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Glucagon-like Peptide-1 Protects Mesenteric Endothelium from Injury During Inflammation

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1. Introduction

Alteration in microvascular fluid permeability is a frequent and often devastating problem in critically ill patients and requires significant resuscitation. Clinically, endothelial hyper-permeability creates generalized edema. This generalized edema, seen frequently in sepsis, shock, and major trauma, contributes to the systemic inflammatory response syndrome (SIRS), abdominal compartment syndrome, and multiple organ dysfunction syndrome (MODS). [25, 27] These complications lead to significant morbidity and mortality.[25,27] Treatment options for these complications of endothelial dysfunction are currently only resuscitative in nature and have little success [14].

Inflammatory mediators impair normal microvascular physiology leading to inter-endothelial gap formation resulting in endothelial hyper-permeability [14,26]. Most of the endothelial hyper-permeability during inflammation occurs at the post-capillary venule [18]. A multitude of mediators attenuate the endothelial hyper-permeability associated with inflammation in experimental models [9,16,29,32,35]. Unfortunately, this has not lead to a clinical treatment for endothelial hyper-permeability or repair of endothelial damage. [14]

Glucagon-like peptide-1 (GLP-1) is a proglucagon-derived hormone secreted by intestinal endocrine cells with multiple local and systemic actions [11]. GLP-1 attenuates ischemia/reperfusion injury to myocytes [3] and inhibits apoptosis in isolated pancreatic beta-cells [12]. GLP-1 directly affects peripheral vascular tissue, improving endothelial function and reducing endothelial-dependent vascular tone [17,31,38].

The GLP-1 receptor is a transmembrane G-protein coupled receptor and has been widely localized in the gastrointestinal tract and recently localized on endothelial cells. [3,5,7] The actions of the GLP-1 receptor are thought to involve cAMP production and protein kinase A (PKA) activation. [23] The role of the GLP-1 receptor and cAMP in the actions of GLP-1 on vascular endothelium has also been suggested [3,19].

We hypothesized that GLP-1 would protect the mesenteric endothelium from injury during inflammation and attenuate the increase in microvascular permeability induced by lipopolysaccharide (LPS). The specific aims of the study were: 1) to determine the effect of GLP-1 on basal microvascular permeability, 2) to determine the effect of GLP-1 on the increase

in microvascular permeability induced by LPS, 3) to determine the involvement of the GLP-1 receptor in the effect of GLP-1 on microvascular permeability, and 4) to determine the involvement of cAMP on the action of GLP-1 on microvascular permeability.

2. Materials and Methods

2.1 Animal Preparation

Adult female Sprague-Dawley rats (250–310 g; Hilltop Lab Animals Inc., Sottdale, PA) were anesthetized with subcutaneous sodium pentobarbital (60 mg/kg body weight). Female rats were used because they have more mesenteric vessels than male rats. The bowel mesentery was gently exposed and positioned on an inverted microscope stage (Diaphot, Nikon, Melville, NY). Body temperature was maintained at 37°C throughout the study. The mesentery was continuously bathed in Ringer's solution.

Mesenteric 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 for control of hydrostatic perfusion pressure.

2.2 Solution Preparation

All studies were approved by and complied with the institutional animal research protocols. Preparation of the animals as well as the mammalian Ringer's solution has been described previously [36]. They are briefly described below.

The test perfusates consisted of rat red blood cell markers, 1% BSA solution, and test mediator (s). Red blood cells are used as flow markers. The velocity of marker red blood cells is measured and used to calculate transmural water flux as detailed in Section 2.3. The red blood cells are harvested from adult female Sprague-Dawley rats (250–310 g; Hilltop Lab Animals Inc., Scottsdale, PA). The blood was centrifuged to remove the buffy coat and then washed three times in 15ml of mammalian Ringer's solution.

The Ringer's solution was prepared daily in distilled deionized water and contained 135 mmol/L NaCl, 4.6 mmol/L KCl, 2.0 mmol/L CaCl_2 , 2.46 mmol/L MgSO_4 , 5.0 mmol/L NaHCO_3 , 5.5 mmol/L dextrose, 9.03 mmol/L Hepes Salt (Research Organics; Cleveland, OH), and 11.04 mmol/L Hepes Acid (Research Organics). A 1% bovine serum albumin (BSA) solution was prepared before each experiment and added to all perfusion solutions (BSA crystallized, Sigma Chemical, St Louis, MO).

