Receptor-mediated vascular and metabolic actions of endothelin-1 in canine small intestine

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King-VanVlack, Cheryl E., Jeffrey D. Mewburn, and Christopher K. Chapler. Receptor-mediated vascular and metabolic actions of endothelin-1 in canine small intestine. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1131–G1136, 1999.—The effects of endothelin-1 (ET-1) infusion on blood flow (QG) and O2 uptake (VO2G) were examined in the small intestine of anesthetized dogs (n = 10). Arterial and venous flows of a gut segment were isolated, and the segment was perfused at constant pressure. Arterial and venous blood samples were taken, gut perfusion pressure and QG were measured, and O2 extraction ratio (OERG) and VO2G were calculated. ET-1 was infused (0.118 µg·kg−1·min−1·ia) throughout the experiment. In group 1 (n = 5), ETA receptors were blocked using BQ-123 (0.143 mg·kg−1·min−1·ia) followed by blockade of ETB receptors with BQ-788 (0.145 mg·kg−1·min−1·ia). The order of ETA and ETB receptor blockade was reversed in group 2 (n = 5). In group 1, the decrease in QG observed with ET-1 infusion was partially reversed with BQ-123; no further change occurred after BQ-788 administration. In group 2, addition of BQ-788 to the infusate further decreased QG, whereas addition of BQ-123 returned QG to a value not different from that with ET-1 infusion alone. These data indicated that ET-1-induced vasoconstriction in the gut was mediated via ETA receptors and that this constriction was buffered by activation of ETB receptors. VO2G decreased in proportion to the decrease in QG with ET-1, decreased further with ET-1 plus ETB receptor blockade (group 2), and increased in proportion to the increases in QG with ETA receptor blockade (both groups). No changes in OERG occurred during ETA and ETB receptor antagonism in either group. This study is the first to demonstrate that a flow-limited decrease in gut VO2G occurred with infusion of ET-1 in gut vasculature. An intriguing and novel finding was that, during O2 limitation, OERG was only 50% of that normally associated with ischemia in this tissue.

The expected vascular response to infusion of endothelin-1 (ET-1) is a transient dilation that occurs over 30–60 s followed by a prolonged constriction; the maximal constrictor response is normally observed by 15 min but varies somewhat between tissues (19, 28, 33). The vasoconstrictor action of ET-1 is primarily mediated through activation of ETA receptors (8, 23, 27, 32, 33) and, in some cases, also ETB receptors located on vascular smooth muscle cells (2, 14, 19, 32). In addition, activation of ETB receptors located on endothelial cells by ET-1 (25) results in the release of the vasodilator substances prostacyclin and/or nitric oxide (NO) (25).

The gut circulation appears to be more susceptible to the vasoconstrictor actions of ET-1 than other peripheral vascular beds. Specifically, the gut vasoconstrictor response to ET-1 is more intense than that observed for iliac flow in anesthetized monkeys (6), renal and carotid flows in anesthetized cats (21), and flow in all nongastrointestinal tissues in the anesthetized rat (17). This sensitivity of the gut vasculature to ET-1 may be of considerable importance in light of the fact that 1) ET-1 levels are elevated in a number of instances, including chronic hypoxia (4, 10), a variety of surgical interventions (11, 12, 30), and sepsis (22), and 2) this tissue has a high resting O2 uptake (20–25 ml·kg−1·min−1) that is normally met by a high blood flow rate (300–500 ml·kg−1·min−1) (7, 9, 24, 29). If blood flow is reduced to the point that gut O2 delivery falls below critical values (30–40 ml·kg−1·min−1), O2 uptake will be compromised (24).

To date, no studies have examined the possible metabolic consequences of an ET-1-induced increase in gut vascular resistance and the accompanying reduction in gut blood flow. We have developed an isolated perfused in vivo canine gut loop preparation to examine simultaneously the effects of ET-1 on both blood flow and O2 uptake in canine small intestine. On the basis of the highly sensitive response of the gut vasculature to ET-1, we hypothesized that ET-1 infusion at concentrations within the moderate range of the dose-response curve (32, 34) would result in a vasoconstriction that would reduce both gut blood flow and gut O2 uptake.

METHODS

Mongrel dogs (23.9 ± 1.2 kg) were anesthetized with pentobarbital sodium (32 mg/kg iv), paralyzed with succinylcholine chloride (30 mg im and 0.1 mg/min iv), and ventilated to maintain arterial PCO2 at ~30 mmHg. A Swan-Ganz catheter was inserted into the right jugular vein and advanced to the pulmonary artery for thermodilution cardiac output determinations (Baxter, COM-2) and withdrawal of mixed venous blood samples. A catheter was placed in the brachial artery for measurement of mean arterial blood pressure and to obtain arterial blood samples.

