Role of endothelin-1 in regulation of the postnatal intestinal circulation

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Nankervis, Craig A., and Philip T. Nowicki. Role of endothelin-1 in regulation of the postnatal intestinal circulation. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G367–G375, 2000.—Newborn intestine is uniquely prone to vasoconstriction in response to a wide variety of perturbations. To test the hypothesis that endothelin (ET)-1 is an important factor in this process, we determined the effects of exogenous ET-1 administration and blockade of endogenous ET-1 in vivo and in vitro in 3- and 35-day-old swine. Intramesenteric artery administration of exogenous ET-1 to vasculally isolated in vivo gut loops (10−9 M/kg bolus) caused vasoconstriction and reduced gut O2 uptake similarly in these age groups. Selective blockade of ETA or ETB receptors with BQ-610 or BQ-788, respectively, in vasculally isolated in vivo gut loops had no effect on gut vascular resistance or O2 uptake in either age group; within in vitro gut loops, BQ-610 significantly increased vasoconstriction when perfusion pressure was reduced below baseline, but only in 3-day-old animals; i.e., it impaired the autoregulatory response to perfusion pressure reduction. Exogenous ET-1 significantly decreased capillary perfusion within in vitro gut loops, as evidenced by a decrease in capillary filtration coefficient, but only in 3-day-old animals; furthermore, blockade of endogenous ET-1 activity with BQ-610 significantly enhanced capillary filtration coefficient in 3-day-old animals and increased O2 extraction ratio. ET-1 did not depress intestinal metabolic rate, as evidenced by its effect on the O2 uptake-blood flow relationship; it did compromise tissue oxygenation because of its effects on intestinal O2 transport. ET-1 concentration in mesenteric venous effluent exceeded arterial concentration, but only in 3-day-old intestine, suggesting production of ET-1 by newborn intestine. We conclude that ET-1 exerts an age-dependent effect on intestinal hemodynamics in postnatal intestine, having a greater impact in 3- than in 35-day-old intestine.

ENDOTHELIN (ET)-1, first isolated by Yanagisawa et al. (29) from the conditioned medium of cultured porcine aortic endothelial cells, is an exceptionally potent vasoconstrictor peptide produced by cardiac myocytes and vascular endothelial cells. Three isoforms of ET have been described: ET-1, ET-2, and ET-3. Each isoform is transcribed from a unique gene, although the ET-1 isoform is responsible for the overwhelming portion of the established biological activity of the peptide family. The vasoconstrictive effect of ET-1 occurs when it binds to ETA receptors on vascular smooth muscle (2). This receptor is a member of the seven-transmembrane-spanning, G protein-coupled family of receptors, the activation of which increases Ca2+ concentration in the intestinal circulation via phospholipase C-mediated production of inositol trisphosphate and diacylglycerol (24). A second class of receptors, designated ETB, are located on the endothelium and vascular smooth muscle (1, 2). Activation of the endothelial ETB receptor causes vasodilation mediated by nitric oxide (NO) and can only be appreciated when ETA receptors are blocked or when the system under study is precontracted. Activation of the vascular smooth muscle ETB receptor causes vasoconstriction. Although an important role for ET-1 has been established in a wide variety of cardiovascular diseases, including hypertension (21), myocardial infarction (25), renal failure (10), and pulmonary hypertension (23), only a few investigations have focused on the role of ET-1 in regulation of the intestinal circulation. Warner and colleagues (26) observed constriction of a buffer-perfused in vitro rat mesenteric arcade preparation with 1–300 pM ET-1, although in a methoxamine-precontracted preparation, 0.1–3 pM ET-1 caused vasodilation. Douglas and Hiley (3) noted constriction in response to 100 pM ET-1; moreover, they demonstrated a significant increase in the contractile effect of ET-1 in preparations devoid of a functional endothelium. Finally, Rakugi and colleagues (19) demonstrated activation of the local endothelial renin-angiotensin system in response to ET-1 infusion, again in a buffer-perfused rat mesentery, as evidenced by an increased concentration of ANG I and II in the mesenteric effluent after ET-1 infusion. This important observation suggests that ET-1 can affect local vascular regulation by modifying other vascular effector systems, as well as by its direct effect. To the best of our knowledge, the role of ET-1 in regulation of the perinatal intestinal circulation has not been studied.

