Endothelin-1 attenuates increases in hydraulic conductivity due to platelet-activating factor via prostacyclin release

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Cureton EL, Strumwasser A, Kwan RO, Dozier KC, Curran B, Sadjadi J, Victorino GP. Endothelin-1 attenuates increases in hydraulic conductivity due to platelet-activating factor via prostacyclin release. J Appl Physiol 110: 717–723, 2011. First published December 23, 2010; doi:10.1152/japplphysiol.00690.2010.—We previously showed that endothelin-1 (ET-1) and prostacyclin (PGI2) similarly attenuate increases in microvascular permeability induced by platelet-activating factor (PAF). This led us to hypothesize that ET-1 attenuates trans-endothelial fluid flux during PAF through PGI2 release. We tested this hypothesis in three phases. First, bovine pulmonary artery endothelial cells were exposed to 0.008–8 μM ET-1 and assayed for PGI2 release. Second, to determine whether increased transmonolayer flux after PAF could be attenuated by ET-1 or PGI2 and reversed by PGI2 synthesis inhibition or PGI2 receptor blockade, we measured endothelial cell transmonolayer flux after cells were exposed to 10 nM PAF plus 10 μM PGI2 or 80 pM ET-1, with or without 500 μM tranylcypromine (PGI2 synthase inhibitor) or 20 μM CAY-10441 (PGI2 receptor blocker). Finally, hydraulic conductivity ($L_p$) was measured in rat mesenteric venules in vivo after exposure to 10 nM PAF and 80 pM ET-1 with or without tranylcypromine (100 and 500 μM) or CAY-10441 (2 and 20 μM). We found that in vitro, ET-1 stimulated a dose-dependent increase in PGI2 production (from 126 to 217 pg/ml, $P < 0.01$). Compared with PAF alone, PGI2 plus PAF and ET-1 plus PAF decreased transmonolayer flux similarly by 52 and 46%, respectively ($P < 0.01$), while tranylcypromine and CAY-10441 reversed these effects by 92 and 47%, respectively ($P < 0.05$). In vivo, PAF increased $L_p$ fourfold ($P < 0.01$) and ET-1 attenuated this effect by 83% ($P < 0.01$). Tranylcypromine and CAY-10441 reversed the ET-1 attenuation in $L_p$ during PAF by 55 and 45%, respectively ($P < 0.01$). We conclude that ET-1 may stimulate endothelial cell PGI2 release to attenuate the increases in transmonolayer flux and hydraulic conductivity secondary to PAF.

transmonolayer flux; hydraulic conductivity; endothelin-1

DURING TRAUMA, ENDOTHELIN-1 (ET-1) and prostacyclin (PGI2) are vasoactive mediators that are released into the systemic vasculature and bind to their respective microvascular endothelial cell receptors (6, 7, 16, 39). Binding to these receptors induces a complex cascade of intracellular events that governs trans-endothelial fluid flux. Because of their physiological importance, ET-1 and PGI2 have clinical applicability. Endothelin blockers have been used to treat pulmonary hypertension and children with congenital heart disease (32). A clinical analog of PGI2, iloprost, has been used to treat pulmonary hypertension and Reynaud’s phenomenon and to diminish platelet aggregation during elevated inflammatory states (1). However, a causal relationship between ET-1 binding and PGI2 release has not been elucidated.

An inter-relationship of ET-1 and PGI2 release in a non-shock setting has been shown by the concomitant secretion of both from human umbilical vein endothelial cells (HUVEC) in the setting of radiographic contrast injury (15). Although not specifically an investigation of microvascular permeability, that study demonstrated that endothelial injury can increase endogenous production of both ET-1 and PGI2. The work of Bentzer et al. (2, 3) on the microvascular effects of ET-1 suggests it may reduce microvascular permeability through the secondary release of PGI2 and that PGI2 restores increased protein permeability after trauma in cat skeletal muscle. Taken together, these findings suggest that ET-1 may regulate microvascular permeability through PGI2 or possibly through an intermediary that involves PGI2.