A previously described isolated gut loop preparation was modified to control arterial perfusion (9, 24). Briefly, a midline abdominal incision was made, and a section of small intestine (ileum or jejunum; 26 ± 1 g average wt) was observed by heparinized (1,000 U/kg), and then a two-channel cannula was placed in the vein draining the gut segment. One channel contained an electromagnetic flow probe (Narco) for measurement of venous outflow, whereas the second allowed for in situ zero calibration of the flow probe without occlusion of venous outflow. Venous outflow was returned to the animal via a reservoir attached to a catheter in the right femoral vein. The distal end of a two-channel catheter was placed in the artery...
serving the vascular arcade while the proximal end was inserted into the right femoral artery. This allowed for autoperfusion of the gut segment through one channel or pump perfusion via the second channel in which an in-line peristaltic pump was located. Venous outflow from the gut segment was isolated to the vein draining the segment by tightening ties around the gut tissue at either end of the area served by the vascular arcade. At one end of the segment, an incision was made into the gut tissue, and a temperature probe was inserted into the lumen to monitor temperature of the exteriorized segment. A heat lamp was used to maintain the gut temperature constant during the experiment. Gut perfusion pressure was measured by a pressure transducer attached to a T branch in the arterial cannula just before insertion into the gut arcade artery. Gut perfusion pressure under autoperfused conditions was determined, the gut was pump perfused, and perfusion pressure was maintained within 5% of control values via an electrical feedback system for the remainder of the experiment. To determine if pump perfusion of the gut segment resulted in edema, dry-to-wet weight ratios were not different between the pump-perfused segments (0.29 ± 0.01) and the autoperfused segments (0.32 ± 0.02).

Once surgical and technical preparations were complete, a 30-min period was allowed for cardiovascular and metabolic parameters to stabilize. In preliminary experiments, we found that infusion of 0.1 mg·kg⁻¹·min⁻¹ ia ET-1 into the canine gut segment vasculature caused an intense vasoconstriction that abolished blood flow. Similarly, Ralevic and Burnstock (26) observed that ET-1 concentrations in excess of 10⁻⁷ M caused severe vasoconstriction that increased perfusion pressure by 120 mmHg in an in vitro-perfused rat mesentry preparation. In the 10 animals used in the current experiments, initial control measures were obtained and then ET-1 was infused sequentially at two different concentrations (0.060 ± 0.001 and 0.118 ± 0.005 μg·kg⁻¹·min⁻¹ ia, respectively) into the gut segment; measurements were taken at 20 min of infusion for each concentration (Table 1). These concentrations of ET-1 were chosen because they evoked physiological increases in gut vascular resistance of 32 and 72%, respectively (Table 1); the magnitude of these changes in gut vascular resistance was similar to that reported by others (1, 32, 34). The protocol was continued with an ongoing infusion of ET-1 at the higher concentration. In group 1, BQ-123 [cyclo-(o-Trp-o-Asp-Pro-o-Val-Leu); 0.143 ± 0.010 mg·kg⁻¹·min⁻¹ ia], a selective ETA receptor antagonist (14), was added to the infusate, and measurements were obtained at 30 min postinfusion. BQ-788 [N-cis-2,6-dimethylpyrroldinocarbonyl-L-γ-methyl-Leu-o-1-(methoxy-carbonyl)-Trp-o-Nle; 0.145 ± 0.014 mg·kg⁻¹·min⁻¹ ia], a selective ETB receptor antagonist (15), was then added to the infusate, and measurements were obtained at 30 min postinfusion of BQ-123 = BQ-788. In group 2, the protocol was identical to that followed in group 1 except that BQ-788 was given first, followed by infusion of BQ-123. Others have reported that the concentrations of BQ-123 and BQ-788 must be 1,000-fold greater than the concentration of ET-1 to produce effective antagonism of the vascular effects of this peptide (25, 33).

At each measurement period, arterial, mixed venous, and gut venous blood samples were taken and whole body and gut perfusion pressure, gut blood flow, and cardiac output were determined. All blood samples were analyzed for PO₂, PCO₂, and pH using a Radiometer BMS MKII, and these values were corrected to the temperature of the dog at the time of sampling. O₂ concentration was determined in all samples using an Instrumentation Laboratories 482 Co-Oximeter. The O₂ concentration values were adjusted for the amount of O₂ in solution using the factor 0.03 ml O₂·dl⁻¹·mmHg (PO₂)⁻¹. Whole body and gut O₂ uptake were determined using the Fick equation, whereas whole body and gut vascular resistance and O₂ extraction ratio were calculated using standard equations.

