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Endothelin-1 and Prostacyclin Attenuate Increases in Hydraulic Permeability due to Platelet-activating Factor in Rats

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Abstract

We have previously documented that endothelin-1 (ET-1) and prostacyclin (PGI₂) decrease basal state hydraulic permeability (L_p). The aim of this study was to investigate the ability of ET-1 and PGI₂ to modulate trans-endothelial fluid flux during situations in which L_p was artificially elevated with platelet activating factor (PAF). We hypothesized that ET-1 and PGI₂ administration before PAF exposure would prevent the increase in L_p secondary to PAF. Additionally, in a potentially more clinically relevant situation, we also hypothesized that ET-1 and PGI₂ administration after PAF exposure would attenuate the increase in L_p secondary to PAF. Microvascular L_p was measured in rat mesenteric post-capillary venules. Exposure to 10 nM PAF increased L_p 4-fold ($p < 0.001$). If the administration of 80 pM ET-1 or 10 μ M PGI₂ was completed prior to PAF exposure, no PAF-associated increase in L_p was observed ($p < 0.001$). The administration of ET-1 or PGI₂ after PAF exposure attenuated the peak increase in L_p due to PAF alone by 55% and 57%, respectively ($p < 0.001$). We conclude that ET-1 and PGI₂ administration prior to PAF exposure prevents PAF-induced elevations in L_p , and in a more clinically relevant situation, ET-1 and PGI₂ administered after PAF exposure attenuates the PAF-induced increase in L_p . ET-1 and PGI₂ receptors may provide important therapeutic targets for decreasing the microvascular fluid leak-associated morbidity resulting from shock and sepsis.

Keywords

microvascular fluid leak; inflammation; shock

Introduction

Endothelial barrier dysfunction transpires from the complex cascade of events that occurs during severe injury and shock. As endothelial dysfunction develops, microvascular permeability increases and intravascular fluid is lost to the interstitium. This endothelial dysfunction and subsequent microvascular fluid leak may decrease intravascular volume, worsen organ perfusion, and lead to the systemic inflammatory response syndrome and multiple organ failure.(1–3) Not only are the clinical consequences of this scenario devastating, but the cost and utilization of resources associated with the care of these patients are tremendous as well.(4)

Two potential pharmacological targets are the endothelial cell receptors for endothelin-1 (ET-1) and prostacyclin (PGI₂). Both ET-1 and PGI₂ are endothelial-derived vasoactive

mediators. ET-1 is a 21-amino acid peptide synthesized by endothelial cells,(5) and PGI₂ is an eicosanoid synthesized from arachadonic acid by vascular endothelial cells and vascular smooth muscle.(6) The pharmacologically stable prostacyclin analogue, iloprost, has been used clinically as a vasodilator to treat pulmonary hypertension and Raynaud's phenomenon, as well as to decrease platelet aggregation.(6,7) Previous studies have shown that ET-1 and PGI₂ are involved in maintaining endothelial barrier integrity and decreasing microvascular hydraulic permeability.(5,8–10) In addition, ET-1 may exert its permeability-decreasing effect by stimulating PGI₂ production.(5,8) Since PGI₂ release appears to be involved in the microvascular permeability-decreasing action of ET-1, (5,8) PGI₂ may also be able to prevent increases in microvascular permeability due to inflammatory mediators. To further investigate the potential role of ET-1 and PGI₂ in minimizing endothelial cell barrier dysfunction, we set out to examine the interaction of ET-1 and PGI₂ with platelet activating factor (PAF).

