (3rd/4th order (B)) arterioles in the small intestine of anesthetized guinea pigs. ○, control group, endothelin (ET)-1 dose response; ●, WEB-2086, pretreatment with PAF inhibition. *Significant difference from control (CON) at \( P < 0.02 \); ‡Significant difference from ET-1 (10^{-10} M) at \( P < 0.02 \); $Significant difference from ET-1 (10^{-9} M) at \( P < 0.02 \); †Significant difference from BQ-123 (10^{-5} M) at \( P < 0.02 \); $Significant difference between CON and WEB-2086 groups at \( P < 0.05 \).

**Fig. 2.** Values are means ± SE for vessel diameter (top) and blood flow (bottom) in submucosal proximal (1st/2nd order (A)) and terminal (3rd/4th order (B)) venules in the small intestine of anesthetized guinea pigs. ○, control group, endothelin (ET)-1 dose response; ●, WEB-2086, pretreatment with PAF inhibition. *Significant difference from CON at \( P < 0.02 \); $Significant difference from ET-1 (10^{-10} M) at \( P < 0.02 \); ‡Significant difference from ET-1 (10^{-9} M) at \( P < 0.02 \); †Significant difference from BQ-123 (10^{-5} M) at \( P < 0.02 \); $Significant difference between CON and WEB-2086 groups at \( P < 0.05 \).
The relative proximal and terminal venular diameter and blood flow responses with ET-1 (10^-8 M) and subsequent changes with ETA, ETB, and ETA+ETB receptor blockade compared with control (1.00) are shown in Fig. 4. The reductions in proximal venule diameter and blood flow with ET-1 were not affected by ETB-receptor blockade, but these values returned to control levels (1.00) with ETA+ETB-receptor blockade. Terminal venule diameter was unaffected by ET-1, ETA, ETB, or ETA+ETB-receptor blockade. Terminal venule blood flow, however, was significantly reduced with ET-1; this response was unaffected by ETB or ETA+ETB-receptor blockade. ETA-receptor blockade in the presence of ET-1 increased terminal venule blood flow to 1.5-fold of control levels.

Effect of ET-1 and PAF Inhibition on Submucosal Vessel Diameter and Blood Flow

PAF inhibition with WEB-2086 had no effect on the ET-1-induced changes in diameter or blood flow in proximal arterioles and venules (Figs. 1A and 2A). In terminal arterioles and venules (Figs. 1B and 2B), the control values for vessel diameter were significantly less with PAF inhibition, but this difference was most likely due to interanimal differences and not to a direct effect of PAF inhibition, because these values were not different before and after PAF inhibition in this group. In both terminal arterioles and venules, PAF inhibition prevented the decrease in blood flow observed with ET-1 at 10^-8 M (Figs. 1B and 2B).
**Submucosal Microvascular Permeability Studies**

Microvascular permeability was assessed by measuring the mean optical intensity (MOI) of FITC-albumin in a selected submucosal area (Fig. 5). In the control group, the average value for MOI was 27.2 ± 0.8. MOI increased 147% above the control in the presence of histamine, a positive control used to indicate a maximal increase in microvascular permeability. ET-1 (10⁻⁸ M) increased microvascular permeability, as indicated by a 49% increase in MOI, a response that was approximately one-third of that observed with histamine. Pretreatment with the ETA-receptor antagonist BQ-123 abolished the ET-1-mediated increase in microvascular MOI; the average value of 29.4 ± 3.0 was not significantly different from the control value. Pretreatment with WEB-2086 resulted in an average value for MOI of 32.4 ± 1.7, a response that was significantly less than that obtained with ET-1 alone and not different from the control value for MOI (P < 0.07). The ET-1-mediated increase in the MOI was not significantly altered with blockade of H1 receptors with diphenhydramine.

