IL-1β alters hemodynamics in newborn intestine: role of endothelin

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Nowicki, Philip T. IL-1β alters hemodynamics in newborn intestine: role of endothelin. Am J Physiol Gastrointest Liver Physiol 291: G404–G413, 2006—Studies were carried out to determine the effects of IL-1β on newborn intestinal hemodynamics. IL-1β increased the release of ET-1 by primary endothelial cells in a dose-dependent manner; as well, it reduced expression of the endothelin (ET) type B (ETB) receptor on endothelial cells and increased expression of the ET type A (ETA) receptor on vascular smooth muscle cells. IL-1β increased endothelial cell endothelial nitric oxide (NO) synthase (eNOS) expression but did not enhance eNOS activity as evidenced by release of NOX into conditioned medium in response to acetylcholine or shear stress. The effects of IL-1β on flow-induced dilation were evaluated in terminal mesenteric arteries in vitro. Pretreatment with IL-1β on ileal oxygenation were then studied using in vivo gut loops. Intramesenteric artery infusion of IL-1β upstream of the gut loop caused ileal vasoconstriction and reduced the arterial-venous O2 difference across the gut loop; consequently, it reduced ileal oxygenation by 60%. This effect was significantly attenuated by pretreatment with BQ610. These data support a linkage between the proinflammatory cytokine IL-1β and vascular dysfunction within the intestinal circulation, mediated, at least in part, by the ET system.

newborn intestine; necrotizing enterocolitis; intestinal blood flow; intestinal oxygenation

THE INTESTINE OF HUMAN INFANTS is uniquely susceptible to a disease termed necrotizing enterocolitis (NEC) (24). Histopathology of gut resected for NEC consistently reveals both inflammation and ischemia (20); moreover, this tissue demonstrates elevated levels of several cytokines, including IL-1β, IL-6, IL-10, and IFN-γ (12, 13, 59). The mechanistic basis for the ischemia relevant to NEC remains an enigma. One possibility, heretofore untested, is that proinflammatory cytokines affect the expression or function of local vascular regulatory systems within the newborn intestine in such a manner as to generate ischemia.

Precedents for the proposed interaction exist. IL-1β upregulates the vasoconstrictor peptide endothelin (ET)-1 in bovine (5) or porcine aortic (66) or human umbilical (38) endothelial cells (ECs) as well as vascular smooth muscle cells (VSMCs) harvested from the human saphenous vein or internal mammary artery (64, 65). In the latter, IL-1β-induced activation of the nuclear transcription factor NF-κB underlies the enhanced transcription of prepro-ET, the precursor of ET-1 (49, 65).

IL-1β also affects the expression of ET receptors. Two subtypes of ET receptors have been identified: ETA and ETB. The former are primarily located on vascular smooth muscle, and their activation leads to smooth muscle contraction and hence vasoconstriction; the latter are primarily located on ECs, where ligand binding activates the endothelial isoform of nitric oxide (NO) synthase (NOS), leading to NO production and vasodilation (9). IL-1β increases 125I-labeled ET-1 binding in VSMC secondary to an increase in expression of the ETA receptor (44), i.e., the receptor responsible for generating ET-1-induced vasoconstriction (40). Simultaneous upregulation of ET-1 and the ETA receptor carries the potential for induction of profound and sustained ischemia capable of generating tissue hypoxia and injury, as has been demonstrated in the liver (15), intestine (48), and skeletal muscle (28).

This putative interaction between IL-1β and the ET system may be particularly applicable to the newborn intestine insofar as ET-1 is an important determinant of vascular resistance therein (41). ETA receptors are present in the newborn intestine (57); moreover, intramesenteric infusion of ET-1 at physiologically relevant levels causes profound sustained ischemia and tissue hypoxia in this tissue (42, 43). Most importantly, however, the intestine resected from human infants with NEC demonstrates an abundance of ET-1, whereas arteries harvested from this tissue demonstrate a basal level of vasoconstriction that is significantly attenuated by ETA receptor blockade (45). The presence of both IL-1β and ETA-1 in tissue removed from human infant NEC justifies further study of their potential interaction within the newborn intestine.