The test mediators included Glucagon-Like Peptide I Amide Fragment 7–36 (5 $\mu\text{mol/L}$), Lipopolysaccharide (LPS, 10mg/kg systemic, 0.5 mg/mL perfused), Exendin Fragment 9–39 (a GLP-1 receptor antagonist, 15 $\mu\text{mol/L}$), 2',5'-dideoxyadenosine (ddA, an adenylate cyclase inhibitor, 10 $\mu\text{mol/L}$), Rolipram (an inhibitor of cAMP-specific phosphodiesterase, PDE4, 10 $\mu\text{mol/L}$), and H-89 (a specific inhibitor of protein kinase A, 15 $\mu\text{mol/L}$). Glucagon-Like Peptide I Amide 7–36, Exendin Fragment 9–39, and LPS were obtained from Sigma-Aldrich Co., St. Louis, MO. 2',5'-dideoxyadenosine, Rolipram, and H-89 was obtained from Biomol Research Lab, Plymouth Meeting, PA. The doses of mediators were based on previously published doses [8,9,30].

2.3 Measurement of Hydraulic Permeability

Single vessel hydraulic permeability (L_p) was determined using the modified Landis micro-occlusion technique. The accuracy, precision, assumptions, and limitations of this model have been previously described [10]. Initial cell velocity (dl/dt) was obtained by recording marker

red blood 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 Starling's equation of fluid filtration: $L_p = (J_v/S)(1/P_c)$, where P_c is the capillary hydrostatic pressure. Control studies that document the stability of this model over time and after multiple recannulations of the study vessels have been previously published [10,36].

The modified Landis micro-occlusion technique specifically measures hydraulic conductivity (L_p), or trans-endothelial water flux, under the conditions when solute concentration is kept constant at physiological concentrations. Unlike other techniques, this *in vivo* technique allows for sensitive measurements of hydraulic conductivity under conditions where other parameters of transport, such as microvascular surface area, hydrostatic pressure, and oncotic pressure, are controlled. These parameters cannot be controlled for in the whole animal preparations or endothelial cell monolayer studies that measure macromolecule permeability.

2.4 Experimental Design

2.4.1 GLP-1 and Basal Microvascular Permeability—The dose effect of GLP-1 at 0.5 $\mu\text{mol/L}$ ($n=3$), 5.0 $\mu\text{mol/L}$ ($n=5$), and 50 $\mu\text{mol/L}$ ($n=3$) on basal microvascular permeability was measured. Mesenteric post-capillary venules were cannulated and perfused with Ringer's/1% BSA for 10 minutes and baseline measurements of L_p were obtained. Postcapillary venules were then continuously perfused with GLP-1. The L_p was measured every 5 minutes for 30 minutes (Fig. 1).

The GLP-1 dose of 5.0 $\mu\text{mol/L}$ was chosen for all further studies. A total of 100 microliters (3.3 $\mu\text{L/min}$) of 5.0 $\mu\text{mol/L}$ of GLP-1 was perfused over the course of each study. Therefore, the total dose of GLP-1 was 5.0 nmol (0.17 nmol/min) in each study.

2.4.2 GLP-1 and Microvascular Permeability Induced by LPS—The effects of LPS on microvascular permeability were measured after bolus injection of LPS (10mg/kg) into the rat femoral vein to induce inflammation. Fifteen minutes after injection, venules were continuously perfused with Ringer's/1% BSA and 0.5 mg/mL of LPS. LPS is systemically injected and perfused to provide an initial exposure to LPS and systemic stimulation with LPS. This is a physiologic representation of sepsis, i.e. continued exposure to an infectious source. The L_p was then measured after 5, 10, 20 and 30 minutes ($n=5$). To measure the effects of GLP-1 on the microvascular permeability induced by LPS, a bolus of LPS (10 mg/kg) was injected into the rat femoral vein. After 15 minutes, microvessels were cannulated and continuously perfused with Ringer's/1% BSA, 0.5 mg/mL of LPS, and 5.0 $\mu\text{mol/L}$ of GLP-1. GLP-1 was administered with LPS and after LPS injection to represent a potential treatment for sepsis-induced endothelial dysfunction. The L_p was then measured every 5 minutes for 30 minutes ($n=5$) (Fig 1).