All data are reported as means ± SE. Because the treatments were identical for all animals during the control period and ET-1 infusions and the data were not different between the two groups at these times, the data are reported for a total of 10 animals for each of these periods. The effects of ETA and ETB receptor blockade were determined within each group (n = 5), with the blocked values being compared with the control and ET-1 values of the five animals within each specific group using a single repeated measures ANOVA (n = 5). Post hoc multiple comparisons of differences between means were achieved by paired t-test analysis within each group of 5 animals with the critical value for significance (P < 0.10) adjusted using the Bonferroni correction (P < 0.017) (31).

RESULTS

The gut blood flow responses are shown in Fig. 1A. Gut blood flow decreased significantly from the control value in all 10 animals with infusion of ET-1. In group 1, gut blood flow then increased above the value observed during ET-1 infusion alone with the addition of BQ-123 to the infusate but remained significantly less than the initial control values. No further effect on gut blood flow was observed with the addition of BQ-788. In group 2, gut blood flow decreased with the addition of BQ-788 to the infusate; the mean value was significantly less than that observed both during the control period and ET-1 infusion alone. When BQ-123 was added to the infusate, gut blood flow increased significantly above that observed with BQ-788 but remained less than that observed during the control period.

The values for gut vascular resistance are shown in Fig. 1B. Gut vascular resistance increased significantly from the control value with infusion of ET-1. In group 1, after addition of BQ-123 to the infusate, the mean value for gut vascular resistance was not different from that of animals. The average values for the dry-to-wet weight ratios were not different between the pump-perfused segments (0.29 ± 0.01) and the autoperfused segments (0.32 ± 0.02).

| Table 1. Hemodynamic responses of canine small intestine to sequential concentrations of ET-1 |
|----------------------------------|---------------|---------------|
| Gut blood flow, ml·kg⁻¹·min⁻¹ | Control 290 ± 22 | Concentration 1 228 ± 20* | Concentration 2 182 ± 14† |
| Gut vascular resistance, PRU    | 0.43 ± 0.04 | 0.57 ± 0.05† | 0.74 ± 0.07‡ |
| Gut perfusion pressure, mmHg    | 118 ± 5 | 121 ± 5* | 124 ± 5‡ |

Values are means ± SE. Concentration 1 = 0.060 ± 0.001 μg·kg⁻¹·min⁻¹; concentration 2 = 0.118 ± 0.005 μg·kg⁻¹·min⁻¹, ET-1, endothelin-1; PRU, peripheral resistance units in mmHg·ml⁻¹·kg·min. *Significant difference from control value. †Significant difference between concentration 1 and concentration 2.
observed during the control period; infusion of BQ-788 had no further effect on gut vascular resistance. In group 2, addition of BQ-788 to the infusate resulted in a significant rise in gut vascular resistance above that observed with ET-1 infusion alone. Addition of BQ-123 to the infusate resulted in a significant decrease in gut vascular resistance to a level not different from that observed during infusion of ET-1 alone. However, this value was significantly greater than that observed during the control period.

The values for gut O₂ uptake are shown in Fig. 2A. Gut O₂ uptake decreased significantly from a value of 19.2 ± 0.7 to 13.1 ± 1.0 ml·kg⁻¹·min⁻¹ with infusion of ET-1. In group 1, after infusion of BQ-123, the value for gut O₂ uptake was not different from that observed during the control period. Subsequent infusion of BQ-788 resulted in a significant fall in gut O₂ uptake below the control value. In group 2, infusion of BQ-788 resulted in a further decrease in gut O₂ uptake from that observed with ET-1 infusion alone. During infusion of BQ-123, the value for gut O₂ uptake rose significantly to a level that was not different from that observed during ET-1 infusion alone but remained significantly less than the control value.

The values for gut O₂ extraction ratio are shown in Fig. 2B. Gut O₂ extraction increased significantly from a value of 0.34 ± 0.03 in the control period to that of 0.41 ± 0.05 with ET-1 infusion. However, the small increase in O₂ extraction was not sufficient to offset the decrease in gut O₂ uptake (Fig. 2A). The values for gut O₂ extraction were not different from those observed during the control period when ETₐ and ETₜ receptor blockade were induced during ET-1 infusion.