Evidence supporting a role for ET-1 and PGI2 in modulating microvascular permeability continues to mount. Both ET-1 and PGI2 attenuate microvascular hydraulic conductivity in cat skeletal muscle and rat mesenteric venules (3, 33, 35). Prostacyclin mitigates cardiac failure in a rat model of endotoxic shock via activation of the PGI2/IP system (19). Prostacyclin has similarly decreased microvascular permeability to albumin in rat gut and lung tissues when LPS was administered to emulate endotoxic shock (14). Most recently, in an experimental PAF model of inflammation, we showed that when ET-1 and PGI2 were administered in rat mesenteric venules before and after PAF, they diminished the PAF-induced increases in trans-endothelial fluid flux (10). While our previous paper (10) demonstrated that ET-1 and PGI2 attenuated the ability of PAF to increase microvascular permeability, no experiments were performed to probe the mechanism of ET-1-induced PGI2 release. Therefore in follow-up to this previous work, we sought to determine whether the hydraulic conductivity-decreasing effect of ET-1 during PAF is due to ET-1-stimulated PGI2 release.

We hypothesized that ET-1, through PGI2 release, attenuates trans-endothelial fluid flux during PAF administration. Our experimental approach consisted of three phases to test this hypothesis: 1) documentation of ET-1-stimulated PGI2 release by in vitro measurement of PGI2 release after endothelial cell exposure to ET-1, 2) determination of transmonolayer flux during PAF administration and ET-1 exposure using a PGI2 synthase inhibitor and a PGI2 receptor antagonist to see whether PGI2 release is responsible for the activity of ET-1 in vitro, and 3) determination of hydraulic conductivity during PAF administration and ET-1 exposure using a PGI2 synthase inhibitor and a PGI2 receptor antagonist to see whether PGI2 release is responsible for the activity of ET-1 in vivo.

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**METHODS**

All studies received institutional approval and complied with animal research protocols.

*Measurement of PGI2 Release After ET-1 Administration*

To determine the impact of ET-1 on PGI2 release we first sought to demonstrate whether ET-1 stimulates release of PGI2 using an ELISA assay in cultured bovine pulmonary endothelial cells. The PGI2 assay was purchased as the 6-keto prostaglandin-F1α, EIA Kit (Cayman Chemical, Ann Arbor, MI). The PGI2 assay is based on the premise that PGI2 is converted to its major metabolite, 2,3-dinor-6-keto (CAY-10441). Tranylcypromine is a specific inhibitor of PGI2 synthesis inhibitor (tranylcypromine) and a PGI2 receptor blocker (29).

**Transmonolayer flux studies** were based on the work of Goldblum et al. (29, 32, 36, 37, 41). Tranylcypromine is a selective competitive blocker of the PGI2 receptor (29).

Transmonolayer flux studies were performed as described above for the transmonolayer flux assay. Again, transwell resistance values confirmed confluence of monolayers before the experiment began, and only transwells exhibiting a monolayer resistance of 150 ohms or greater were used. The transwells were then carefully aspirated and washed three times with PBS to remove residual media in both apical and basolateral compartments. The apical and basolateral compartments were aspirated free of PBS after each wash until negligible PBS volume remained in either compartment. In seven transwells, 300 μl of warmed PBS (37°C) was added to each of the basolateral compartments. The apical compartment of these seven transwells was prepared as follows: **well 1)** 100 μl of control PBS, **well 2)** 100 μl control PBS with 20 μg/ml bovine immunotyped BSA, **wells 3–7)** 100 μl mediator-enriched PBS with bovine immunotyped BSA. In the solutions designed to contain mediator (wells 3–7), one of the following combinations was also added to the apical compartment: 1) PAF-16 (1 μM) (Calbiochem, EMD Biosciences, San Diego, CA), 2) ET-1 (80 nM) plus PAF-16 (1 μM), 3) PGI2 (100 μM) (Biomol Research Lab, Plymouth Meeting, PA) plus PAF (1 μM), 4) ET-1 (80 nM) plus tranylcypromine (500 μM) (Biomol Research Lab) plus PAF (1 μM), and 5) ET-1 (80 nM) plus CAY-10441 (20 μM) (Cayman Chemical, Ann Arbor, MI) plus PAF (1 μM).