The physiologic responses to PAF observed in the laboratory mimic clinical sepsis, shock, and multiple organ failure. Because of this similarity, it has been suggested that PAF plays a major role in the pathophysiology of sepsis and shock.(11,12) PAF has been associated with dramatic increases in vascular permeability and extravascular fluid leak,(11–13) and the dysfunction of the endothelial barrier that results from PAF has been described in several studies.(14–16) It is thought that PAF may modulate endothelial barrier functions by directly activating endothelial cells.(14–16)

The aim of this study was to investigate the ability of ET-1 and PGI₂ to modulate trans-endothelial fluid flux during situations in which hydraulic permeability was elevated with PAF. In particular we were interested in whether the pretreatment with ET-1 and PGI₂ would prevent the detrimental endothelial effect of PAF. In addition, because clinical therapies are usually started after the pathophysiology has already set in, we were interested in whether the administration of ET-1 and PGI₂ after PAF exposure would attenuate the detrimental endothelial effect of PAF. We hypothesized the following: 1) ET-1 and PGI₂ pretreatment prevents the increase in hydraulic permeability observed from subsequent PAF exposure, and 2) ET-1 and PGI₂ attenuate PAF-induced increases in hydraulic permeability even when administered after PAF exposure.

Materials and Methods

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

Animal and solution preparations

Preparation of the animals as well as preparation of the mammalian Ringer's solution has been described previously.(17) They are briefly described below.

Red blood cells that are used as flow markers were harvested from female Golden Syrian hamsters (140–180g; Harlan, Indianapolis, In.). The blood was centrifuged to remove the plasma and buffy coat, and then washed three times in 15 ml of mammalian Ringer's solution.

The Ringer's 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% bovine serum albumin (BSA crystallized, Sigma Chemical, St Louis, MO) Ringer's solution was prepared prior to each experiment by adding the appropriate amount of BSA to the Ringer's. This was used as the perfusate.

The test perfusates consisted of hamster red cell markers and test mediator(s) in a 1% BSA Ringer's solution. The mediators included endothelin-1 (American Peptide Co, Sunnyvale, CA), prostacyclin (Biomol Research Lab, Plymouth Meeting, PA), and platelet-activating factor-16 (Calbiochem, EMD Biosciences Inc, San Diego, CA). The doses for the various mediators were determined from previous studies on microvascular permeability and confirmed by our own dose response data using endothelin-1, prostacyclin, and platelet-activating factor.(5,8,9,18–20)

Animal Preparation

Adult female Sprague-Dawley rats (250–310 g; Hilltop Lab Animals Inc., Scottsdale, Pa.) were anesthetized with subcutaneous sodium pentobarbital (60 mg/kg body weight). 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's solution.

Postcapillary venules, 20 to 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 for control of hydrostatic perfusion pressure.

Measurement of Hydraulic Permeability

Single vessel L_p was determined using the modified Landis micro-occlusion technique. The assumptions and limitations of this model have been previously described.(11) Initial cell velocity (dl/dt) was obtained by recording marker cell position as a function of time. Transmural water flux per unit area (J_v/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. Determination of hydraulic permeability (L_p) was based on a modified version of Starling's equation of fluid filtration: $L_p = J_v/S[(P_c - P_i) - \sigma\Delta\pi]$, where P_c is the capillary hydrostatic pressure, P_i is the interstitial hydrostatic pressure, σ is the osmotic reflection coefficient, and $\Delta\pi$ is the osmotic pressure gradient. Assuming P_i was near zero and $\sigma\Delta\pi$ remained constant at 3.78 for 1% BSA Ringer's solution, L_p was calculated from the slope of the regression of J_v/S on P , where $P = (P_c - 3.78)$. This was derived from several occlusions at three different perfusate 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 6 different vessels were studied in 6 different rats. Control studies that document the stability of this model over time and after multiple recannulations of the vessels have been previously reported.(17)

Experimental Design

PAF controls—We have previously reported a 5-fold increase in L_p due to continuous PAF perfusion.(19) The recannulation and double exposure of PAF to postcapillary venules was necessary as appropriate controls for some study groups involving recannulation and double exposure of PAF (figures 4–8). In this set of control studies, baseline L_p measurements were obtained, and then each microvessel was perfused for 5 minutes with 10 nM PAF in Ringer's/BSA solution ($n=6$). Study vessels were recannulated and again perfused with 10 nM PAF in Ringer's/BSA solution for the remainder of the 30 minute study period. Measures of L_p were obtained at three to five minute intervals during of the perfusion period. For study groups involving a single exposure of PAF, the single PAF exposure controls were used for comparison (figures 2 and 3).