The goal of this study was to determine whether ET-1 participates in resistance and exchange vessel regulation in postnatal intestine. The working hypothesis of the study was that the effect of ET-1 on intestinal hemodynamics and oxygenation is age dependent, being greater in newborn intestine. This hypothesis was based on our prior work, which consistently demonstrated newborn intestine to be prone to intense vasoconstriction over a wide range of experimental circum-

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stances (15–18); thus we hypothesized that ET-1 might contribute to these vasoconstrictive events. Two types of experiments were carried out: those in which exogenous ET-1 was administered to the intestinal circulation and those in which blockade of the endothelin ET$_{A}$ and ET$_{B}$ receptors was carried out using the highly selective antagonists BQ-610 and BQ-788, respectively (8). Studies were carried out under in vivo and in vitro conditions in 3- and 35-day-old swine, the latter to facilitate observation of postnatal developmental changes. Finally, the concentration of ET-1 in arterial and in systemic and mesenteric venous blood was determined.

METHODS

Care and Handling of Experimental Animals

Studies were conducted on 3-day-old (newborn group, age range 2–5 days) and 35-day-old (weanling group, age range 30–40 days) swine obtained from a local farm on the day before study. At the end of postnatal month 1, swine have been removed from the sow, weaned from milk, and placed on a grain-based diet. Although not an adult, the 35-day-old weanling piglet has undergone significant postnatal matura-

tion, particularly from the standpoint of intestinal function (22). The baseline levels of blood flow and tissue O$_2$ uptake are also substantially less in weanling than in newborn animals (16). Animals were kept in a large run dedicated to swine and fed an age-appropriate diet until 12 h before surgery, when food, but not water, was withheld. Anesthesia was induced with xylazine (5 mg/kg) and telazol (7.5 mg/kg) and maintained with pentobarbital sodium (5 mg kg$^{-1}$·h$^{-1}$). Animal care was provided in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publ. No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, Bethesda, MD 20832]. All protocols were approved by the Institutional Animal Care and Use Committee of the Children’s Research Institute.

In Vivo Hemodynamic Experiments

Preparation. Animals were anesthetized and ventilated to maintain normal blood gas tensions. A femoral artery-vein pair was cannulated. The arterial catheter was connected to a pressure transducer to measure systemic pressure. The venous catheter served as the inflow site for crystalloid (5% dextrose in 0.9% saline at 15 ml·kg$^{-1}$·h$^{-1}$), additional anesthetic, and return of the venous effluent from the in vivo gut loop. A segment of distal small bowel ~25 cm long was vascu-

larly isolated from the remainder of the gut and mesentery. Care was taken to avoid trauma to periarterial nerves and lymphatics. The mesenteric vein draining the segment was cannulated, and the catheter was directed to a beaker primed with ~50 ml of swine blood obtained from a prior study. Blood was pumped back to the animal at a rate equal to venous outflow, thus maintaining euvo1emia within the animal. The height of the venous catheter was set so that venous pressure was ~0 mmHg. A fine needle (27 gauge) attached to a can-

nula was inserted into the mesenteric artery leading to the isolated gut loop and fixed in place with glue. A con-

tinuous infusion of 0.9% saline was administered through this needle after placement to ensure patency (0.1 ml/min). A temperature probe inserted into the lumen of the isolated gut loop was connected to a thermostat, which regulated output from heating elements, while the gut loop and entire abdo-

minal incision were covered with plastic wrap to minimize evaporative heat and water loss. Tissue temperature was kept at 38°C. An electromagnetic flowmeter and pressure transducer set in line with the mesenteric vein catheter allowed continuous measurement of flow and venous pres-

sure, respectively. Paired arteriovenous blood samples (0.5 ml) were taken at various time points for measurement of O$_2$ content by means of a Lel-O$_2$-Con.

Protocol 1. The goal of protocol 1 was to determine the effects of a single dose of exogenous ET-1 on intestinal hemodynamics and oxygenation under in vivo conditions. Baseline measurements were obtained once steady-state conditions, defined as fluctuation of systemic pressure and intestinal blood flow less than ±5% for 10 min, were achieved. Thereafter, 10$^{-9}$ M/kg ET-1 was administered as a single bolus dose into the mesenteric artery catheter. This dose was determined to be the ET-1 giving half-maximal response in pilot studies carried out in mesenteric artery rings suspended for isometric tension recording in standard myographs. Serial measurements were taken over the ensuing 1 h. In separate groups of 3- and 35-day-old animals, protocol 1 was run after pretreatment with the selective ET$_{B}$ receptor antagonist BQ-788. This blocking agent was deliv-

ered into the mesenteric artery (5 × 10$^{-9}$ M/kg or 0.5 ml) as a single bolus dose 15 min before the administration of ET-1. The blocking dose for BQ-788 was determined in pilot studies, in which mesenteric rings were suspended for isometric tension recording in standard myographs.