These experiments approached this problem by testing two hypotheses. The first hypothesis was that IL-1β upregulates the ET system in the newborn intestinal circulation and thus exerts a deleterious effect on intestinal hemodynamic regulation. The first part of this hypothesis was tested by determining the effects of IL-1β on ET-1 production and ET receptor expression at the cellular level, whereas the second was examined by noting the effects of IL-1β flow-induced dilation in terminal mesenteric arteries (TMAs) harvested from newborn swine and studied in vitro. TMAs (~150 μm) reside immediately upstream from the intramural gut microcirculation and are resistance vessels, a circumstance that renders them an excellent site to study intestinal hemodynamic regulation (53). The choice of flow-induced dilation as a benchmark of hemodynamic regulation was driven by the importance of this response in determining downstream O2 transport and hence tissue oxygenation (56), whereas an in vitro approach was selected to afford precise manipulation of environmental stimuli (41). The second hypothesis was that IL-1β compromises intestinal tis-
sue oxygenation via upregulation of the ET system. This hypothesis was tested by measuring the effects of IL-1β on hemodynamics and oxygenation within isolated ileal gut loops studied under in vivo conditions. This approach was selected to provide data to confirm the physiological relevance of in vitro observations.

MATERIALS AND METHODS

Animal Acquisition and Care

The Institutional Animal Care and Use Committee of Columbus Children’s Research Institute approved this work. Piglets were studied within 8 h of birth and were never allowed to initiate feeding before use to eliminate the confounding variable of nutrient absorption on newborn intestinal hemodynamics (7). Anesthesia was achieved by an intramuscular injection of 7.5 mg/kg telezol and 5.0 mg/kg xylazine and was sustained by pentobarbital sodium (5 mg/kg iv). Piglets were euthanized via an intravenous infusion of Succin (1 ml/kg) while still anesthetized.

Pharmacological Reagents

All pharmaceuticals were obtained from Sigma (St. Louis, MO) and included ET-1, BQ610 (ETα receptor antagonist), and BQ788 (ETβ receptor antagonist). All agents were dissolved in Krebs buffer of the following composition (in mM): 118 NaCl, 4.5 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25.0 NaHCO3, 11.0 glucose, and 0.026 EDTA; pH was 7.4 when the buffer was aerated with 21% O2–5% CO2-balance N2 at 37°C. This buffer also served as the vehicle given to generate control data as well as the suffusate and perfusate of TMAs.

EC and VSMC Studies

Harvest and growth of ECs. ECs were harvested from the mesenteric artery via collagenase digestion using the method of Kobayashi et al. (25). Cells were seeded onto gelatin coated six-well plates in media of the following composition: medium 199 with 5 mM l-glutamine, 20% fetal bovine serum, 5,000 U/ml heparin, 0.5% EC growth supplement, 105 U/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml amphotericin. Confirmation of cell type was made by positive staining for von Willebrand factor (26). The principal contaminating cell type was vascular smooth muscle. This contamination was assessed by staining for smooth muscle α-actin.

Experimental protocol. Primary EC cultures at 90% confluence were placed into quiescent medium for 24 h before study (composition: DMEM with 2 mM Na pyruvate, 6 mM l-glutamine, 0.1% BSA, 105 U/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml amphotericin). Thereafter, medium was aspirated and replaced with fresh medium containing study drugs or vehicle. If the study end point was EC release of NO or ET-1, medium was stored at 80°C and the cell count per well was determined. If the study end point required immunoblotting, cells were lysed [lysis buffer composition: HEPES buffer (pH 7.4), containing 50 mM β-glycerophosphate, 2 mM EGTA, 1 mM DTT, 10 mM NaF, 1 mM Na orthovanadate, 1% Triton X-100, 10% glycerol, 200 mM phenylmethylsulfonyl fluoride, 100 μM leupeptin, 10 mM okadaic acid, and 200 kallikrein inhibitory units of aprotinin], and the supernatant was recovered.

Generation of shear stress. Some cells were exposed to the mechano-stimulus of shear stress (15 dyn/cm² for 1 h). This perturbation was achieved using a cone-plate viscometer device that permits simultaneous application of radial shear to all wells of a six-well plate; the magnitude of shear stress generated by this instrument has been validated (60). A shear rate of 15 dyn/cm² was selected to duplicate the wall shear stress generally noted within small arteries (60).

Harvest and growth of VSMCs. VSMCs were harvested using the explant method and were grown in media consisting of 50% DMEM, 50% Ham’s F-12 medium, 10% fetal bovine serum, 2 mM l-glutamine, 105 U/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml amphotericin. After 10 days, VSMCs were separated from the remains of the minced aorta and replated in six-well plates (single passage). Confirmation of cell type was made by positive staining for smooth muscle α-actin (23).

Experimental protocol. Single-passage VSMCs at 90% confluence were placed into serum-free medium for 48 h to generate a differentiated (spindle-shaped) phenotype. At the onset of the experiment, the quiescent medium was aspirated and replaced with fresh medium containing the pharmacological agents under study. At the completion of the protocol, the medium was discarded and cells were lysed using the buffer previously described, and the supernatant was used for immunoblotting.