2.4.3 GLP-1 Receptor Antagonism and Microvascular Permeability Induced by LPS—The effect of exendin (9–39), a specific antagonist to the GLP-1 receptor, on basal microvascular permeability was measured. Mesenteric post-capillary venules ($n=3$) were cannulated and perfused with Ringer's/1% BSA for 10 minutes and baseline measurements of L_p were obtained. Postcapillary venules were then continuously perfused with 15.0 $\mu\text{mol/L}$ exendin (9–39). The L_p was measured every 5 minutes for 30 minutes.

The effect of exendin (9–39) on microvascular permeability induced by LPS was then measured. Study venules were cannulated and continuously perfused with Ringer's/1% BSA and 15.0 $\mu\text{mol/L}$ of exendin (9–39) for 30 minutes. The pretreatment with exendin (9–39) was three times that of GLP-1 to insure complete antagonism of the GLP-1 receptor prior to infusion

of GLP-1. A bolus of LPS (10mg/kg) was then injected into the rat femoral vein to induce inflammation. Fifteen minutes after the LPS injection, postcapillary venules were then continuously perfused with Ringer's/1% BSA, 15.0 $\mu\text{mol/L}$ of exendin (9–39), 0.5 mg/mL of LPS, and 5.0 $\mu\text{mol/L}$ of GLP-1. The L_p was measured every 5 minutes for 30 minutes (n=5) (Fig 1).

2.4.4 cAMP Involvement in the Effect of GLP-1 on Microvascular Permeability—

We have previously published data demonstrating the effects of cAMP synthesis inhibition, cAMP degradation inhibition, and protein kinase A inhibition on baseline microvascular permeability [8,9]. Infusion of a cAMP synthesis inhibitor (ddA) alone initially increases microvascular permeability fourfold, but this effect is short lived with microvascular permeability returning to baseline by 15 minutes of infusion. After 15 minutes, infusion of a cAMP degradation inhibitor (Rolipram) alone decreases microvascular permeability to levels half of baseline. Infusion of a PKA inhibitor (H-89) alone increases microvascular permeability 2.5 fold, but this effect is short lived with microvascular permeability returning to baseline by 25 minutes of infusion. In collecting our current data, vessels were pretreated with the test mediators for 30 minutes (Fig 1) alleviating any initial increase in microvascular permeability due to the test mediator during the study period.

Study venules were cannulated and continuously perfused with Ringer's/1% BSA and a test mediator for 30 minutes. The following test mediators were used: 1) 10 $\mu\text{mol/L}$ of ddA, a cAMP synthesis inhibitor via adenylate cyclase inhibition; 2) 10 $\mu\text{mol/L}$ of Rolipram, a cAMP degradation inhibitor via phosphodiesterase (PDE4) inhibition; and 3) 15 $\mu\text{mol/L}$ of H-89, a protein kinase A (PKA) inhibitor. Previous studies in our laboratory with ddA, rolipram and H-89 showed that each mediator has a transient effect on microvascular permeability. Therefore the pretreatment with ddA, Rolipram, or H-89 was done to insure inhibition of adenylate cyclase, PDE4, and PKA, respectively, while eliminating any potential effect of each of these mediators alone on microvascular permeability. A bolus of LPS (10mg/kg) was then injected into the rat femoral vein to induce inflammation. Fifteen minutes after the LPS injection, postcapillary venules were then continuously perfused with Ringer's/1% BSA, 10.0 $\mu\text{mol/L}$ of ddA, 0.5 mg/mL of LPS, and 5.0 $\mu\text{mol/L}$ of GLP-1. The L_p was measured every 5 minutes for 30 minutes (n=3) (Fig 1).