Protocol 2. The goal of protocol 2 was to compare the potency of the contractile effect of ET-1 with another constrictor agent, norepinephrine. To this end, an equimolar dose of norepinephrine (10$^{-9}$ M/kg), instead of ET-1, was adminis-

tered as a single bolus dose into the mesenteric artery catheter.

Protocol 3. The goal of protocol 3 was to determine the effects of selective ET$_{A}$ and ET$_{B}$ receptor blockade on intestinal hemodynamics and oxygenation under in vivo conditions. Baseline measurements were obtained; thereafter, BQ-610 (5 × 10$^{-9}$ M/kg) or BQ-788 (5 × 10$^{-9}$ M/kg) was administered as a single bolus dose into the mesenteric artery. Serial measurements were obtained over the ensuing 2 h. The blocking dose for BQ-610 was determined in pilot studies in which mesenteric rings were suspended for isometric tension recording in standard myographs.

In Vitro Hemodynamic Experiments

Preparation. This preparation required two animals for each experiment: one to provide the in vitro gut loop and the other to provide blood for the extracorporeal perfusion appa-
ratus. Blood donor animals were ~90 days old and had an estimated blood volume of 4 liters. These animals were anesthetized and ventilated, and catheters were placed into both common carotid arteries and one jugular vein. One arterial catheter was used to withdraw blood, and the other was used to monitor systemic pressure; the venous catheter provided a portal to administer 0.9% saline during phle-

botomy. After heparinization (500 U/kg), ~2 liters of blood were removed from the donor while an equal volume of 0.9% saline was returned to maintain systemic pressure. These steps were taken to minimize the activation of a humoral pressor response to acute blood loss in the donor (e.g., angiotensin, norepinephrine, vasopressin), inasmuch as these agents could present confounding variables during in vitro perfusion. The donor subject was euthanized at the comple-
tion of the phlebotomy. Blood was filtered twice (40-µm mesh), and its hematocrit was adjusted to 35% with swine plasma, if necessary. The blood was placed into a collecting flask, which was then placed within a water bath (38°C) situated over a...
stir plate. Blood in the collecting flask was circulated (100 ml/min) through a 0.6-m² membrane oxygenator (Scimed, Minneapolis, MN) gassed with 95% air-5% CO₂ to maintain normal blood gas tensions. Once the extracorporeal perfusion apparatus was primed, the experimental animal that was to provide the gut loop was prepared. A segment of distal jejunum-proximal ileum ~25 cm long was isolated as previously described. The single artery-vein pair serving the segment was cannulated, extracorporeal perfusion of the segment was initiated, and the segment was removed from the study animal. The gut lumen was cleansed by repetitive, gentle instillation of warm saline followed by air. The in vitro gut loop was covered with saline-soaked gauze, wrapped in plastic, and placed on a wire mesh pan. A temperature probe was inserted into the study animal. The gut lumen was covered with saline-soaked gauze, wrapped in plastic, and placed on a wire mesh pan. A temperature probe placed into the lumen of the segment was connected to a servo-controlled heating element set to keep temperature at 38°C.

Extracorporeal perfusion apparatus and measurement techniques. A small volume of blood was continuously withdrawn from the collecting flask and directed to a sealed arterial reservoir, which was also positioned within a water bath and over a stir plate. Blood flow from the arterial reservoir to the gut loop could be achieved in two ways. The arterial reservoir could be pressurized with 95% air-5% CO₂ by means of a low-range air pressure regulator. This action provided tight control over perfusion pressure (~1 mmHg) and established pulseless, controlled-pressure perfusion. Alternatively, the arterial circuit could be drawn through a peristaltic pump that established controlled-flow perfusion. Venous pressure was adjusted by altering the position of the venous drainage catheter with respect to the gut loop. Blood collected in the venous reservoir could be pumped back into the collecting flask, if necessary, to sustain the duration of the perfusion. The use of multiple pump heads creates the risk of mechanical hemolysis; accordingly, K⁺ concentration within the collecting flask was measured hourly and never rose >4.0 meq/dl. Use of heterologous blood is feasible in swine, inasmuch as this species does not express strong red blood cell antigens. Arterial and venous circuit pressures were measured by standard pressure transducers. Flow through the gut loop was measured by an electromagnetic flowmeter placed in the venous circuit.