After the plates were prepared and the mediators were added, the 24-well plate was incubated for 1 h at 37°C. At this point, 100 μl of the solution in each basolateral compartment was aliquoted in triplicate to a 96-well NUNC plate. The 96-well NUNC plate was then allowed to sit at room temperature for 1 h. The plate wells were then blocked with 400 μl blocking buffer (Tris buffered saline plus 0.1 g/ml bovine serum albumin, Sigma Chemical) and then left at room temperature for 2 h. The plate wells were then emptied of residual blocking buffer and washed with 200 μl of adhesion buffer (Tris buffered saline plus 1 mg/ml bovine serum albumin), and then incubated for 30 min with 100 μl of streptavidin-horseradish peroxidase conjugated at 1 μg/ml. The plate wells were then emptied of residual adhesion buffer and streptavidin-horseradish peroxidase and washed again with 200 μl of adhesion buffer. Next, 100 μl of TMB reagent (containing equal quantities of TMB substrate reagent A and TMB substrate reagent B) were added to each plate well, and when the wells developed appropriately (indicated by turning blue), the reaction was stopped by adding 100 μl of 2.5 M H2SO4 (Sigma-Aldrich). The absorbance was measured at 450 nm (Tecan-Genios).

*In Vivo Measurement of Hydraulic Conductivity*

In the third phase of our experiments, we used the modified Landis micro-occlusion technique to explore the in vivo effects of PAF plus ET-1 on hydraulic conductivity during PGI2 synthesis inhibition and during PGI2 receptor antagonism.

**Animal and solution preparations.** Red blood cells that are used as flow markers were harvested from female Sprague-Dawley rats (250–310 g; Hilltop Lab Animals, Scottsdale, PA). The blood was centrifuged to remove the plasma anduffy coat and then washed three times in 15 ml of mammalian Ringer solution. The Ringer solution was prepared daily in distilled deionized water and contained 135 mM NaCl, 4.6 mM KCl, 2.0 mM CaCl2, 2.46 mM MgSO4, 5.0 mM NaHCO3, 5.5 mM Dextrose, 9.03 mM Hepes salt (Research Organics; Cleveland, OH), 11.04 mM Hepes acid (Research Organics). A 1% BSA (fraction V, fatty acid free, Pierce Biotechnology, Rockford, IL) Ringer solution was prepared before each experiment by adding the appropriate amount of BSA to the Ringer. This was used as the perfusate.

The test perfusates consisted of red cell markers and test mediator(s) in a 1% BSA Ringer solution. The mediators included ET-1, PGI2, and PAF. The doses for the various mediators were determined from previous studies and confirmed by our own dose-response data using endothelin-1, prostacyclin, and platelet-activating factor (2, 4, 5, 29, 32, 36, 37, 41).

Adult female Sprague-Dawley rats were anesthetized with subcutaneous pentobarbital sodium (60 mg/kg body wt). The bowel mesentery was gently exposed and positioned on an inverted microscope stage (Diaphot, Nikon, Melville, NY). The animal’s body temperature was maintained at 37°C throughout the study. The mesentery was continuously bathed in Ringer solution.

Postcapillary venules, 20–30 μm in diameter and at least 400 μm in length, were identified based on flow patterns. Vessels with no evidence of leukocyte adherence or side branches were chosen. The vessels were cannulated with micropipettes attached to a water manometer to control hydrostatic perfusion pressure.

**Measurement of Hydraulic Permeability**

Single vessel Lp was determined using the modified Landis micro-occlusion technique (21). The assumptions and limitations of this
technique have been previously described (11). Because this technique measures water flux at the level of the postcapillary venule, it effectively eliminates any effect on water flux due to changes in arteriolar vasconstriction or vasodilation. Moreover, because postcapillary venules are mostly devoid of smooth muscle cells, we do not appreciate any change in diameter of the venules during the experiments, and irrespective of whether test mediators have vasodilatory or vasoconstrictive effects, this does not influence the measurable changes in hydraulic permeability. Initial cell velocity (dl/dt) was determined by recording marker cell position as a function of time. Transmural water flux per unit area (Jv/S) was calculated by the equation: 