Immunoblotting. Protein separation was carried out by electrophoresis in 7% SDS-polyacrylamide gels (10 μg protein/lane) and then transferred overnight to polyvinylidene difluoride membranes. After being blocked for 1 h in 5% dried milk in PBS-Tween buffer, the membranes were incubated with primary antibody for ETα or ETβ (1:500, Alomone Lab, Jerusalem, Israel) or endothelial NOS (eNOS) or inducible NOS (iNOS; 1:1,000, BD Biosciences Pharmigen, San Jose, CA) diluted in PBS-Tween buffer for 1 h. Membranes were rinsed and incubated in horseradish peroxidase-conjugated secondary antibody (1:2,500; Calbiochem, San Diego, CA) and developed using enhanced chemiluminescence (Pierce, Rockford, IL). Membranes were then stripped and reprobed with primary antibody for β-actin. Membranes were scanned, and quantity of the protein of interest was expressed as a ratio with the β-actin signal.

Measurement of NO. NO is rapidly oxidized to NO2 and NO3, hereafter designated NOx. NOx within conditioned medium was reduced to NO using 0.1 M vanadium chloride and 1.0 M HCl at 95°C. This NO was directly delivered to a Sievers NO analyzer (Sievers Instruments, Boulder, CO) by vacuum through a bubble trap collector containing 1 M NaOH. NO was reacted with analyzer-generated ozone in a chemiluminescence reaction and the product was measured. The resulting signal was fitted to a standard curve to calculate NO concentration, expressed in picograms per 10⁵ cells (52).

Measurement of ET-1. Protein concentration of the supernatant was determined by the bichinonic acid method (Pierce). Peptide extraction was carried out on conditioned medium added to 1 ml of 1% trifluoroacetic acid applied to C-18 columns preequilibrated with 100% acetonitrile. After being washed three times with 1% trifluoroacetic acid, peptides were eluted from the column with 60% acetonitrile in 1% trifluoroacetic acid. Elutants were dried on a centrifugal concentrator without heat. ET-1 concentration was determined by ELISA (Peninsula Labs, San Carlos, CA).

In Vitro Studies of TMAs

Experimental apparatus. TMAs were mounted between glass cannulae set inside a Plexiglas chamber (CH/2/AS, Living Systems, Wellington, VT) that was continuously suffused with warmed (37°C) aerated Krebs buffer. Vessels were perfused with the same buffer. Pressure and flow within the vessel were established by two pressure-servo systems (PS/200/Q, Living Systems) placed at the inflow and outflow cannulae so that the axis of symmetry was set as the midpoint of the vessel. Inflow (Pi) and outflow (Po) pressures, adjusted separately, could be set to so that the vessel was pressurized in the absence of flow (i.e., Pi = Po) or so that flow moved through the vessel (i.e., Pi > Po).

Hemodynamic measurements. Pi and Po were measured with microtransducers at the inflow and outflow cannulae (PS/200/Q; Living Systems). Flow was measured with a microflowmeter (FM1; Living Systems). The chamber was mounted on the stage of an inverted microscope set in line with a video camera. Artery diameter was determined by a video dimension analyzer (V94; Living Systems).
IL-1β and Newborn Intestinal Hemodynamics

TMA stabilization and viability determination. P₁ and P₀ were initially set at 0 mmHg for 60 min after vessels were mounted. P₁ and P₀ were simultaneously increased to 25 mmHg and then to 45 mmHg. The second pressure was selected to duplicate pressures noted in situ (53). TMA diameter increased following both steps; however, in response to the second pressurization step, the vessel exhibited vasoconstriction after initial dilation, i.e., it displayed myogenic vasoconstriction (41). Phenylephrine (10⁻⁵ M) was added to the perfusion buffer to further contract the vessel, followed by acetylcholine (10⁻⁷ M) to confirm the presence of an intact endothelium. Vessels that failed to demonstrate a myogenic response, that failed to contract >40% to phenylephrine, or that failed to subsequently dilate >25% following acetylcholine were excluded from further study. The perfusion buffer was then changed, and the protocol was begun.

Experimental protocol. Hemodynamic measurements were first made under zero-flow conditions with P₁ and P₀ set at 45 mmHg. P₁ was then raised, and P₀ was lowered by equal amounts as necessary to generate a flow rate of 100 µl/min across the vessel; the simultaneous changes in P₁ and P₀ kept mean pressure within the vessel (Pₙₐ₅) at ~45 mmHg [determined as (P₁ + P₀)/2]. The pressure gradient was then widened in a similar manner to raise the flow rate to 200 µl/min. This protocol was carried out under control conditions or following exposure of the vessel to a single pharmacological agent. Studies that involved IL-1β required a 4-h incubation with the cytokine. To account for this, all vessels were kept in buffer, with or without IL-1β, for 4 h before study.