Protocol 1. The goal of protocol 1 was to determine the effect of exogenous ET-1 or BQ-610 on the relationship between blood flow and O₂ uptake. To this end, in vitro gut loops were perfused under controlled-flow conditions. The gut loop weight was adjusted so that at a baseline pump rate of 10 ml/min the adjusted tissue flow rates were similar to those under in vivo conditions, i.e., 90 and 50 ml·min⁻¹·100 g⁻¹ for gut loops from 3- and 35-day-old animals, respectively. Flow rate was then raised or lowered by altering the pump rate, in increments or decrements of 20 ml·min⁻¹·100 g⁻¹, until measurements were taken at three different flow rates above or below baseline. Each new flow rate was maintained until initial steady-state conditions were attained, which occurred within 3–5 min after adjustment. The flow rate was then restored to baseline, and a continuous infusion of ET-1 (10⁻⁶ M/min) or BQ-610 (5 × 10⁻⁶ M/min) was begun into the arterial circuit to provide arterial perfusate concentrations of 10⁻⁹ or 5 × 10⁻⁹ M, respectively. Once new steady-state conditions were attained during drug infusion, the flow rate was varied in the manner previously described. The rate of drug infusion was adjusted with each change in flow rate to keep the concentration of the peptide within the arterial perfusate constant.

Protocol 2. The goal of protocol 2 was to determine the effect of exogenous ET-1 on capillary perfusion. To this end, in vitro gut loops were perfused under controlled pressure conditions, and the perfusion pressures were set at age-appropriate levels, i.e., 65 and 80 mmHg for gut loops from 3- and 35-day-old animals, respectively. Capillary perfusion was estimated by measuring the capillary filtration coefficient (Kᶜ) by the flow equilibration technique of Granger and colleagues (7). The gut loop was placed on a Grass FT03 force transducer. When the tissue was isogravimetric, venous pressure was rapidly elevated from 0 to 10 mmHg. This action caused a initial reduction in venous outflow from the gut loop, but then venous flow rate attained steady state at a new, lower level. The point at which this new steady state occurs is flow equilibration and represents the end of the blood volume shift within the tissue consequent to acute venous hypertension; thus the rate of tissue weight increase from this point onward represents capillary filtration and is used to calculate Kᶜ. Estimation of Kᶜ by this method requires an accurate assessment of capillary pressure. This measurement was made using the stop-flow technique of Granger et al. (6), in which venous flow is rapidly occluded. The subsequent change in venous pressure has two phases: an early rapid increase followed by a slower rise. The inflection point between these slopes is capillary pressure. Hemodynamic measurements were obtained before and during infusion of ET-1 into the arterial limb to achieve a drug concentration of 5 × 10⁻⁹ M within the arterial perfusate.

Protocol 3. The goal of protocol 3 was to determine the effects of ETₐ and ET₉ receptor blockade on the relationship between pressure and flow in postnatal intestine. The study was conducted using in vitro gut loops perfused under controlled-pressure conditions. Arterial pressure was initially set at age-appropriate levels, as described above; venous pressure was set at 0 mmHg. Arterial pressure was then decreased to 35 or 50 mmHg for gut loops from 3- or 35-day-old animals, respectively. When hemodynamic conditions reached their initial steady-state level after pressure reduction, the arterial pressure was increased in successive 10-mmHg increments. Each new pressure was held until the initial steady state was attained, 3–5 min after adjustment. Venous pressure was restored to 0 mmHg after each arterial pressure change. On completion of the pressure ramp, arterial pressure was restored to the age-appropriate baseline and BQ-610 or BQ-788 was added to the arterial reservoir to a final concentration of 5 × 10⁻⁹ M to duplicate the drug concentrations used in the controlled-flow protocol. These agents had no effect on vascular resistance and, thus, flow rate at baseline arterial pressures. Fifteen minutes after drug addition, arterial pressure was reduced and the pressure ramp was carried out as previously described.

Protocol 4. The goal of protocol 4 was to determine the effects of ETₐ and ET₉ receptor blockade on capillary perfusion. The work was carried out under controlled-pressure perfusion. Arterial pressure was set at an age-appropriate level, as previously described; venous pressure was set at 0 mmHg. After baseline measurements were made, including Kᶜ infusions of BQ-610 or BQ-788 (5 × 10⁻⁶ M) were begun into the arterial circuit; in each instance, the drug infusion rate was adjusted to provide a concentration of 5 × 10⁻⁹ M within the arterial perfusate. Repeat measurements were obtained after new steady-state conditions were achieved after blockade.