\[ J_v/S = (dl/dt)(r/2l) \]

where \( r \) is the capillary radius and \( l \) is the initial distance between the marker cell and the occluded site. Hydraulic permeability (Lp) was determined by using a modified version of Starling’s equation of fluid filtration:

\[ L_p = J_v/S \cdot (P_c - P_o) / \sigma \Delta \pi \]

where \( P_c \) is the capillary hydrostatic pressure, \( P_o \) is the interstitial hydrostatic pressure, \( \sigma \) is the osmotic reflection coefficient, and \( \Delta \pi \) is the osmotic pressure difference. Assuming \( P_o \) was near zero and \( \sigma \Delta \pi \) (units = cmH₂O) remained constant at 3.78 for 1% BSA Ringer solution, \( L_p \) was calculated from the slope of the regression of \( J_v/S \) on \( P_c \), where \( P = (P_c - 3.78) \). This was derived from several occlusions at three different perfuse pressures. Each \( n \) signifies that a single vessel was cannulated and the \( L_p \) serially measured in a single rat, such that an \( n = 6 \) means that six different vessels were studied in six different rats. Control studies that document the stability of this model over time and after multiple recanalizations of the vessels have been previously reported (34).

In all the studies, each individual venule was initially cannulated and perfused with 1% BSA/mammalian Ringer solution for 10 min, and \( L_p \) measurements were obtained as baseline controls. To determine the effect of PAF on \( L_p \) during PG12 synthase inhibition in postcapillary venules, we first measured \( L_p \) at baseline and every 5 min during the continuous administration of 10 nM PAF alone for 30 min (\( n = 6 \)). Next, to determine whether ET-1 reverses PAF-induced increases in hydraulic conductivity, we measured \( L_p \) in another group of rats during continuous perfusion of 80 pM ET-1 plus 10 nM PAF for 30 min (\( n = 6 \)). Then, to test the effects of PG12 synthesis inhibition on the effects of ET-1, in another group of rats, venules were continuously perfused for 30 min with the PGI2 synthesis inhibitor tranylcypromine at concentrations of 100 \( \mu \)M (\( n = 3 \)) and 500 \( \mu \)M (\( n = 3 \)). We then administered 80 pM ET-1 plus 10 nM PAF continuously for 30 min and measured \( L_p \) every 5 min. Finally, to determine the effect of PAF plus ET-1 on \( L_p \), during PGI2 receptor blockade in postcapillary venules, in another group of rats, we measured \( L_p \) at baseline. Venules were then continuously perfused for 40 min with the PGI2 receptor antagonist CAY-10441 at concentrations of 2 \( \mu \)M (\( n = 3 \)) and 20 \( \mu \)M (\( n = 3 \)).

**Statistical Analysis**

To measure PG12 release after ET-1 administration, sequential doses of ET-1 were compared using Pearson correlation coefficients. Comparisons between control and sample means for in vitro endothelial transmonolayer flux measurements and the in vivo Landis calculations were made with single-factor ANOVA. Differences between measures of \( L_p \) were evaluated with a paired Student’s t-test or repeated-measures ANOVA, as appropriate. Percentages are expressed as means ± SE based on a normal distribution. P values <0.05 were considered statistically significant. Values for \( L_p \) are expressed as means ± SE × 10⁻⁷ cm/s·cm H₂O⁻¹, except when fold increases or percentage change are reported.

**RESULTS**

**ET-1 Produced a Dose-Dependent Increase in Prostacyclin Release**

Prostacyclin release increased in a dose-dependent manner as ET-1 concentration increased from 0.008 to 8 \( \mu \)M (Fig. 1). Prostacyclin concentrations varied at baseline from 126 ± 25 (control) to 217 ± 43 pg/ml (\( P < 0.01 \)) and significantly correlated with increasing concentrations of ET-1 (\( r = 0.76, P = 0.003 \)).