In Vivo Study of Ileal Gut Loops

Preparation of gut loops. The piglet was anesthetized (telezol and xylazine) and ventilated. Catheters were placed into a femoral artery-vein pair for monitoring of blood gas tensions and systemic arterial pressure. Catheters were also placed into the ileal gut loop were measured by a Lex-O₂-Con (Chestnut Hill, MA). O₂ consumption was calculated by the Fick equation, i.e., as the product of blood flow rate and the arterial-venous O₂ concentration difference (a-VO₂) across the gut loop. A segment of the ileum ~15 cm long was isolated from the remainder of the gut so that it was perfused by a single artery-vein pair. After heparinization (500 U/kg), the vessel was cannulated and the cannula was directed to a saline-filled reservoir; blood was returned to the animal via a femoral vein at a rate equal to the mesenteric venous outflow. The artery leading to the gut loop as well as the mesenteric nerves were left intact so that the ileal loop was autoperfused and innervated. An electromagnetic flowmeter and pressure transducer were placed within the venous circuit to measure mesenteric venous pressure, respectively (46). The O₂ concentrations of blood samples obtained from the artery-vein pair perfusing and draining the ileal gut loop were measured by a Lex-O₂-Con (Chestnut Hill, MA). O₂ consumption was calculated by the Fick equation, i.e., as the product of blood flow rate and the arterial-venous O₂ concentration difference (a-VO₂) across the gut loop. A cannula was inserted in a jejunal artery branch and advanced retrograde until its tip was immediately proximate to the main mesenteric artery trunk, just upstream from the ileal artery branch that served the isolated gut loop. Drugs were infused through this cannula. Ileal hemodynamics (pressure, flow, O₂ consumption) generated under these conditions (i.e., in an anesthetized, ventilated, acutely prepared piglet) are similar to those noted in awake, spontaneously breathing, chronically cannulated piglets (47).

Experimental protocol The ileal gut loop was allowed sufficient time (minimum 2 h) to attain steady-state conditions, defined as fluctuation of blood flow and a-VO₂ of <5%. Baseline measurements were made; thereafter, a continuous infusion of IL-1β (or an equal volume of Krebs buffer) was begun into the mesenteric artery just upstream of the ileal artery serving the study gut loop. The drug infusion rate was set to generate an initial IL-1β concentration of 1 ng/ml arterial blood, based on the existing arterial flow rate at the onset of drug infusion. The infusion was continued for 1 h, and then measurements were made over the ensuing 4 h. In some studies, a single bolus infusion of BQ610 (50 nM/kg) or BQ788 (50 nM/kg) was given into the mesenteric artery just before the IL-1β infusion. These doses are sufficient to block ETₐ or ETₜ receptors, respectively, under these in vivo conditions (42).

Statistical Methods

ANOVA was used to determine the significance within each data set. One- or two-way ANOVA formats were used depending on the number of variables under consideration. If the F-statistic of the ANOVA was significant (P < 0.05), then post hoc Tukey’s B tests were carried out to determine the sites of significance at the P < 0.05 level.

RESULTS

IL-1β Effects on ECs and VSMCs

IL-1β increased ET-1 production by ECs in a dose-dependent manner over the range of 0.1–10 ng/ml, with the maximal effect noted at 1 ng/ml (Table 1). This effect was also time dependent over the range of 1–6 h, with a maximal effect noted at 4 h (data not shown). The mechanostimulus of wall shear stress has been demonstrated to enhance endothelial ET-1 production (18). To determine whether prior exposure to IL-1β altered the effects of shear on ET-1 production, ECs were pretreated with IL-1β and then exposed to shear stress. Under control conditions, shear stress (15 dyn/cm² applied for 1 h) reduced EC release of ET-1 into the medium. IL-1β reversed this trend, i.e., IL-1β-pretreated cells released significantly more ET-1 into the medium in response to shear; moreover, the amount released significantly exceeded that generated by IL-1β alone (Table 2).