Measurement of ET-1 in Arterial, Mesenteric Venous, and Systemic Venous Blood

ET-1 was measured by RIA on blood samples obtained from each of the animals studied under in vivo conditions. Whole blood (2 ml) was drawn simultaneously from the femoral
artery, femoral vein, and mesenteric vein catheters into chilled syringes and transferred into iced polypropylene tubes containing 2 mg of EDTA and 100 kallikrein-inactivating units of aprotinin. Tubes were centrifuged at 1,600 g for 15 min at 4°C, and the plasma was decanted. An equal volume of 1% trifluoroacetic acid (TFA) was added to the plasma, and the tube was centrifuged again at 10,000 g for 20 min at 4°C. Sep columns filled with 200 mg of C18 were washed with 1 ml of 60% acetonitrile in 1% TFA, then twice with 3 ml of 1% TFA. Acidified plasma was loaded onto the column and washed with 3 ml of 60% acetonitrile in 1% TFA. The elutant was evaporated to dryness in a centrifugal concentrator. The resulting pellet was resuspended in RIA buffer (0.5 M sodium phosphate with 0.1% BSA, fraction V, pH 7.50). RIA was carried out using a kit purchased from Peninsula Laborato-

Fig. 2. Effects of a single bolus dose of norepinephrine ($10^{-9}$ M/kg) on intestinal hemodynamics and oxygenation in postnatal swine. Norepinephrine was rapidly administered into mesenteric artery of vascularly isolated, but innervated and autoperfused, in situ gut loops (volume 0.5 ml). Peak response is maximal change after agonist; steady state is value after agonist infusion when measured variables first remained constant (i.e., ±5%). ○, 3-day-old animals; ●, 35-day-old animals. Values are means ± SD; n = 5 for all points. a P < 0.01 vs. baseline within each group.

Fig. 1. Effects of a single bolus dose of endothelin-1 (ET-1, $10^{-9}$ M/kg) on intestinal hemodynamics and oxygenation within in vivo postnatal swine. ET-1 was rapidly administered into mesenteric artery of vascularly isolated, but innervated and autoperfused, gut loops (volume 0.5 ml) in control animals and animals pretreated with selective ETB receptor antagonist BQ-788 ($5 \times 10^{-9}$ M/kg, also given as a bolus dose into mesenteric artery). Peak response is maximal change after agonist administration in each animal, which occurred ~2 min after drug was given; steady state is value after agonist infusion when measured variables first remained constant (i.e., ±5%). Open symbols, 3-day-old animals; solid symbols, 35-day-old animals; circles, control data; triangles, animals pretreated with BQ-788. a-v, Arteriovenous difference. Values are means ± SD; n = 5 for all points. a P < 0.01 vs. baseline within each group; b P < 0.01 vs. 3-day-old group.
Fig. 3. Relationship between intestinal blood flow and O₂ uptake within in vitro gut loops from 35-day-old swine. Control data (n = 8); ○, ET-1 (n = 4). Gut loops were perfused under controlled-flow conditions, and flow rate was directly varied above and below established in vivo flow rate of 50 ml·min⁻¹·100 g⁻¹ by altering pump speed. Each flow rate was maintained until initial steady-state conditions were attained, which occurred 3–5 min after flow rate adjustment. After collection of control data, infusion of ET-1 (10⁻⁹ M/min) was begun during perfusion at baseline flow rate; drug infusion rate was adjusted proportionately with blood flow rate to keep perfusate drug concentration steady as flow rates were adjusted again. Values are means ± SD. *P < 0.01 vs. control.

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Table 1. O₂ extraction data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Peak Response</th>
<th>Steady State</th>
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<tr>
<td>3-Day-old group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25 ± 2</td>
<td>17 ± 1*</td>
<td>20 ± 1*</td>
</tr>
<tr>
<td>BQ-788</td>
<td>27 ± 2</td>
<td>19 ± 2*</td>
<td>21 ± 1*</td>
</tr>
<tr>
<td>35-Day-old group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32 ± 2†</td>
<td>22 ± 2*</td>
<td>25 ± 1*</td>
</tr>
<tr>
<td>BQ-788</td>
<td>32 ± 2†</td>
<td>22 ± 3*</td>
<td>23 ± 2*</td>
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Values are means ± SD; n = 5. O₂ extraction was calculated as ratio of arteriovenous O₂ difference to arterial O₂ content and expressed as a percentage. Blood samples were obtained under baseline conditions, after peak flow response to endothelin-1 (ET-1) was attained, and after new steady-state flow conditions were attained, i.e., fluctuation in flow ±5%. ET-1 was rapidly administered as a single bolus dose (10⁻⁹ M/kg) into mesenteric artery perfusing an innervated, autoperfused in vivo gut loop in control animals and animals pretreated with BQ-788 (5 × 10⁻⁹ M/kg, via mesenteric artery). *P < 0.01 vs. baseline; †P < 0.01 vs. 3-day-old group.