**Endothelial Transmonolayer Flux**

Transmonolayer flux of biotinylated albumin increased after PAF was administered, and ET-1 and PGI2 attenuated this increase. Prostacyclin synthesis inhibition and prostacyclin receptor blockade reversed the attenuating effects of ET-1. In the presence of 10 nM PAF, transmonolayer flux increased 1.7-fold from control to peak absorbance (controls = 0.167 ± 0.01; PAF = 0.288 ± 0.06; Fig. 2). Adding 80 pM ET-1 to 10 nM PAF reduced absorbance by 46% from PAF alone (to 0.158 ± 0.01, \( P < 0.01 \)). Similarly, adding 10 \( \mu \)M PGI2 to 10 nM PAF reduced absorbance by 52% from PAF alone (to 0.110 ± 0.01, \( P < 0.01 \)).
0.139 ± 0.02, *P < 0.01). Pretreatment with 500 μM tranylcypromine reversed the attenuating effects of ET-1 plus PAF by 92% [ET-1 + PAF + tranylcypromine (500 μM) = 0.278 ± 0.01 vs. 0.158 ± 0.01, *P < 0.01]. Finally, pretreatment with 20 μM CAY-10441 followed by exposure to 80 pM ET-1 plus 10 μM PAF reversed the attenuating effects of ET-1 by 47% (to 0.219 ± 0.01, *P < 0.05; Fig. 2).

**In Vivo Measurement of Hydraulic Conductivity**

Next, we investigated the effects of ET-1 and PGI2 on rat mesenteric venules exposed to PAF by using an in vivo method of measuring hydraulic conductivity. With PAF 10 μM alone, \( L_p \) increased by fourfold over controls (peak \( L_p \) PAF = 4.50 ± 0.95, peak \( L_p \) controls = 1.00 ± 0.09, *P < 0.01). In contrast, adding 80 pM ET-1 to 10 μM PAF decreased \( L_p \) compared with PAF alone (peak \( L_p \) ET-1 + PAF = 0.76 ± 0.09, *P < 0.01). Similarly to ET-1, PGI2 (10 μM) added to 10 μM PAF decreased \( L_p \) compared with PAF alone (peak \( L_p \) PGI2 + PAF = 0.72 ± 0.11, *P < 0.01; Fig. 3). Except when reporting fold increases or percentage change, the units for \( L_p \) values are means ± SE \( \times 10^{-7} \) cm·s\(^{-1}\)·cmH\(_2\)O\(^{-1}\).

In the rat in vivo model, pretreatment with tranylcypromine or CAY-10441 before administering ET-1 reversed the hydraulic conductivity-attenuating effect of ET-1 during PAF. Pretreatment with tranylcypromine (100 and 500 μM) before adding 80 pM ET-1 plus 10 nM PAF reversed the leak-attenuating effects of ET-1 during PAF administration (Fig. 4). When PAF effects peaked at 10 min, 100 μM tranylcypromine attenuated the decrease in \( L_p \) induced by ET-1 during PAF exposure by 39% (peak \( L_p \) tranylcypromine 100 μM = 2.20 ± 0.31, *P < 0.01). At 500 μM, tranylcypromine attenuated the decrease in \( L_p \) by 55% (peak \( L_p \) tranylcypromine 500 μM = 2.81 ± 0.67, *P < 0.01). Similar studies with CAY-10441 showed that pretreatment with CAY-10441 at the higher dose (20 μM) had a similar \( L_p \)-attenuating effect as tranylcypromine during ET-1 plus PAF (Fig. 5). At peak effect, CAY-10441 (20 μM) reversed the decrease in \( L_p \) by ET-1 during PAF by 45% (\( L_p \) = 2.46 ± 0.01, *P < 0.01). CAY-10441 pretreatment at the lower dose (2 μM) had no effect (\( L_p \) = 0.85 ± 0.04, *P = 0.56).