IL-1β increased EC expression of eNOS, and the maximal effect was noted at 1 ng/ml and a 4-h exposure (Fig. 1); in contrast, the cytokine had no effect on iNOS expression in these cells (data not shown). To determine whether IL-1β

Table 1. Effect of IL-1β on ET-1 release by ECs

<table>
<thead>
<tr>
<th>Condition</th>
<th>ET, pM/10⁵ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>IL-1β (0.1 ng/ml)</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>IL-1β (1 ng/ml)</td>
<td>4.9±0.6*</td>
</tr>
<tr>
<td>IL-1β (10 ng/ml)</td>
<td>4.7±0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, primary cell cultures from 4 piglets. IL-1β was applied to endothelial cells (ECs) in quiescent (0.1% serum) medium for 4 h. Release of endothelin (ET)-1 into the medium was determined by ELISA and was expressed as picomoles of ET per 10⁵ cells. *P < 0.05 vs. control or 0.1 ng/ml IL-1β.

Table 2. Effects of IL-1β on EC release of ET-1 in response to shear stress

<table>
<thead>
<tr>
<th>Condition</th>
<th>ET, pM/10⁵ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>IL-1β (1 ng/ml; 5 h)</td>
<td>4.2±0.3*</td>
</tr>
<tr>
<td>Shear (15 dyn/cm²; 1 h)</td>
<td>1.5±0.2*</td>
</tr>
<tr>
<td>IL-1β + shear</td>
<td>6.2±0.4*†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, primary EC cultures from 4 piglets. ECs were treated with IL-1β or shear stress at the doses and exposure times indicated. *P < 0.05 vs. control; †P < 0.05 vs. IL-1β alone.
affected eNOS function, ECs were pretreated with IL-1β and then which are exposed to acetylcholine or shear stress, established stimulants of eNOS activity (1, 6). Release of NOx into the medium was used as an indication of eNOS activity. Despite the increased expression of eNOS generated by IL-1β, release of NOx in response to acetylcholine was unchanged; moreover, release of NOx in response to shear stress was significantly blunted by IL-1β (Table 3).

IL-1β significantly reduced expression of ETB receptors on primary newborn ECs (Fig. 2). This receptor is primarily located on the endothelium in the newborn swine mesenteric artery (57), and ligand binding activates eNOS (40); accordingly, additional studies were carried out to determine whether IL-1β altered the effect of ET-1 on EC NO production. Pre-treatment with IL-1β significantly attenuated the release of NOx into conditioned medium in response to ET-1 (Table 3).

Finally, the effects of IL-1β on ETA receptor expression in VSMCs was determined. The ETA receptor mediates ET-induced vasoconstriction, and it is primarily localized to vascular smooth muscle in the swine intestine (40, 57). IL-1β significantly increased ETA expression in vascular smooth muscles in a dose-dependent manner (Fig. 3). The effect was also time dependent, with a maximal effect noted after a 4-h exposure. IL-1β had no effect on iNOS expression in these cells (data not shown).

Effects of IL-1β on Flow-Induced Dilation in Vitro

Flow-induced dilation was clearly present in newborn TMA. Vessel diameter at zero flow (P1 and P0 at 45 mmHg) was 149 ± 7 μm (means ± SD, n = 8). Generation of a flow rate of 100 μl/min required a change in pressure (ΔP) of 12 ± 2 mmHg [a-v pressure (Pav) 46 ± 2 mmHg] and caused vessel diameter to increase to 217 ± 9 μm. Further widening the ΔP to 15 ± 1 mmHg (Pav 45 ± 2 mmHg) caused diameter to increase to 249 ± 12 μm. Resistance across the vessel fell significantly (P < 0.05) from 0.11 ± 0.01 to 0.07 ± 0.02 mmHg·μl⁻¹·min⁻¹ when flow was increased from 100 to 200 μl/min.

IL-1β reduced the TMA diameter noted under zero-flow conditions and also when flows of 100 and 200 μl/min were
established (Fig. 4). The percent changes in TMA diameter noted in response to flows of 100 and 200 μl/min were significantly less that noted in control vessels; as well, IL-1β increased the ΔP requisite to establish flow, increased the resistance noted at each flow rate, and eliminated the reduction in resistance noted in control vessels when flow was increased from 100 to 200 μl/min (Table 4). Interestingly, the hemodynamic changes induced by IL-1β were sustained; stated otherwise, the response to flow could not be restored by washout of IL-1β. This sustained effect lasted for at least 6 h.

On the basis of the effects of IL-1β on ET-1 and ETA receptor expression in ECs and VSMCs, we next sought to determine whether the IL-1β-induced attenuation of flow-induced dilation was mediated by ET-1. BQ610 (50 nM) had no effect on flow-induced dilation when given alone; however, when given before IL-1β, it nearly eliminated IL-1β-induced attenuation of the vascular response to flow (Fig. 5 and Table 5). BQ788 (50 nM) had no effect on flow-induced dilation.