Statistics

Data were analyzed by ANOVA for repetitive measures. With one exception, data were analyzed without transformation. The exception was resistance data from the in vivo ET-1 infusion experiments, which were expressed as a function of baseline to more readily demonstrate differences between age groups. In most instances, a three-way ANOVA was carried out initially. This analysis utilized age (3 days vs. 35 days), condition (no drug vs. drug), and time (various flow rates or pressures or experimental sequence) as main events. If the F statistic was significant, i.e., <0.01, then post hoc Tukey B tests were carried out to determine the sites of significance within each data set.

RESULTS

Direct infusion of ET-1 into the mesenteric artery upstream from in vivo gut loops caused a slowly developing vasoconstriction that peaked ~2 min after drug administration in both age groups. The constrictor effect waned thereafter, although vascular resistance remained significantly greater than baseline at the conclusion of the protocol, 1 h after ET-1 infusion (Fig. 1). The relative magnitude of resistance increase was similar in both age groups; however, blockade of ETB receptors with BQ-788 before ET-1 administration significantly increased the extent of intestinal vasoconstriction in 3-day-old, but not in 35-day-old, animals. An equimolar dose of norepinephrine caused an increase in intestinal vascular resistance that was similar in magnitude to that after ET-1 in both age groups, although the contraction developed much faster and was not sustained (Fig. 2). Exogenously administered ET-1 significantly affected intestinal oxygenation in both groups. O₂ delivery, calculated as the product of flow rate and arterial O₂ content, decreased to the same extent as flow was reduced (data not shown). Also, O₂ extraction by the intestine decreased after ET-1 administration, as evidenced by the reduction in the arteriovenous O₂ content difference across the intestine [(a-vO₂)] and also by a fall in the O₂ extraction ratio (Table 1). Consequent to these changes, the rate of O₂ uptake by the intestine was compromised by ET-1 administration.

The basis for the effect of exogenous ET-1 on intestinal O₂ uptake was pursued in two additional experi-
ments carried out using in vitro gut loops. First, the relationship between blood flow and $O_2$ uptake was determined before and during infusion of ET-1. Under control conditions, intestine from both age groups demonstrated the characteristic biphasic flow-uptake relationship; i.e., $O_2$ uptake was independent of flow when the rate of perfusion was at or above baseline, but uptake decreased when flow was reduced below baseline. In gut loops from newborn animals, infusion of ET-1 eliminated this biphasic relationship, as $O_2$ uptake became dependent on flow across the entire range of flow rates tested (Fig. 3). The level of $O_2$ uptake was significantly depressed during ET-1 infusion; however, it was possible to restore $O_2$ uptake to baseline by increasing the flow rate to a level substantially above baseline. This effect was significantly less pronounced in weanling animals (Fig. 4). In the second experiment the effects of exogenous ET-1 on capillary perfusion were determined by measuring the $K_{f,c}$. ET-1 significantly decreased $K_{f,c}$ and $(a-vO_2)$, and increased mesenteric venous $PO_2$ in both age groups, although the relative magnitudes of change were consistently greater in newborn animals (Fig. 5).

Blockade of $ET_A$ or $ET_B$ receptors under in vivo conditions did not significantly affect the intestinal resistance vasculature in either age group when hemodynamic conditions were normal (data not shown). To further evaluate the effect of ET-1 on intestinal resistance vessel function, blockade studies were repeated under in vitro conditions that allowed independent adjustment of perfusion pressure above and below baseline. BQ-610 and BQ-788 altered the pressure-flow relationship, but only at pressures below baseline and only in 3-day-old animals (Fig. 6). Blockade of $ET_A$ receptors did not affect the relationship between pressure and flow in 35-day-old intestine (Fig. 7). Blockade of $ET_A$ receptors significantly increased the perfused capillary density in in vitro newborn gut loops perfused under controlled-pressure conditions, indicating that endogenous ET-1 exerts a tonic, constrictor effect on the intestinal exchange vasculature (Table 2). Simultaneously, a modest, albeit consistently observed, increase in $(a-vO_2)$ occurred, accompanied by a reduction in mesenteric venous $PO_2$. Changes in these $O_2$ transport variables did not attain statistical significance; however, when the data were expressed as an $O_2$ extraction ratio, a significant increase in $O_2$ extraction was noted within in vitro newborn gut loops after $ET_A$ receptor blockade. Blockade of $ET_B$ receptors had

![Fig. 5. Effects of a single bolus dose of ET-1 ($10^{-9}$M) on hemodynamics and oxygenation within in vitro gut loops from postnatal swine. ET-1 was rapidly administered into arterial limb of an extracorporeal perfusion circuit (volume 0.5 ml). Venous blood gas and $O_2$ content and capillary filtration coefficient ($K_{f,c}$) were measured when peak contractile response to ET-1 was noted. $P_{VO_2}$, venous $PO_2$; $a$, 3-day-old animals; $b$, 35-day-old animals. Values are means $\pm$ SD; $n = 5$. $aP < 0.01$ vs. baseline within each group; $bP < 0.01$ vs. 3-day-old group.](http://ajpgi.physiology.org/Downloaded from 10.220.33.4 on September 27, 2017)
no effect on $K_{i,C}$ ($\alpha$-vO$_2$), or mesenteric venous PO$_2$ in either age group.