The effect of ETB-receptor blockade on ET-1-induced permeability was examined using two different protocols; however, the data are not shown in Fig. 5. In one protocol, ETB-receptor blockade was induced, and ET-1 was then suffused for 20 min and followed by administration of FITC to determine permeability changes (n = 3). In this instance, vascular permeability decreased from a control value of 28.2 (MOI) to 23.0 (P < 0.37). In the second protocol, ETB-receptor blockade, ET-1, and FITC were administered concurrently (n = 3). Under these circumstances, vascular permeability increased from a control value of 25.7 to 27.8 (MOI; P < 0.10).

**DISCUSSION**

The above findings support our hypothesis that ET-1 exerts both vasoconstrictor and proinflammatory actions in the submucosal microcirculation and provided novel evidence for differential hemodynamic and proinflammatory effects of ET-1 in submucosal proximal and terminal microvessels, respectively. Specifically, ET-1 suffusion resulted in vasoconstriction and a reduction in blood flow in the first- and second-order arterioles and venules. In contrast, blood flow was reduced, and in most instances, stasis occurred in third- and fourth-order arterioles and venules with no change in vessel diameter. PAF inhibition abolished the reduction in blood flow in the terminal microvessels with ET-1 suffusion but had no effect on ET-1-mediated reductions in vessel diameter and blood flow in the proximal microvessels. Furthermore, ET-1 increased microvascular permeability, which was approximately one-third the magnitude of that observed with histamine. This increase in microvascular permeability was abolished with ETB-receptor blockade and with PAF inhibition. The physiological relevance of these findings is twofold. First, ET-1 affects the volume of flow entering the microcirculation by its vasoconstrictor action in proximal microvessels, a response not unexpected in a reaction to stress. The proinflammatory action of ET-1, however, increases vascular permeability, which may be associated with the development of regions of stasis within terminal microvessels. The functional outcome may be an increased heterogeneity of microvascular flow distribution, which ultimately increases diffusion distances for O₂ and results in a reduced O₂ extraction capacity.

ET-1 induced significant vasoconstriction in first- and second-order arterioles and venules but not in third- and fourth-order vessels. This was a consistent finding within each group of animals studied. Vessel diameter decreased 54 and 31% in proximal arterioles and venules, respectively at an ET-1 concentration of 10⁻⁸ M but did not change significantly in terminal arterioles and venules. Few studies have assessed the submucosal microcirculatory response to ET-1 at each level of the microcirculation; however, Wilson et al. (30) reported that vessel diameter decreased 40 and 75% in first- and third-order arterioles, respectively, in response to ET-1 (10⁻⁸ M) in the ileal submucosa of the guinea pig. These findings correlate well with our results. ET-1 may be an increased heterogeneity of microvascular flow distribution, which increases vascular permeability and ultimately decreases O₂ extraction.

![Fig. 5](http://ajpgi.org) Values are means ± SE for mean optical intensity (no units) of FITC-albumin in the measured area of interest in guinea pig submucosal microcirculation. The data in the first 3 histograms were obtained during CON experiments, with histamine administration (HIST; 10⁻⁵ M) and suffusion with ET-1 alone (10⁻⁸ M). The data in the last 3 histograms were obtained in animals pretreated with BQ-123 (ET + BQ; 10⁻⁵ M), WEB-2085 (ET + WEB; 25 mg/kg iv), pretreatment with dihyraphenamine (ET + H1; 20 mg/kg iv), followed by 20 min of suffusion with ET-1 (10⁻⁸ M). *Significant difference from CON at P < 0.05; †significant difference from ET-1 at P < 0.05.
anesthetized rat. The similarity in initial diameters in the rat versus guinea pig submucosa for these branch order of vessels averaged 63 and 19 μm in A1 and A3 vessels, respectively, in the rat and 72 and 29 μm, respectively, in the current study, suggesting that vessel size would not be a factor in the different response to ET-1. Preliminary data from hamster submucosal proximal and terminal microvessels replicate the responses to ET-1 that we observed in the guinea pig, supporting the reproducibility of this response across species in our laboratory.