Effect of ET-1 on L_p when given prior to PAF—The impact of ET-1 on L_p when administered prior to PAF exposure was evaluated ($n=6$). After baseline L_p measurements were obtained, microvessels were first perfused for 5 minutes with 80 pM ET-1 in Ringer's/BSA

solution. Next, the venules were recannulated and perfused with 80 pM ET-1 plus 10 nM PAF in Ringer's/BSA solution for the remainder of the 30 minute study period. Measurements of L_p were obtained at three to five minute intervals for the duration of the perfusion period.

Effect of PGI₂ on L_p when given prior to PAF—Next we examined the impact of PGI₂ (10 μ M) on L_p when administered prior to PAF exposure (n=6). After baseline L_p measurements were obtained, microvessels were first perfused for 5 minutes with 10 μ M PGI₂ in Ringer's/BSA solution. Next, the venules were recannulated and perfused with 10 μ M PGI₂ plus 10 nM PAF in Ringer's/BSA solution for the remainder of the 30 minute study period. Measurements of L_p were obtained at three to five minute intervals for the duration of the perfusion period.

Effect of ET-1 on L_p in microvessels previously exposed to PAF—A more clinically relevant scenario is the exposure of endothelial cells to PAF before treatment with ET-1. The effect of ET-1 perfusion after endothelial cell activation with PAF was evaluated in another set of studies (n=6). After baseline L_p measurements were obtained, microvessels were first perfused for 5 minutes with 10 nM PAF in Ringer's/BSA solution. Next, the venules were recannulated and continuously perfused with 80 pM ET-1 plus 10 nM PAF in Ringer's/BSA solution for the remainder of the 30 minute study period. Measurements of L_p were obtained at three to five minute intervals for the duration of the perfusion period.

Additionally, to investigate the effect of ET-1 alone after an initial PAF exposure, the experiment was repeated in another set of animals without the continuation of PAF. After an initial perfusion for 5 minutes with 10 nM PAF in Ringer's/BSA solution, the venules were recannulated and perfused with 80 pM ET-1 alone in Ringer's/BSA solution for the remainder of the 30 minute study period (n=3). Measurements of L_p were obtained at three to five minute intervals for the duration of the perfusion period.

Effect of PGI₂ on L_p in microvessels previously exposed to PAF—The same set of experiments as above was performed using PGI₂ (10 μ M) in place of ET-1 in order to examine the impact of PGI₂ on L_p in microvessels previously exposed to PAF (n=6). After baseline L_p measurements were obtained, microvessels were first perfused for 5 minutes with 10 nM PAF in Ringer's/BSA solution. Next, the venules were recannulated and continuously perfused with 10 μ M PGI₂ plus 10 nM PAF in Ringer's/BSA solution for the remainder of the 30 minute study period. Measurements of L_p were obtained at three to five minute intervals for the duration of the perfusion period.

Additionally, to investigate the effect of PGI₂ alone after an initial PAF exposure, the experiment was repeated in another set of animals without the continuation of PAF. After an initial perfusion for 5 minutes with 10 nM PAF in Ringer's/BSA solution, the venules were recannulated and perfused with 10 μ M PGI₂ alone in Ringer's/BSA solution for the remainder of the 30 minute study period (n=3). Measurements of L_p were obtained at three to five minute intervals for the duration of the perfusion period.

Effect of simultaneous PAF, ET-1 and PGI₂ on L_p when given after PAF—Next we examined the impact of ET-1 plus PGI₂ on L_p when administered in combination after to PAF exposure (n=3). After baseline L_p measurements were obtained, microvessels were first perfused for 5 minutes with 10 nM PAF in Ringer's/BSA solution. Next, the venules were recannulated and continuously perfused with 80 pM ET-1 and 10 μ M PGI₂, plus 10 nM PAF in Ringer's/BSA solution for the remainder of the 30 minute study period. Measurements of L_p were obtained at five minute intervals for the duration of the perfusion period.