The concentration of ET-1 in arterial and in mesenteric venous blood was significantly greater in newborn than in weanling animals (Table 3). The concentration of ET-1 was significantly greater in the mesenteric venous effluent from in vivo gut loops in 3-day-old intestine than in the arterial or systemic venous concentration. This observation was consistently noted in all animals in this age group but was not noted in any animal from the 35-day-old group.

**DISCUSSION**

Data obtained from these experiments generally supported our working hypothesis. Blockade of $ET_A$ or $ET_B$ receptors did not alter resistance vessel tone in either age group, suggesting that this peptide does not participate in setting basal vascular tone when circulatory conditions are normal. However, ET-1 contributed to vasoconstriction in 3-day-old intestine when perfusion pressure was reduced below baseline; thus endogenous ET-1 participates in resistance vessel regulation, albeit to a limited extent and in an age-specific manner. Endogenous ET-1 clearly participates in regulation of intestinal exchange vessel regulation, and this effect was significantly greater in newborn than in weanling intestine, as evidenced by the increase in $K_{i,C}$ that occurred after blockade of $ET_A$ receptors with BQ-610 in younger animals and also by the greater effect of exogenous ET-1 on the perfused capillary density, O$_2$ extraction, and mesenteric venous PO$_2$ that occurred in this group. The circulating concentration of ET-1 is greater in younger than in older animals, and there is an efflux of the peptide into the mesenteric venous effluent, but only in younger animals. Each of these observations will be discussed in turn.

The effect of exogenous ET-1 on intestinal vascular resistance was similar in both age groups and was also similar to the effect of an equimolar concentration of norepinephrine. The latter observation was surprising, inasmuch as previous reports describe the relative potency of ET-1 as significantly greater than that of other constrictor agents (29). Most of that work was carried out in adult animals, however, making direct comparison to the present data a tenuous enterprise. An important caveat should be mentioned in interpretation of these data: specifically, observations were made after a single bolus dose of the constrictor agents, not during a continuous infusion. Data presented in Figs. 1 and 2 represent the peak contractile response to ET-1 and norepinephrine within each subject. The increased contractile response to ET-1 after selective blockade of $ET_B$ receptors in 3-day-old, but not 35-day-old, animals suggests that $ET_B$ receptors are present within the resistance vasculature of younger animals. Ligand binding to $ET_B$ receptors on endothelial cells can lead to generation of NO by stimulation of the endothelial isoform of NO synthase (14, 27). This action provides a dilator force that offsets, to some extent, the contractile force generated by ligand binding to vascular smooth muscle $ET_A$ receptors.

![Graph](image_url)
Although endogenous ET-1 does not participate in setting basal intestinal vascular resistance in either age group, ETA receptor blockade enhanced pressure-flow autoregulation within gut loops from younger animals when perfusion pressure was reduced below baseline. Autoregulation does not occur in the newborn intestinal circulation, even when hemodynamic conditions are specifically manipulated to optimize observation of autoregulatory capacity (16, 18). The present data indicate that locally produced ET-1 contributes to the vasoconstriction noted when perfusion pressure is reduced below baseline in 3-day-old intestine, as evidenced by the enhancement of autoregulatory capacity after ETA receptor blockade with BQ-610. How might this action occur? Significant reduction of flow might diminish washout of ET-1, causing a relative increase in the peptide’s concentration within the microvasculature. Alternatively, the binding affinity of the ETA or ETB receptors might change when perfusion pressure and, thus, flow rate decrease, possibly consequent to interactions with other vasoactive effector systems. For example, the binding affinity of the bradykinin BK2 receptor, another member of the seven-transmembrane-spanning family of receptors, decreases in the presence of NO, because NO interferes with receptor-G protein coupling (13). Constitutive production of NO by the newborn intestinal vascular endothelium is highly contingent on flow rate (20). We speculate that flow reduction could reduce local NO production and thus enhance the binding affinity of the ETA receptors. This speculation is supported by our observation that sustained exposure to low-flow conditions decreases the dose giving half-maximal response for several constrictor agents, including ET-1 (15). Additional studies are necessary to clarify the interactions between ET-1 and NO, particularly as regards their effects on autoregulation.