Table 4. Dose-response effect of IL-1β on TMA hemodynamics

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>100 μl/min</th>
<th>200 μl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ΔP</td>
<td>12 ± 2</td>
<td>15 ± 1*</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.11 ± 0.01</td>
<td>0.07 ± 0.02*</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>45 ± 4</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>IL-1β (0.1 ng) ΔP</td>
<td>12 ± 3</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.11 ± 0.01</td>
<td>0.08 ± 0.02*</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>40 ± 4</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td>IL-1β (1.0 ng) ΔP</td>
<td>19 ± 3†</td>
<td>33 ± 4†</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.18 ± 0.02†</td>
<td>0.18 ± 0.04†</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>15 ± 2†</td>
<td>3 ± 1†</td>
</tr>
<tr>
<td>IL-1β (10 ng) ΔP</td>
<td>21 ± 3†</td>
<td>34 ± 3†</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.18 ± 0.04†</td>
<td>0.19 ± 0.03†</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>16 ± 3§</td>
<td>6 ± 1†</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 8. ΔP is given in mmHg and resistance as mmHg·μl⁻¹·min⁻¹. %ΔDiameter is the relative change in terminal mesenteric artery (TMA) diameter following elevation of flow rate from 0 to 100 and then 100 to 200 μl/min. Measurements were made on each TMA under only one condition (i.e., control or after a single dose of IL-1β), and all IL-1β exposures were carried out for 4 h before hemodynamic measurements. * P < 0.05 vs. 100 μl/min (within group); † P < 0.05 vs. control.

Table 5. Effects of BQ610, BQ788, ET-1, and phenylephrine on TMA hemodynamics

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>100 μl/min</th>
<th>200 μl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β + BQ610 ΔP</td>
<td>13 ± 1‡</td>
<td>18 ± 1*‡</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.12 ± 0.02‡</td>
<td>0.08 ± 0.01*‡</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>39 ± 4‡</td>
<td>13 ± 2*‡</td>
</tr>
<tr>
<td>IL-1β + BQ788 ΔP</td>
<td>19 ± 1†</td>
<td>30 ± 3*†</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.18 ± 0.02†</td>
<td>0.19 ± 0.03†</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>18 ± 3§</td>
<td>5 ± 2†</td>
</tr>
<tr>
<td>ET-1 ΔP</td>
<td>21 ± 3†</td>
<td>32 ± 4†</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.21 ± 0.03†</td>
<td>0.15 ± 0.03*†</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>38 ± 7</td>
<td>14 ± 4*</td>
</tr>
<tr>
<td>Phenylephrine ΔP</td>
<td>20 ± 3†</td>
<td>34 ± 4*</td>
</tr>
<tr>
<td>Resistance</td>
<td>0.20 ± 0.03†</td>
<td>0.16 ± 0.02†</td>
</tr>
<tr>
<td>%ΔDiameter</td>
<td>38 ± 6</td>
<td>17 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 8. ΔP is given in mmHg and resistance as mmHg·ml⁻¹·min⁻¹. %ΔDiameter is the relative change in TMA diameter following elevation of flow rate from 0 to 100 and then 100 to 200 μl/min. Measurements were made on each TMA under only one condition (i.e., control or after a single dose of IL-1β), and all IL-1β exposures were carried out for 4 h prior to hemodynamic measurements. * P < 0.05 vs. 100 μl/min (within group); † P < 0.05 vs. control data, given in Table 4; ‡ P < 0.05 vs. IL-1β 1 ng/ml data, given in Table 4.

**Fig. 4.** Effect of IL-1β on flow-induced dilation in terminal mesenteric arteries (TMA). Flow rate was increased from 0 to 100 μl/min and then to 200 μl/min by generating a change in pressure (ΔP) across the vessel without inducing a concomitant change in arteriovenous pressure (Pav). TMAs were exposed to IL-1β or vehicle for 4 h before measurements, and each vessel received only a single treatment. Values are means ± SD; n = 8. * P < 0.05 vs. control.