The ET-1-receptor studies provided novel insight into the microvascular actions of ET-1 in the small intestine submucosa. In proximal arterioles and venules, ET_A and ET_A + ET_B but not ET_B-receptor blockade restored vessel diameter to control values, indicating that vasoconstriction in these vessels was entirely mediated through ET_A-receptor activation. Our findings are consistent with those of others in which ET_A-receptor blockade with BQ-123 prevented the vasoconstrictor action of ET-1 in third-order branches of the rat mesenteric artery (24) and in the rat in vitro mesentery preparation (8). Our data from the dog gut loop preparation also demonstrated that the constrictor response to ET-1 was entirely mediated via ET_A receptors (14).

The vascular responses of the terminal vessels to ET-1 provided a much more complicated and intriguing situation. Terminal vessel (arteriole and venule) diameter did not change with ET-1 suffusion. ET_B-receptor blockade resulted in a reduction in vessel diameter, whereas ET_A-receptor blockade increased vessel diameter; ET_A + ET_B-receptor blockade had no effect on vessel diameter. These are the first data to indicate that a substantial vasodilator influence of ET-1 is present at the lower level of the microcirculation in small intestine submucosa. The enhanced vasoconstriction to ET-1 in the presence of ET_B-receptor blockade has been reported by others for the canine small intestine mesenteric vessels and rat third-order mesenteric arteries (24), suggesting that ET_B-receptors located on vascular smooth muscle cells mediated a portion of the constrictor response to ET-1 (13, 24). In the terminal vessels, there was no evidence of ET_B receptor-mediated constriction, because ET_A + ET_B blockade totally reversed the constriction to ET-1, whereas ET_A-receptor blockade only partially reversed the constriction in guinea pig mesenteric veins and rat third-order mesenteric arteries (24), suggesting that ET_B-receptors located on vascular smooth muscle cells mediated a portion of the constrictor response to ET-1 (13, 24). In the terminal vessels, there was no evidence of ET_B receptor-mediated constriction, because ET_A + ET_B blockade did not influence vessel diameter. However, ET_A-receptor blockade resulted in a substantial increase in vessel diameter, providing evidence for significant ET-1-mediated vasodilation, most likely via activation of ET_B receptors on the vascular endothelial cells (3, 18). To our knowledge, this is the first evidence provided to indicate that the seeming lack of a constrictor response of terminal vessels to ET-1 was actually the result of a balance of vasoconstriction mediated through ET_A receptors and vasodilation mediated through ET_B1 receptors.

The decrease in terminal microvessel blood flow with ET-1 was most likely the result of one of two things: 1) the reduction in flow in the upstream proximal vessels or 2) a proinflammatory action of ET-1. We hypothesized that inflammation would increase protein extravasation and fluid loss from the vascular compartment, resulting in a hyperviscosity in the terminal vessels, which, in turn, would increase resistance to flow and reduce RBC velocity in these vessels, a theory supported by Menger et al. (23). To determine which of the above conjectures was correct, animals were pretreated with the PAF inhibitor WEB-2086. We chose to use PAF inhibition to eliminate or reduce the inflammatory response with ET-1, because others (11, 19) have demonstrated the contribution of PAF to ET-1-associated inflammatory responses using Evan’s blue dye protein extravasation studies. In the current study, PAF inhibition had no effect on the response of proximal order vessels to ET-1. However, pretreatment with PAF inhibition prevented the decrease in blood flow in terminal-order vessels with ET-1 suffusion. These findings indicate that the decrease in flow in terminal microvessels was not a result of lower flow in higher order vessels but was associated with an ET-1-mediated inflammatory response via PAF activation. To confirm our hypothesis that the decrease in terminal vessel flow was associated with protein extravasation and fluid loss with resultant hyperviscosity, we also examined vascular permeability of these vessels using FITC-albumin. ET-1 significantly increased vascular permeability, which was totally abolished with ET_A-receptor blockade and with PAF inhibition. These data indicate that in the guinea pig submucosal preparation, the inflammatory response to ET-1 is mediated via ET_A-receptor activation and PAF activation. Our findings are in agreement with others that have identified an inflammatory action of ET-1. Protein extravasation with ET-1 was entirely prevented by ET_A-receptor blockade (10) or with PAF inhibition (11, 19) in anesthetized rats. Other investigations have used increased leukocyte rolling and adherence as evidence of an inflammatory action of ET-1. In these cases, increased leukocyte rolling and adherence were increased in rat submucosal postcapillary venules at ET-1 concentrations of 1.0–1.0 nmol/kg iv (4), a response which was partially reversed with ET_A-receptor blockade.