Statistics

Comparisons between baseline measurements and test solution measurements of L_p were made with paired Student's *t*-test. Group means of sequential measurements were analyzed with repeated measures ANOVA with post hoc analysis. Comparisons between measurements of L_p from different groups were made using unpaired *t*-test. Statistical significance was set at an alpha error of 5%. All values for L_p are represented as mean $\pm$ SEM $\times 10^{-7}$ cm $\cdot$ s $^{-1}$ ·cmH $_2$ O $^{-1}$.

Results

PAF controls

Consistent with a one-time exposure of PAF continuously over 30 minutes,(19) the re-exposure of PAF after 5 minutes of initial PAF perfusion resulted in a 4-fold increase in L_p compared to baseline (4.67 ± 0.43 compared to 1.27 ± 0.14 , $p < 0.001$). The elevation in L_p persisted for approximately 25 minutes ($p < 0.001$, Figure 1).

Effect of ET-1 on L_p when given prior to PAF

ET-1 administrative for 5 minutes before PAF exposure prevented the 4-fold increase in L_p seen with PAF alone ($p < 0.001$). After initial perfusion of ET-1, there was no elevation in L_p during the subsequent perfusion with ET-1 plus PAF for the following 25-minute period. The L_p never increased above baseline levels of 1.06 ± 0.04 in venules pre-treated with ET-1. Compared to a peak L_p of 4.67 ± 0.43 after 20 minutes of perfusion with PAF alone, ET-1 administration followed by PAF exposure resulted in an L_p of only 0.86 ± 0.07 ($p < 0.001$, Figure 2). Each data point represents the group mean L_p at that particular time point.

Effect of PGI $_2$ on L_p when given prior to PAF

Almost identical to ET-1, the administration of post-capillary venules with PGI $_2$ blocked any increase in L_p during subsequent PAF exposure. Compared to peak L_p elevations of 4-fold with PAF alone ($L_p = 4.67 \pm 0.43$), PGI $_2$ administration followed by PAF exposure resulted in an L_p measurement that was significantly reduced to 0.89 ± 0.04 ($p < 0.001$, Figure 3). In other words, PGI $_2$ prevented the increase in L_p caused by PAF.

Administration of ET-1 in microvessels previously exposed to PAF

Administration of ET-1 after PAF exposure started to decrease L_p from PAF alone levels at 12 minutes. This effect reached a peak at 15 minutes and continued to the end of the experiment. At 15 minutes, PAF alone increased L_p 4-fold while treatment with ET-1 attenuated this by 55%. This corresponded to a peak L_p elevation of 4.67 ± 0.46 with PAF alone which was significantly lower at 2.11 ± 0.09 with ET-1 treatment ($p < 0.001$, Figure 4). When the experiment was repeated administering ET-1 alone after initial, but not continued PAF exposure, L_p at 15 minutes was still significantly lower than PAF alone at 1.86 ± 0.10 ($p = 0.0005$, Figure 6).

Administration of PGI $_2$ in microvessels previously exposed to PAF

PGI $_2$ administration had a similar effect on PAF-induced elevations in L_p as ET-1 treatment. Administration of PAF and PGI $_2$ after PAF exposure started to decrease L_p from PAF alone levels at 10 minutes. This effect reached a peak at 15 minutes and continued to the end of the experiment. At 15 minutes, PAF alone increased L_p 4-fold while treatment with PAF and PGI $_2$ attenuated this by 57%. This corresponded to a peak L_p elevation of 4.67 ± 0.46 with PAF alone which was significantly lower at 2.00 ± 0.21 with PGI $_2$ treatment ($p < 0.001$, Figure 5). When the experiment was repeated administering PGI $_2$ alone after initial, but not continued

PAF exposure, L_p at 15 minutes was still significantly lower than PAF alone at 2.37 ± 0.10 ($p = 0.003$, Figure 7).

Effect of simultaneous PAF, ET-1 and PGI₂ on L_p when given after PAF

Administration of simultaneous PAF, ET-1 and PGI₂ after PAF had a similar effect on as either ET-1 or PGI₂ after PAF pretreatment. Compared to a peak L_p elevation of 4.67 ± 0.43 with PAF alone at 15 minutes, L_p was 2.51 ± 0.16 with PAF, ET-1 and PGI₂ after PAF ($p = 0.005$, Figure 8).