During the last segment of the experiment, the ETₐ and ETₜ receptors were blocked in both groups. The values for gut blood flow, resistance, O₂ uptake, and O₂ extraction ratio were not different between the two groups under these conditions.

The values for whole body hemodynamic and metabolic variables and blood gas data are listed in Table 2. There was no expectation that the local infusion of ET-1, BQ-123, or BQ-788 into the gut segment would have an effect on whole body metabolic or cardiovascular...
Table 2. Whole body vascular and metabolic responses and blood gas status during ET-1 infusion in canine small intestine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ET-1</th>
<th>ANT</th>
<th>ANT + ANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, ml·kg⁻¹·min⁻¹</td>
<td>119 ± 10</td>
<td>111 ± 8</td>
<td>96 ± 6*</td>
<td>89 ± 6*‡‡</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>121 ± 6</td>
<td>115 ± 5</td>
<td>125 ± 4†</td>
<td>127 ± 4</td>
</tr>
<tr>
<td>Total peripheral resistance, PRU</td>
<td>1.08 ± 0.08</td>
<td>1.07 ± 0.07</td>
<td>1.32 ± 0.09†</td>
<td>1.47 ± 0.08‡</td>
</tr>
<tr>
<td>Arterial PO₂, mmHg</td>
<td>5.8 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Arterial PCO₂, mmHg</td>
<td>62 ± 2</td>
<td>63 ± 2</td>
<td>61 ± 2</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.33 ± 0.01</td>
<td>7.33 ± 0.01</td>
<td>7.34 ± 0.01</td>
<td>7.33 ± 0.01</td>
</tr>
<tr>
<td>Gut venous PO₂, mmHg</td>
<td>31 ± 1</td>
<td>30 ± 2</td>
<td>28 ± 2</td>
<td>30 ± 2</td>
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</tbody>
</table>

Values are means ± SE. The ET-1 concentration was 0.118 ± 0.005 μg·kg⁻¹·min⁻¹. For group 1, antagonist (ANT) = BQ-123 and ANT + ANT = BQ-123 + BQ-788. For group 2, ANT = BQ-788 and ANT + ANT = BQ-788 + BQ-123. *Significant difference from control value. †Significant difference from ET-1. ‡Significant difference between ANT and ANT + ANT.

DISCUSSION

This is the first study to examine the metabolic consequence of ET-1-induced flow reduction in the small intestine in vivo. As others have reported, we also found that ET-1-induced vasoconstriction was mediated solely through ETA receptors and that ETB receptor activation buffered the magnitude of ETA receptor-mediated vasoconstriction. The new and important findings in our study were that O₂ uptake fell in proportion to the fall in blood flow during ET-1 administration and that little, if any, compensatory increase in gut O₂ extraction occurred to offset this flow limitation. The latter observation is in striking contrast to the response normally observed in this tissue during local or whole body stagnant hypoxia.

In group 1 as gut vascular resistance increased during ET-1 infusion, gut O₂ uptake and blood flow decreased 32 and 37%, respectively, during ET-1 administration. When gut blood flow returned to control levels during ETA receptor blockade, so did gut O₂ uptake. A similar pattern emerged in group 2; as gut blood flow decreased further (64%) with ETB receptor blockade, gut O₂ uptake also decreased (62%). Subsequent ETA receptor blockade resulted in small but significant increases in both blood flow and gut O₂ uptake, but both values remained significantly less than those observed in the control period. Gut O₂ uptake during infusion of ET-1 in both groups and during infusion of ET-1 and BQ-788 in group 2 appeared to be flow limited. It was surprising that little, if any, compensatory increase in gut O₂ extraction occurred to offset this flow limitation. The average values for gut O₂ extraction ratio ranged from a minimum of 0.34 during control to a maximum value of 0.41 during ET-1 infusion. Previous studies using the same in situ canine gut loop preparation have established that the gut relies heavily on compensatory O₂ extraction responses during periods of reduced O₂ supply (9) such that under conditions of low flow, gut O₂ extraction increased to values ranging from 0.60 to 0.70 (7, 29). Peak and/or critical values for O₂ extraction in this gut preparation during ischemia have been reported to reach 0.80 (24). It is not unreasonable to postulate that the failure of the gut to increase O₂ extraction during ET-1 infusion and ETA and ETB receptor blockade was the result of redistribution of gut blood flow away from exchange vessels. Another possibility is that ET-1 administration may have reduced gut O₂ demand through a direct action on cellular metabolism. The latter seems unlikely in light of in vitro studies that have demonstrated that ET-1 causes contraction of guinea pig ileal longitudinal smooth muscle via activation of ETB receptors located on the longitudinal smooth muscle cells (3, 13, 20, 35). This direct inotropic action of ET-1, if present in situ, would be expected to result in an increase rather than a decrease in gut metabolism and O₂ demand.