2.5 Statistical Analysis

The L_p measurements are expressed as the group mean $\pm$ SEM $\times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$. Group means of sequential measurements were analyzed by repeated measures ANOVA with post-hoc analysis. Measurements of L_p were plotted over time and the area-under-the-curve (AUC) was calculated. Group means of AUC measurements were analyzed by ANOVA with post-hoc analysis. Statistical significance was considered an alpha error of 5%. StatView (SAS Institute, Cary, NC) was used for statistical analysis.

3. Results

3.1 Effects of GLP-1 on Basal Microvascular Permeability

Administration of GLP-1 had no effect on basal L_p at 0.5 $\mu\text{mol/L}$, 5.0 $\mu\text{mol/L}$, or 50 $\mu\text{mol/L}$ of GLP-1 infused (Fig 2). The baseline microvascular permeability AUC was 27 ± 1.4 . The microvascular permeability after GLP-1 administration remained unchanged at 0.5 $\mu\text{mol/L}$ (25 ± 0.4 , $p = 0.5$), 5.0 $\mu\text{mol/L}$ (31 ± 0.4 , $p = 0.08$), or 50 $\mu\text{mol/L}$ (32 ± 1.5) of GLP-1.

A GLP-1 dose of 5.0 $\mu\text{mol/L}$ was chosen for all further studies. With infusion of 5.0 $\mu\text{mol/L}$ microvascular permeability remained close to baseline throughout the study (Fig 2).

3.2 Effects of GLP-1 on the Increase in Microvascular Permeability Induced by LPS

Systemic administration of LPS increased the microvascular permeability of post-capillary venules two-fold over baseline (AUC: baseline = 27 ± 1.4 , LPS = 54 ± 1.7 , $p < 0.0001$). Administration of GLP-1 after LPS-induced inflammation returned L_p back to levels from GLP-1 alone by 15 minutes (Fig 3A). Over the 30-minute period, perfusion of GLP-1 attenuated the LPS-induced increase in microvascular permeability by 75% (AUC: LPS = 54 ± 1.7 , LPS+GLP-1 = 34 ± 1.5 , $p < 0.0001$) (Fig 3B).

3.3 Involvement of the GLP-1 Receptor in the Effect of GLP-1 on Microvascular Permeability

Administration of the specific GLP-1 receptor antagonist, exendin (9–39), alone had no effect on basal L_p . The baseline microvascular permeability AUC was 27 ± 1.4 . The microvascular permeability after GLP-1 receptor antagonist infusion remained unchanged (27 ± 1.4 , $p = 0.95$). Microvascular permeability remained at baseline throughout the study.

Perfusion with the specific GLP-1 receptor antagonist, exendin (9–39), decreased the ability of GLP-1 to attenuate the microvascular permeability induced by LPS (Fig 4A). Perfusion with the GLP-1 receptor antagonist increased microvascular permeability in comparison to perfusion with LPS plus GLP-1, however, decreased microvascular permeability compared to LPS alone (AUC: LPS+GLP-1 = 34 ± 1.5 , LPS+GLP-1+GLP-1 receptor antagonist = 46 ± 2.0 , LPS = 54 ± 1.7 , $p < 0.0009$) (Fig 4B). GLP-1 antagonism reduced the effects of GLP-1 on the LPS-induced increase in microvascular permeability 60%.

3.4 Involvement of cAMP in the Effect of GLP-1 on Microvascular Permeability

Perfusion with the cAMP synthesis inhibitor, ddA, decreased the ability of GLP-1 to attenuate the microvascular permeability induced by LPS (Fig 5A). Perfusion with the cAMP synthesis inhibitor increased microvascular permeability in comparison to perfusion with LPS plus GLP-1, however, decreased microvascular permeability compared to LPS alone (AUC: LPS +GLP-1 = 34 ± 1.5 , LPS+GLP-1+cAMP synthesis inhibitor = 46 ± 1.5 , $p < 0.0001$) (Fig 5D). The effect of cAMP synthesis inhibition was apparent throughout the study period, though the effect was diminished after 20 minutes of GLP-1 perfusion. Over the 30-minute study period, inhibition of cAMP synthesis reduced the effect of GLP-1 on the LPS-induced increase in microvascular permeability 60%.