Discussion

PAF plays a major role in the pathophysiology of sepsis and shock.(11,12) It has been associated with dramatic increases in vascular permeability and extravascular fluid leak,(11–13) as well as endothelial barrier dysfunction.(14–16) Cell surface receptor studies have identified that the PAF receptor is a seven-transmembrane, G-protein associated receptor (12,21) that activates protein kinase C, stimulates tyrosine kinase, and induces depolymerization and redistribution of F-actin.(12,21) These mechanisms may be responsible for the increase in microvascular hydraulic permeability seen with PAF. Endothelial cell receptors for ET-1 are also G-protein associated and increase cyclic nucleotide second messengers as well as stimulate PGI₂ production ultimately leading to protein kinase activation and cytoskeletal changes.(5,8,18,22) In contrast to PAF, both ET-1 and PGI₂ decrease hydraulic permeability.(5,8–10) We have previously shown that ET-1 diminishes elevations in hydraulic permeability that are secondary to adenosine triphosphate and bradykinin.(9) Meanwhile, others have demonstrated decreases in albumin and protein permeability with PGI₂ administration after elevating permeability with tumor necrosis factor, histamine, and surgical trauma.(23,24) In addition, ET-1 has been shown to decrease hydraulic permeability through the stimulation of PGI₂ synthesis.(5,8)

Given the similarities between ET-1's and PGI₂'s receptors intracellular signaling, and their effect on hydraulic permeability, we hypothesized that ET-1 and PGI₂ would prevent and even reverse the increase in hydraulic permeability induced by PAF. The purposes of this series of experiments were: 1) to examine the effect of ET-1 or PGI₂ administered prior to PAF exposure on PAF-induced elevations in hydraulic permeability, and 2) to determine the individual effects of ET-1 and PGI₂ on hydraulic permeability after endothelial cell activation with PAF. We found that ET-1 and PGI₂ pre-treatment blocked PAF-induced elevations in hydraulic permeability, and in a more clinically relevant scenario, ET-1 and PGI₂ administration reversed the PAF-induced increase in hydraulic permeability.

Even though pre-treatment of venules with ET-1 and PGI₂ prevented the permeability-increasing effects of PAF, pre-treatment is usually not feasible clinically. Therefore, in a more clinically relevant scenario, we also activated postcapillary venules with PAF to simulate an inflamed state and explored the effects of ET-1 or PGI₂ administration in the activated endothelium. After activating the endothelial cells and increasing the L_p with PAF, ET-1 blunted the PAF-induced elevation in L_p by 55%. PGI₂ also reduced the 4-fold increase in L_p due to PAF by 57%.

ET-1 and PGI₂ may blunt the effects of subsequent PAF exposure and prevent elevations in hydraulic permeability through a MLCK-independent pathway. Studies on human umbilical vein endothelial cells (HUVEC), bovine pulmonary artery endothelial cells (BPAEC), and coronary venules have demonstrated both MLCK-dependent and MLCK-independent pathways that alter endothelial cell permeability. The MLCK-dependent mechanism involves the following sequential steps: 1) an elevation in intracellular calcium concentration; 2) activation of MLCK; 3) increased myosin light chain (MLC) phosphorylation; 4) increased

actin stress fibers; 5) increased intercellular gap formation; and 6) an elevation in endothelial cell permeability to bovine serum albumin.(16,25) In contrast, the MLCK-independent pathway utilizes other kinases such as Rho kinase, Raf-1 kinase, and extracellular signal-regulated kinases to increase actin stress fibers, intercellular gap formation, and endothelial cell permeability.(26–29)