We have obtained preliminary data that may provide some insight into the mechanism responsible for the failure of gut O₂ extraction to increase in the face of reduced O₂ delivery following infusion of ET-1. Using intravital videomicroscopy, we observed mesenteric arterial (diameter of ~20 µm) microvascular responses during ET-1 infusion (0.1 µg·kg⁻¹·min⁻¹ iv) in anesthetized rats (n = 3). No changes in vessel diameter were observed; however, mean red blood cell velocity and blood flow were significantly reduced at 20 min of ET-1 infusion, and stasis occurred by 25 min of ET-1 infusion (Table 3). The mesenteric vessels employed in these experiments were similar in size to third-order arterioles in the submucosal layer. These preliminary findings suggest that ET-1 infusion may result in microvascular plugging independent of changes in vessel diameter, which would significantly reduce the number of capillaries receiving nutritive flow with a resultant decrease in O₂ extraction. Further experiments must be performed to confirm these initial findings.

Our vascular findings that infusion of ET-1 into the gut vasculature at concentrations of ~0.06 and 0.12 µg·kg⁻¹·min⁻¹ increased gut vascular resistance 32 and 72%, respectively, were in general agreement with values of 33% (32) and 100% (26) reported by others.
EFFECT OF ENDOTHELIN IN CANINE SMALL INTESTINE

Allcock et al. (1) was unable to demonstrate any effect of ETB receptor blockade with PD-142893. In contrast, Allcock et al. (1) for vascular resistance in the rat small intestine. These data indicate that the vasoconstrictor response of the small intestine to ET-1 is substantially modulated by the simultaneous activation of ETB receptors. The mechanism underlying the magnitude of this buffering capacity is not clear but may be due to the loss of release of dilator substances such as NO or a reduced clearance rate of ET-1 (25). The latter seems most probable because the dilator response may be of short duration (16). ETB receptors are the primary method by which ET-1 is cleared from the system (25), and blockade of these receptors may reduce the clearance rate and therefore enhance the constrictor action of ET-1.

Our findings have demonstrated that the modest increase in vascular resistance that occurred during ET-1 administration resulted in gut ischemia that caused a substantial limitation in gut O2 uptake. These findings may have direct clinical implications for those situations that promote the release of endogenous ET-1. Plasma ET-1 levels have been shown to double during chronic hypoxia (4, 10), increase 2- to 7-fold during surgeries including small bowel transplantation, abdominal aortic aneurysm resection, and coronary artery bypass grafting (11, 12, 30), increase 8-fold with chronic peritonitis (5), and increase 15-fold with septic shock (22). Furthermore, teVelthuis et al. (30) demonstrated that the increase in plasma ET-1 levels during coronary artery bypass surgery was associated with increased circulating endotoxin concentrations. It is possible that the prolonged duration of reduced gut oxygenation as a result of ET-1-induced gut ischemia may contribute to mucosal dysfunction in these conditions.

In conclusion, the findings of the current study demonstrate that ET-1-induced constriction in the canine small intestine is mediated solely by ETA receptors and second that this constrictor response is substantially modulated by ETB receptor activation. Finally, this study is the first to demonstrate that a flow-limited decrease in gut O2 uptake occurred with infusion of ET-1 in gut vasculature. An intriguing and novel finding was that, during the period of O2 limitation, O2 extraction ratio was only 50% of that normally associated with ischemia in this tissue.

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Table 3. Mesenteric arterial microvascular responses to ET-1 infusion in anesthetized rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, µm</td>
<td>15 ± 1</td>
<td>16 ± 2*</td>
<td>15 ± 3</td>
<td>14 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Mean red blood cell velocity, mm/s</td>
<td>2.5 ± 0.4</td>
<td>1.9 ± 0.5*</td>
<td>1.7 ± 0.3*</td>
<td>1.5 ± 0.3*</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>Blood flow, mm3/s</td>
<td>0.47 ± 0.08</td>
<td>0.38 ± 0.12</td>
<td>0.32 ± 0.12</td>
<td>0.25 ± 0.08*</td>
<td>0.11 ± 0.05*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>117 ± 12</td>
<td>141 ± 10*</td>
<td>144 ± 11*</td>
<td>140 ± 11*</td>
<td>133 ± 7*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3. *Significant difference from control at P < 0.05.
REFERENCES