For comparison, an integrated area under the curve (AUC) analysis was performed for the \( L_p \) data obtained with the PGI2 synthesis inhibitor and PGI2 receptor antagonist (Fig. 6). The AUC increased for PAF-induced changes in hydraulic conductivity [AUC = 83 ± 17 (10\(^{-7}\) cm·s\(^{-1}\)·cmH\(_2\)O\(^{-1}\))·min]. Subsequent addition of ET-1 or PGI2 diminished hydraulic conductivity, manifesting as a 73 and 76% decreased AUC, respectively, compared with PAF (AUC: ET-1 + PAF = 22.5 ± 2.7, PGI2 + PAF = 19.8 ± 2.2, *P < 0.05). Tranylcypromine at 100 and 500 μM reversed the hydraulic permeability-
attenuating effect of ET-1 during PAF by 34 and 58%, respectively (AUC: ET-1 + PAF + tranylcypromine 100 μM = 42.6 ± 3.8, ET-1 + PAF + tranylcypromine 500 μM = 57.1 ± 1.1, P < 0.01). Although CAY-10441 at 2 μM had no effect compared with ET-1 + PAF, CAY-10441 at 20 μM reversed the hydraulic permeability-attenuating effect of ET-1 during PAF by 41% (AUC: CAY-10441 20 μM = 47.2 ± 7.9, P < 0.05).

DISCUSSION

The vasoactive mediators ET-1 and PGI2 have been previously demonstrated to decrease hydraulic permeability (2, 3, 23, 33). We recently found that pretreatment of rat mesenteric venules with either ET-1 or PGI2 completely abolished the increases in hydraulic conductivity observed after PAF administration, and adding ET-1 and PGI2 after PAF attenuated hydraulic conductivity by 52 and 57%, respectively (10). The novel findings of this paper suggest that the mechanism of ET-1 attenuation of hydraulic conductivity in vivo during PAF may be due to PGI2 release stimulated by ET-1. In this study, we provide the following evidence suggesting that ET-1 mediates its permeability-decreasing effects during PAF: 1) ET-1 induces a dose-dependent increase in PGI2 production in cultured bovine pulmonary endothelial cells; 2) in vitro administration of ET-1 during PAF attenuates transmonolayer flux of biotinylated albumin, and this effect is subsequently reversed when either a prostacyclin synthesis inhibitor (tranylcypro- 
mime) or prostacyclin receptor blocker (CAY-10441) is added; 3) in a rat in vivo model, pretreatment with a prostacyclin synthesis inhibitor (tranylcypro- 
mime) or prostacyclin receptor blocker (CAY-10441) before administering ET-1 reversed the hydraulic conductivity-attenuating effect of ET-1 during PAF. Taken together, these findings suggest that ET-1 decreases microvascular permeability during PAF by stimulating PGI2 release.

Experimental evidence observed in vitro suggests a causal relationship between ET-1 binding and PGI2 release. In rat aortic endothelial cells, ET-1 induces PGI2 production through a mechanism mediated by calcium and PKC, and calcium antagonism decreases downstream PGI2 production in the presence of ET-1 (24). In peripheral-lung vascular smooth muscle cells exposed to antagonists of ETA and ETB receptors, ET-1 regulates cyclooxygenase via ETA stimulation and phosphorylation of p38 and p44/42 mitogen-activated protein kinase, which may regulate downstream PGI2 production (8). Finally, in human umbilical vein endothelial cells (HUVEC) the addition of quercetin (an ET-1 antagonist) decreased the downstream production of PGI2 (40).

Other in vitro studies also suggest that ET-1 and PGI2 release may be linked. A sickle cell anemia model using activated endothelial cells demonstrated that during sickling, both ET-1 and PGI2 release were increased, but in the presence of inflammatory stimuli (IL-1), ET-1 release stayed constant while PGI2 release increased. This disparate finding suggests there may be alternative pathways leading to prostacyclin release in vitro (31). In cultured retinal microvascular endothelial cells subjected to variations of pulsatile flow, ET-1 and PGI2 increased with increased flow rates, but the regulatory activity of nitric oxide (NO) synthase was the trigger for PGI2 release, not ET-1 (38). These findings from both studies suggest that ET-1 can directly and indirectly stimulate PGI2 release, yet few investigators have been able to directly demonstrate a dose-response relationship to the production of PGI2 via ET-1. However, in 2002, an in vitro model of HUVEC incubated with varying levels of ET-1 provided evidence that ET-1 increased production of PGI2 (30). These results are consistent with the results of our in vitro experiments, which demonstrate that a dose-dependent increase of PGI2 occurs with increasing dosages of ET-1.