Further studies on the MLCK-independent pathway have demonstrated the role of cAMP and protein kinase A in maintaining and reducing elevations in endothelial cell permeability.(22, 27,29) The MLCK-independent pathway of ET-1 and PGI₂ may also involve increasing cAMP synthesis and activating protein kinase A. Earlier studies demonstrated the protective roles of cAMP and protein kinase A against increases in endothelial cell permeability caused by thrombin.(26) More recently, Qiao et al (28) linked the effects of cAMP on endothelial cell permeability to protein kinase A, while Birukova et al (22) demonstrated that the cAMP/protein kinase A pathway for preventing increases in endothelial cell permeability is mediated through decreased Rho kinase activity and MLC phosphorylation levels by a MLCK-independent pathway. Consistent with these studies, data from our laboratory showed that ET-1 exerts its permeability-decreasing effects through PGI₂ and subsequent increases in cAMP synthesis and cAMP-specific protein kinase A activation.(8,18) ET-1 and PGI₂ may increase the synthesis of cAMP followed by cAMP-specific protein kinase A activation. This would lead to decreased Rho kinase activity and MLC phosphorylation levels and ultimately result in reduced actin stress fiber formation, endothelial cell gap formation, and a decrease in hydraulic permeability.

ET-1 has been found clinically at a concentration of 2–6 pg/ml and have been shown to increase two to eight times above normal during pathological conditions, (5) including shock and sepsis. (10) The dosing of ET-1 in this study is similar to our and others' previous investigations (5, 8–10,18,20). This is higher than concentrations which been measured in human plasma, however, it is estimated that concentrations seen locally can be much higher than those measured centrally. Prostacyclin concentrations in humans are reported to be 1 pg/ml at baseline and can increase to 200 pg/ml during administration of prostacyclin at 2 ng/kg/min. (6) Our dosing is similar to our and others' previous investigations.(6,8)

ET-1 and PGI₂ receptors may therefore provide important therapeutic targets for decreasing the microvascular fluid leak-associated morbidity resulting from shock and sepsis. Due to vasodilatory and platelet aggregation activity, PGI₂ analogs such as iloprost are beneficial in patients with pulmonary artery hypertension and Raynaud's phenomenon. (6,7) Iloprost, like PGI₂, decreases microvascular permeability induced by histamine,(6,30) tumor necrosis factor- α and ischemia-reperfusion.(31,32) The use of iloprost and other PGI₂ analogues may therefore be a novel strategy to decrease the microvascular leak occurring in patients after inflammatory insults.(33,34)

Interestingly, endothelin-1 *antagonists* are also used therapeutically in pulmonary hypertension (7) and have shown potential in the treatment of septic shock(35). It may be that endothelin's vasoconstrictive effects on arteries are separate from its leak-reducing properties in postcapillary venules. This may be related to the distinct anatomical expression of ET-1's two different receptors; the ET_A subtype receptor located on vascular smooth muscle cells which regulates arterial vasoconstriction, and the ET_B, subtype receptor located primarily on endothelial cells which may be responsible for the microvascular permeability-decreasing effects of ET-1. Furthermore, through an ET_A receptor-dependent process, ET-1 is also a potent secretagogue for atrial natriuretic peptide (ANP). Both ET-1 and ANP are known to have a hemoconcentrating effect in whole animal studies and both increase hydraulic permeability in perfused single vessels. These observations suggest that ANP is a secondary mediator that may contribute to the response ET-1.(20) Taken together, the observations regarding ET-1's subtype receptor distribution and the influence on ANP release may explain the conflicting results of

some studies that examined the effect of ET-1 on vascular permeability (10) and the potentially detrimental vasoconstrictive effects of ET-1 in sepsis.(18)

Uncontrolled fluid extravasation in severely injured and septic patients have been linked to adult respiratory distress syndrome, multiple organ failure, and systemic inflammatory response syndrome.(3) Pharmacologic interventions do not exist to prevent or reverse the increase in microvascular hydraulic permeability seen in these scenarios. By further understanding the mechanistic pathways and mediator interactions, a treatment for pathologic increases in fluid extravasation may be developed in the future. The ability of ET-1 and PGI₂ to decrease third space fluid loss may be of benefit as a treatment option for trauma and septic patients in shock and may be amenable to pharmacologic manipulation.

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Figure 1.