Because either ET_A-receptor blockade or PAF inhibition eliminated the stasis and increased vascular permeability with ET-1 administration, it is likely that these two events are linked to produce the overall outcome. The mechanism(s) through which ET_A receptors and PAF interact are not clear. Primary locations of PAF synthesis are neutrophils, monocytes, and endothelial cells (26). ET_A receptors are present on neutrophils, which, when activated, increase intracellular calcium (20), and this, in some manner, activates PAF. Furthermore, ET_A-receptor activation includes phosphokinases as second messengers, and this may be the

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route of stimulus for PAF synthesis. In endothelial cells, PAF is translocated to the plasma membrane and retained on the cell surface. In this instance, PAF acts as an intracellular messenger and stimulates polymorphonuclear cells as part of a juxtacrine system (26). ET-1, through ETA-receptor stimulation, can result in neutrophil (20) and mast cell (25) activation; however, the next question to be asked is whether this action is mediated through ETA receptors located on the endothelium, on neutrophils, or both? This, in turn, may help to identify whether the inflammatory response is tissue specific, as is ETA-receptor density, or if it is more global, as is the distribution of neutrophils.

We originally hypothesized that microcirculatory dysfunction with ET-1 administration would increase diffusion distance for oxygen and thereby limit the ability of the tissue to extract oxygen. No direct measures of O2 utilization were performed in this study, and few microcirculatory measures of ET-1 effects on oxygenation are available. One recent report (2) demonstrated that ET-1 produced a “patchy pattern” of both an increase and decrease in PO2 across a zone in rat liver acini, but when vasoconstriction was induced with phenylephrine, a homogenous decrease in tissue PO2 occurred within a zone. These findings are consistent with our O2 extraction limitation hypothesis.

The next question to address is whether the concentrations used in the current study reflect physiological or pharmacological values of ET-1. ET-1 is produced in the endothelium, with the majority moving abluminal; the resulting concentration being 100-fold greater than that in blood (21). Young healthy males ventilated with a gas mixture to achieve 75–80% oxygen saturation, a situation similar to that in individuals with cor pulmonale, had serum ET-1 concentrations of 2.1 pM (5). If the abluminal concentration is 100-fold greater, then this value would be ~0.2 nM, which is slightly less than the concentrations at which the greatest effects of ET-1 were observed in the present study. In liver transplant recipients, serum ET-1 levels were 15 pg/ml (7), translating to an abluminal concentration of 6 × 10^-10 M. We saw substantial vascular effects of ET-1 at 10^-8 M; however, this was the sulfusate concentration, which is slightly greater than our estimates of abluminal ET-1 concentration. We do not have direct measures of interstitial ET-1 concentrations in our studies. The interstitial ET-1 concentration in our experiments may be slightly lower, given diffusion through tissue layers. This would place our findings in a similar ET-1 concentration range as that identified in certain pathological conditions in which serum ET-1 is elevated.

In summary, we have shown for the first time in a single preparation that a differential action of ET-1 occurs in submucosal microvessels in guinea pig small intestine. In first- and second-order vessels, ET-1 (10^-8 M) decreased vessel diameter and blood flow. In third- and fourth-order vessels, no vasoconstriction occurred, yet a substantive decrease in blood flow was observed. We also demonstrated that ET-1 increased vascular permeability, an effect mediated by ETA-receptor activation and PAF stimulation. In terminal microvessels, the inflammatory action of ET-1 most likely decreased blood flow due to increased resistance to flow as a result of fluid loss from the vascular compartment and blood hyperviscosity. The precise series of events involved in the ET-1-mediated inflammatory response is not clear, and critical steps in this process are yet to be identified. These findings have potential significance in defining the microvascular responses observed in conditions/diseases in which ET-1 is elevated.

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