**Fig. 5.** Effects of ET receptor blockade on IL-1β-induced change in flow-induced dilation in TMAs. Flow rate was increased from 0 to 100 μl/min and then to 200 μl/min by generating a ΔP across the vessel without inducing a concomitant change in Pav (see text). Control and IL-1β data are repeated from Fig. 4 to facilitate comparison. TMAs were exposed to either vehicle (Krebs buffer, control), IL-1β (1 ng/ml for 4 h), or IL-1β (1 ng/ml for 4 h) plus a single receptor-blocking agent, either BQ610 (ETa receptor antagonist; 50 nM for 15 min) or BQ788 (ETa receptor antagonist; 50 nM for 15 min), before hemodynamic manipulation. Each TMA was exposed to a single treatment. Control; ○, IL-1β alone; △, IL-1β + BQ610; □, IL-1β + BQ788. Values are means ± SD; n = 8. * P < 0.05 vs. IL-1β alone; † P < 0.05 for 100 vs. 0 μl/min; ‡ P < 0.05 for 200 vs. 100 μl/min.
when given alone, nor did it alter the effect of IL-1β on flow-induced dilation (Fig. 5 and Table 5).

Interestingly, administration of exogenous ET-1 did not mimic the effects of IL-1β on the TMA response to flow. ET-1 (1 nM) decreased TMA diameter under zero-flow conditions and shifted the diameter-flow curve downward but did not eliminate flow-induced dilation; thus the percent change in diameter from zero-flow baseline following application of flow was similar in ET-1-treated vessels compared with controls. Phenylephrine, which induces vasoconstriction via activation of α-receptors, had an effect similar to ET-1 (Fig. 6 and Table 5).

**Effects of IL-1β on Intestinal Hemodynamics and Oxygenation In Vivo**

Intramesenteric artery infusion of IL-1β for 1 h caused a subsequent progressive vasoconstriction within ileal gut loops and hence reduced perfusion; as well, the cytokine reduced the arteriovenous O2 concentration gradient across the gut loop (Fig. 7). These changes conspired to reduce the calculated O2 consumption by this tissue. Administration of BQ610, either before or after 4 h of IL-1β infusion, significantly attenuated the IL-1β-induced compromise in ileal perfusion and tissue oxygenation, whereas BQ788 had no effect.

**DISCUSSION**

Data collected in these experiments support the proposed hypotheses as follows: 1) IL-1β increased the release of ET-1 by ECs, upregulated ETA receptors on VSMCs, and downregulated EC ETB receptors; 2) IL-1β significantly attenuated the intrinsic vascular response of flow-induced dilation, due in part to ETA receptors; and 3) IL-1β compromised intestinal oxygenation in vivo, due in part to ETA receptors.

**Effects of IL-1β at a Cellular Level**

The maximal stimulatory effect of IL-1β on ET-1 release was observed at a cytokine concentration of 1 ng/ml, a dose lower than that reported for adult bovine (5) and porcine (29, 30).
ET-1 production by shear may reflect the concomitant effect this stimulus had on NO production. Shear more than doubled NO release into conditioned medium, and Bourlanger and Luscher (3) reported that NO reduces ET-1 production. IL-1β doubled ET-1 release by ECs under static conditions; subsequent exposure of these cells to shear caused an additional, significant rise in ET-1 release. Thus the effect of shear on EC ET-1 release was dependent on the presence of IL-1β. In the absence of this cytokine, shear reduced ET-1 release; in its presence, shear increased ET-1 release. This observation is consistent with Bodin et al. (2).

IL-1β had a discrepant effect on ET receptors: downregulation of ETβ receptors on ECs vs. upregulation of ETA receptors on VSMCs, and this difference may contribute to the effects of IL-1β on gut hemodynamics. Ligand binding to the VSMC ETA receptor increases intracellular Ca$^{2+}$, induces cell contraction, and hence generates vasoconstriction of the vessel (49). In contrast, ligand binding to the EC ETβ receptor activates eNOS to generate NO and hence induces vasodilation (58); moreover, NO directly interferes with ETA receptor function, thus further limiting ET-1-induced vasoconstriction (54). Normally, expression of endothelial ETβ receptors exceeds expression of ETA receptors on smooth muscle in the intestinal circulation of 1-day-old swine (57), a circumstance that likely contributes to the relatively vasodilated state of this circulation (41). The physiological effects of perturbation of ETβ receptor expression by IL-1β was evidenced by the effects of the cytokine on endothelial NOx release. Thus a significant reduction in NOx release in response to exogenous ET-1 occurred, which likely reflected downregulation of endothelial ETβ receptors. Release of NOx by ECs in response to shear stress was also compromised by IL-1β, whereas the release of ET-1 was increased. Once again, these observations may reflect a relative loss of ETβ receptors; hence, shear-induced release of ET-1 failed to generate a concomitant rise in NOx release insofar as ETβ-induced activation of eNOS was blunted by the effect of IL-1β on ETβ receptor expression.