Increases in PGI2 release after ET-1 administration have also been suggested by in vivo models. In rat and pig lung, release of PGI2 and thromboxane are induced by ET-1 (12), and PGI2 inhibition via indomethacin diminishes ET-1-mediated stimu-
latory activity in rat mesenteric arteries (27). In a murine model, markers of platelet aggregation inhibition after ET-1 administration were dependent on PGI2 production and regulated by COX-2 and NO production (20). In perfusates of cat skeletal-muscle vasculature subjected to varying levels of ET-1, the capillary fluid coefficient (CFC) was reduced with ET-1, and tranylcypro- 
mime and ETB antagonism enhanced the CFC (23). The authors of that study also demonstrated in a similar animal model that, at low doses, PGI2 decreases permeability to albumin across the microvasculature by 25–30% and concluded that PGI2 was the key mediator in reducing the CFC and that its actions were mediated by stimulation of the ETB receptor (3, 23). We previously demonstrated that ET-1 decreases postcapillary water flux via PGI2 release (33, 35). In those studies, hydraulic conductivity measured in rat mesenteric venules was attenuated with PGI2, and when PGI2 synthesis was inhibited, ET-1 hydraulic conductivity-attenuating effects were reversed. We recently showed that ET-1 and PGI2 similarly decreased hydraulic conductivity before and after PAF administration (10). Our present study adds to this growing body of evidence that ET-1 decreases microvascular permeability through PGI2 release by demonstrating, in vitro and in vivo, that PGI2 synthesis inhibition and PGI2 receptor
blockade reverses the transmonolayer flux and hydraulic conductivity attenuating effects of ET-1 during PAF.

The mechanism that signals ET-1-induced PGII2 release remains unknown. Endothelial cell receptors for ET-1 are associated with G protein and increase cyclic nucleotide secondary messengers that stimulate PGII2 production, ultimately leading to protein kinase activation and dramatic cytoskeletal changes (3, 33). We and others have implicated cGMP as a central mechanism to ETB1 function and concomitant PGII2 release in vitro and in vivo (13, 22, 28). These experimental models demonstrate that proinflammatory stimuli induce production of ET-1, activating phospholipase C (PLC), which hydrolyzes membrane phosphoinositides to inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 and DAG then act as intracellular secondary messengers that function to liberate arachidonic acid from endothelial membrane phospholipids. IP3 and DAG increase the downstream release of intracellular calcium, which may regulate release of PGII2 directly or through NO (9, 11, 30). These results suggest that multiple mechanisms may exist for PGII2 production from arachidonic acid, which may be responsible for the attenuation in microvascular permeability seen in a proinflammatory state after PAF administration. There is some evidence that PGII2 production shares the PLC and IP3/DAG pathway, and this may be another mechanism whereby ET-1 stimulates PGII2 release (26). Another pathway where arachidonic acid-second messenger systems implicate ET-1-mediated PGII2 release is via protein kinase C (PKC; Ref. 25). ET-1 may activate PKC, which activates phospholipase A2 (PLA2), which directly liberates arachidonic acid and subsequently increases PGII2 production and release (25). However, protein kinase activity may have other intracellular functions that can alternatively govern the release of PGII2, not just through activation of the PKC-PLA2-arachidonic acid-PGII2 pathway.

Uncontrolled fluid extravasation in severely injured and septic patients has been linked to adult respiratory distress syndrome, multiple organ failure, and the systemic inflammatory response syndrome (41). No pharmacologic interventions exist to prevent or reverse the increase in microvascular permeability or capillary leak syndrome observed in these scenarios. Furthering our understanding of the mechanistic pathways and mediator interactions could lead to the development of a treatment for pathologic increases in fluid extravasation. The ability of ET-1 and PGII2 to decrease fluid extravasation from the intravascular compartment into tissue interstitial spaces may be amenable to pharmacologic manipulation and may be a treatment option for patients in septic shock or after trauma.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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