After initially perfusing with PAF for 5 minutes, PAF was reintroduced and perfused for 25 more minutes. This is compared to a continuous one-time exposure of PAF for 30 minutes. Similar to a single exposure of PAF (—■—), the double exposure of PAF (---●---) increased L_p by almost 4-fold compared to baseline. (The * represent statistical significance comparing sequential L_p measurements to baseline, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7}$ cm/sec/cmH₂O; $n=6$)

Figure 2.

After perfusing with ET-1 for 5 minutes, the introduction of PAF failed to increase L_p (—■—). Compared to a peak L_p of 4.67 ± 0.43 after 20 minutes of perfusion with PAF alone (---●---), ET-1 pre-treatment followed by PAF exposure resulted in an L_p of only 0.86 ± 0.07 ($p < 0.001$) after 20 minutes of perfusion. (The * represent a statistical significance between the two groups, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7} \text{ cm/sec/cmH}_2\text{O}$; $n=6$)

PGI₂ Followed by PGI₂+PAF

Figure 3.

Compared to peak L_p elevations to 4.67 ± 0.43 with PAF alone (---●---), the measurements of L_p with PGI₂ pre-treatment and subsequent PAF exposure (—■—) was significantly lower at 0.89 ± 0.04 ($p < 0.001$). (The * represent a statistical significance between the two groups, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7}$ cm/sec/cmH₂O; $n=6$)

Figure 4.

Compared to peak L_p elevations to 4.67 ± 0.46 with PAF alone (---●---) which occur at 15 minutes, the measurements of L_p at the same time point with PAF pre-treatment and subsequent PAF and ET1 exposure (—■—) was significantly lower at 2.11 ± 0.09 ($p < 0.001$). (The * represent a statistical significance between the two groups, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7} \text{cm/sec/cmH}_2\text{O}$; $n=6$)

PAF Followed by PAF+PGI₂

Figure 5.

Compared to peak L_p elevations to 4.67 ± 0.46 with PAF alone (---●---) which occur at 15 minutes, the measurements of L_p at the same time point with PAF pre-treatment and subsequent PAF and PGI₂ exposure (—■—) was significantly lower at 2.00 ± 0.21 ($p < 0.001$). (The * represent a statistical significance between the two groups, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7}$ cm/sec/cmH₂O; $n=6$)

PAF followed by either PAF+ET-1 or ET-1 alone

Figure 6.

Compared to peak L_p elevations to 4.67 ± 0.46 with PAF alone (---●---) which occur at 15 minutes, the measurements of L_p at the same time point with PAF pre-treatment and subsequent exposure to ET-1 alone (---▲---) was significantly lower at 1.86 ± 0.10 ($p=0.0005$). (The * represent a statistical significance between the groups with ET-1 exposure versus PAF alone, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7} \text{cm/sec/cmH}_2\text{O}$; $n=3$)

Figure 7.

Compared to peak L_p elevations to 4.67 ± 0.46 with PAF alone (—●—) which occur at 15 minutes, the measurements of L_p at the same time point with PAF pre-treatment and subsequent exposure to PGI_2 alone (---▲---) was significantly lower at 2.37 ± 0.10 ($p=0.003$). (The * represent a statistical significance between the groups with PGI_2 exposure versus PAF alone, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7} \text{ cm/sec/cmH}_2\text{O}$; $n=3$)

Figure 8.

Compared to peak L_p elevations to 4.67 ± 0.46 with PAF alone (---●---) which occur at 15 minutes, the measurements of L_p at the same time point with PAF pre-treatment and subsequent exposure to simultaneous PAF, ET-1, and PGI₂ (—■—) was significantly lower at 2.51 ± 0.16 ($p = 0.005$). Exposure to simultaneous PAF, ET-1, and PGI₂ after PAF pretreatment results in a similar decrease to exposure to PAF+ET-1 or PAF+ PGI₂ alone. (The * represent a statistical significance between the groups with PGI₂ exposure versus PAF alone, $p < 0.05$; the error bars denote $\pm$ standard error; L_p units are $\times 10^{-7}$ cm/sec/cmH₂O; $n=3$)