**Effects of IL-1β on Flow-Induced Dilation in TMAs In Vitro**

IL-1β significantly impaired the normal hemodynamic response of TMAs to the mechanostimulus of flow, as evidenced by 1) lessened TMA response to flow; 2) exigency for a greater ΔP to establish a specified flow rate; and 3) increased vascular resistance during flow. The cytokine effect was mediated, at least in part, by the changes in ETA receptor expression insofar as its selective blockade attenuated the effect; hence, BQ610 nearly restored the TMA response to flow to baseline. In contrast, the increased endothelial release of ET-1 noted following IL-1β was, by itself, insufficient to eliminate flow-induced dilation. Hence, exogenous ET-1, given at a concentration slightly greater that the $K_d$ of the newborn swine ETβ (0.36 ± 0.14 nM) and ETA (0.51 ± 0.24 nM) receptors, shifted the diameter-flow curve downward, whereas the percent changes in diameter and resistance in response to flow rates of 100 and 200 μl/min were unchanged from control (40). Phenytoin, given at the concentration necessary to duplicate ET-1 vasoconstriction, had a similar effect. Vasoconstriction per se was not culpable. These observations are generally consistent with published reports on the effects of IL-1β on mesenteric vascular reactivity in adult rats (10, 63).

The present understanding of the basis for flow-induced dilation is that the phenomenon is mediated by several factors, including eNOS-derived NO (6, 51), oxidants, particularly $\text{H}_2\text{O}_2$ (27, 35), and $\text{K}^+$ channels (36, 37). In this context, the effects of IL-1β were certainly more complex that singular adjustments in ET receptor expression. For example, IL-1β caused upregulation of endothelial eNOS expression, yet had no effect on NOx release under control conditions or following acetylcholine, and actually blunted NOx release in response to shear; stated otherwise, the functional activity of eNOS, as evidenced by NOx release, did not follow its expression. eNOS activity requires posttranslational modification, most importantly targeting of the enzyme to calveolae (55); as well, coupling of the reductase and oxidase domains of eNOS via tetrahydrobiopterin must be intact lest eNOS produces superoxide anion in lieu of NO (17). The increased eNOS expression in the absence of greater eNOS activity suggests compromise of eNOS function by IL-1β in TMAs. Existing reports have indicated that IL-1β enhances oxidant production (4, 33); moreover, ligand binding by the ETA receptor generates $\text{H}_2\text{O}_2$ (61). IL-1β-induced oxidant production might be predicated to enhance flow-induced dilation, although oxidant stress can uncouple eNOS, reducing NOx production (39). This putative interplay among eNOS, oxidants, and the ETA receptor is made more complex in the newborn intestine insofar as antioxidant systems are developmentally regulated, i.e., some systems are not fully functional at birth (8).

**Effects of IL-1β on Intestinal Oxygenation In Vivo**

IL-1β was infused directly into the mesenteric artery at a dose designed to approximate the in vitro levels requisite for alteration of cellular expression of the ET system and in vitro TMAs hemodynamics. This infusion caused a 60 ± 5% reduction in ileal $\text{O}_2$ consumption, as determined by the Fick equation. Reductions of both blood flow (31 ± 6%) and $\text{a-VO}_2$ (50 ± 5%) contributed to the compromise of ileal oxygenation. The latter effect likely reflects reduction of the perfused capillary density secondary to ETA-induced closure of the precapillary arterioles.
illary sphincter as under normal conditions an inverse relationship between levels of flow and $\alpha$-VO$_2$ exist so that tissue oxygenation is controlled (16). ET$_A$ ligand binding causes vasoconstriction and reduces capillary surface area in vivo (42). On the basis of in vitro findings, interpretation of the in vivo data suggests that IL-1$\beta$ enhanced ET$_A$ receptor expression and local (intestinal vascular) ET-1 production; indeed, this explanation is strengthened by the partial reversal of cytokine-induced hemodynamic effects by selective blockade with BQ610.

BQ788, a selective ET$_B$ receptor antagonist, did not alter IL-1$\beta$-induced changes in ileal hemodynamics or oxygenation in the in vivo model nor did it affect flow-induced dilation in the in vitro model (42). On the basis of in vitro findings, interpretation of the in vivo data suggests that IL-1$\beta$ (42). If IL-1$\beta$-induced downregulation of EC ETB receptors in newborn intestine elicits vasoconstriction and reduces capillary surface area in vivo (16). ET A ligand binding causes vasoconstriction and reduces capillary surface area in vivo (16). ET A ligand binding causes vasoconstriction and reduces capillary surface area in vivo (16). ET A ligand binding causes vasoconstriction and reduces capillary surface area in vivo (16).


