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).

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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 determined in the pump-perfused segment and in an autoperfused, non-treated gut segment in one group 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).

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 10⁻⁹ M caused severe vasoconstriction that increased perfusion pressure by 120 mmHg in an in vitro-perfused rat mesentery 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-(D-Trp-D-Asp-Pro-D-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-dimethylpiperidino-carbonyl-L-γ-methyl-Leu-o-1-(methoxy-carbonyl)-Trp-d-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 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 during both 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

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Table 1. Hemodynamic responses of canine small intestine to sequential concentrations of ET-1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Concentration 1</th>
<th>Concentration 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut blood flow, ml·kg⁻¹·min⁻¹</td>
<td>290 ± 22</td>
<td>228 ± 20*</td>
<td>182 ± 14†</td>
</tr>
<tr>
<td>Gut vascular resistance, PRU</td>
<td>0.43 ± 0.04</td>
<td>0.57 ± 0.05*</td>
<td>0.74 ± 0.07†</td>
</tr>
<tr>
<td>Gut perfusion pressure, mmHg</td>
<td>118 ± 5</td>
<td>121 ± 5*</td>
<td>124 ± 5†</td>
</tr>
</tbody>
</table>

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⁻¹. *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 0.41 ± 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 ET₁_A and ET₁_B receptor blockade were induced during ET-1 infusion.

During the last segment of the experiment, the ET₁_A and ET₁_B 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...
infusion in canine small intestine

responses and blood gas status during ET-1

Table 2. Whole body vascular and metabolic findings in our study were that O2 uptake fell in mediated vasoconstriction. The new and important nous PO2 reflected the O2 extraction responses of the gut.

experiment (13). Arterial PO2, PCO2, and pH remained decreased 32 and 37%, respectively, during ET-1 administration. When gut blood flow returned to control levels during ET-A 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 ET-A 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 ET-A and ET-B 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 ET-B 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 arteries 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

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>ET-1 (0.1 µg·kg⁻¹·min⁻¹)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Diameter, µm</td>
<td>15 ± 1</td>
<td>16 ± 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>
</tr>
<tr>
<td>Blood flow, mm³/s</td>
<td>0.47 ± 0.08</td>
<td>0.38 ± 0.12</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>117 ± 12</td>
<td>141 ± 10*</td>
</tr>
</tbody>
</table>

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

during infusion of ET-1 at similar concentrations in the perfused rat mesentery. Furthermore, whole body infusion of ET-1 (10 pmol·kg⁻¹·min⁻¹) in anesthetized rats increased small intestine vascular resistance fivefold (1). Finally, bolus intravenous administration of ET-1 of 0.1 µg/kg in anesthetized monkeys did not affect mesenteric blood flow, whereas higher concentrations of 0.3, 1.0 and 3.0 µg/kg resulted in decreases in mesenteric blood flow of 20, 40, and 60%, respectively (6).

Our data indicated that ET-1-induced vasoconstriction in the gut vasculature was mediated through ETA receptors. This conclusion was based on the findings that gut vascular resistance returned to levels that were not significantly different from control and gut blood flow also returned to control values when ETA receptor blockade was superimposed during ET-1 infusion. Furthermore, subsequent ETB receptor blockade did not alter gut blood flow or gut vascular resistance. If the constrictor actions of ET-1 had been partially mediated by ETB receptors, then further changes in both gut blood flow and vascular resistance would have been expected with ETB receptor blockade. Our results were consistent with those of Warner et al. (32), in which the maximal increase in perfusion pressure following administration of ET-1 (10⁻⁹ M) in the in vitro-perfused rat mesentery bed was reduced ~70% by ETA receptor blockade with BQ-123, whereas no further decrease in perfusion pressure was observed with ETA and ETB blockade with PD-142893. In contrast, Allcock et al. (1) were unable to demonstrate any effect of ETA receptor blockade with BQ-123 or ETA and ETB receptor blockade with PD-145065 on ET-1-induced vasoconstriction in the small intestine of anesthetized rats. However, in the latter study, the rats were pretreated with hexamethonium and ET-1 (10 pmol·kg⁻¹·min⁻¹) was administered systemically, two factors that may have accounted for the differences between the studies. Our findings and those of Warner et al. (32) clearly demonstrated that ETB receptor blockade caused no further attenuation of ET-1-mediated constriction in the mesenteric vasculature and suggested that ETA receptor activation was the primary mechanism of ET-1-induced vasoconstriction in the mesenteric circulation.

The current study also established that there was a substantial contribution of ETB receptor activation to vascular tone in canine small intestine during ET-1 administration. In group 2, gut vascular resistance, which increased significantly during ET-1 administration, increased an additional 62% with ETB receptor blockade (BQ-788), an effect similar to that reported by 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 endothelin 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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