Endogenous ET-1 exerts a tonic constrictor effect on intestinal exchange vessels, or capillaries, in newborn, but not weanling, intestine. The primary evidence of this effect was the increase in $K_{fc}$ after blockade of ETA receptors with BQ-610 within in vitro gut loops from 3-day-old animals. The rise in $K_{fc}$ after BQ-610 administration was most likely a direct effect of the antagonist on capillary perfusion, rather than an indirect effect mediated by a metabolic feedback signal secondary to a stimulatory effect of the agent on intestinal metabolic rate. Support for this contention is provided by noting the effect of BQ-610 on the blood flow-$O_2$ uptake curve. ETA receptor blockade shifted the curve leftward without altering the maximal level of $O_2$ uptake; these effects are consistent with an agent that increases capillary perfusion without having a direct effect on tissue metabolic rate (5, 11, 12). ET-1 shifted the curve downward and caused $O_2$ uptake to become dependent on flow over the entire range of flow rates tested. These effects are consistent with a constrictor agent that decreases capillary perfusion without directly affecting tissue metabolic rate (5, 11, 12). Also, exogenous ET-1 significantly decreased $K_{fc}$, an established marker of capillary perfusion. These effects were of substantially greater magnitude in newborn than in weanling intestine.

An unexpected finding was the efflux of ET-1 into the mesenteric vein in the newborn group. It is most likely that the source of ET-1 production within the newborn intestine is the vascular endothelium, although the present data are insufficient to exclude other sources. Why would newborn intestine produce ET-1 to this extent? As just discussed, endogenously produced ET-1 participates in exchange vessel regulation in newborn intestine, thus local production might be anticipated to facilitate this vascular regulatory role. Another possibility is that the peptide is being expressed to facilitate angiogenesis and vascular remodeling, processes in which ET-1 plays an important stimulatory role (4, 9). The rate of intestinal growth during the immediate postnatal period in swine is tremendous, inasmuch as the mass of intestinal tissue increases ~10-fold be-

Table 2. Effects of ETA or ETB receptor blockade on capillary perfusion within in vitro gut loops from postnatal swine

<table>
<thead>
<tr>
<th></th>
<th>3-Day-Old Group</th>
<th>35-Day-Old Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BQ-610</td>
</tr>
<tr>
<td>$P_c$, mmHg</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>$K_{fc}$, cm·min⁻¹·mmHg·100g</td>
<td>0.355 ± 0.031</td>
<td>0.421 ± 0.028*</td>
</tr>
<tr>
<td>a-$vO_2$, ml/dl</td>
<td>3.1 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>$O_2$ extraction, %</td>
<td>26 ± 2</td>
<td>31 ± 1*</td>
</tr>
<tr>
<td>$PvO_2$, mmHg</td>
<td>52 ± 2</td>
<td>49 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5. Measurements were made within in vitro gut loops perfused under controlled-pressure conditions. Arterial pressure was set at an age-appropriate level, and venous pressure was set at 0 mmHg. Measurements were made at baseline and after receptor blockade; each gut loop received only 1 blocking agent. $P_c$, capillary pressure; $K_{fc}$, capillary filtration coefficient; a-$vO_2$, arteriovenous $O_2$ difference; $PvO_2$, mesenteric venous $O_2$.

Table 3. Concentrations of ET-1 in arterial, mesenteric venous, and systemic venous plasma

<table>
<thead>
<tr>
<th></th>
<th>ET-1 Concentration, pg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-Day-old group</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>109 ± 9</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>80 ± 7</td>
</tr>
<tr>
<td>Mesenteric vein</td>
<td>156 ± 6†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 20. Blood samples were drawn simultaneously from femoral artery and vein catheters and mesenteric vein catheter. *P < 0.01 vs. 3-day-old group; †P < 0.01 vs. femoral artery and femoral vein.
tween postnatal days 1 and 30, whereas the total body mass increases 3-fold (28). It might be anticipated that factors that stimulate vessel growth are present in abundance in rapidly growing tissue.

We conclude that ET-1 exerts an age-dependent effect within postnatal intestine. Endogenously produced ET-1 participates in regulation of resistance vessel tone only within postnatal intestine, although this effect is only noted at perfusion pressures below baseline; in addition, this peptide exerts a tonic constrictor effect on capillary perfusion in newborn, but not weanling, intestine. Unopposed ET-1-induced vasoconstriction would clearly compromise intestinal oxygenation in newborn intestine.

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