Perfusion with the cAMP degradation inhibitor, Rolipram, enhanced the ability of GLP-1 to attenuate the microvascular permeability induced by LPS (Fig 5B). Administration of GLP-1 and the cAMP degradation inhibitor after LPS-induced inflammation returned L_p back to levels from GLP-1 alone by 5 minutes. Perfusion of the cAMP degradation inhibitor with LPS and GLP-1 did not change microvascular permeability in comparison to perfusion with LPS plus GLP-1 over the 30-minute study period (AUC: LPS+GLP-1 = 34 ± 1.5 , LPS+GLP-1+cAMP degradation inhibitor = 32 ± 1.5 , $p = 0.5$) (Fig 5D).

Perfusion with the PKA inhibitor, H-89, completely blocked the ability of GLP-1 to attenuate the microvascular permeability induced by LPS (Fig 5C). Perfusion of the PKA inhibitor with LPS and GLP-1 increased microvascular permeability in comparison to perfusion with LPS plus GLP-1, increasing microvascular permeability to levels induced by LPS alone (AUC: LPS +GLP-1 = 34 ± 1.5 , LPS+GLP-1+PKA inhibitor = 56 ± 1.5 , $p < 0.0001$) (Fig 5D). The effect of PKA inhibition was apparent by 10 minutes and remained throughout the study period. Over the 30-minute study period, inhibition of PKA reduced the effect of GLP-1 on the LPS-induced increase in microvascular permeability 100%.

4. Discussion

GLP-1 is a proglucagon-derived peptide secreted from gut endocrine cells with multiple paracrine and systemic actions [11]. The incretin actions of GLP-1 are the basis of the diabetic medication Exanetide (Byetta), a synthetic exendin-4 that acts as an agonist to the GLP-1 receptor. Evidence is mounting about the ability of GLP-1 to protect different cell types during stress. Injury to myocytes and pancreatic beta-cells after an ischemic insult is attenuated by GLP-1 [3,12,13,33]. GLP-1 also directly affects peripheral vascular tissue, improving endothelial function after ischemic insult and reducing endothelial-dependent vascular tone [17,31,38]. The presence of the GLP-1 Receptor throughout the large and small bowel has been demonstrated [5,7]. The presence of GLP-1 Receptors in the microvascular endothelium has also been established [3,31]. We hypothesized that GLP-1 would protect the mesenteric endothelium from injury during inflammation, reducing microvascular fluid permeability. We demonstrated that: 1) GLP-1 alone does not affect basal microvascular permeability, 2) GLP-1 attenuates the increase in microvascular permeability induced by LPS, 3) the effect of GLP-1 to attenuate LPS-induced microvascular permeability is reduced, but not eliminated, by a GLP-1 receptor antagonist, and 4) the effect of GLP-1 to attenuate LPS-induced microvascular permeability is partially mediated through cAMP signaling pathways.

To our knowledge this is the first study to demonstrate that GLP-1 has an effect on microvascular permeability and the first to demonstrate an anti-inflammatory effect of GLP-1 in an in-vivo model. There are several potential mechanisms for these effects. The ability of GLP-1 to attenuate the increase in microvascular permeability during inflammation may be due to an endothelial protective action that prevents apoptosis or by direct cell receptor mediated signaling that affects inter-endothelial cell gap formation. During ischemic injury, GLP-1 enhances myocyte glucose uptake [28] and increases levels of antiapoptotic proteins in pancreas β -Cells and myocytes [12,34]. Since LPS induces apoptosis in endothelial cells [4] and endothelial cell apoptosis has been proposed as a mechanism for microvascular permeability [2], these actions may explain the protective effect of GLP-1 on mesenteric endothelium during LPS-induced inflammation. We have shown that the anti-inflammatory actions of GLP-1 on mesenteric endothelium are mediated in part through the GLP-1 receptor and cAMP signaling pathways.

The actions of the GLP-1 receptor is thought to involve cAMP production and protein kinase A activation [23]. The role of the GLP-1 receptor and cAMP in the actions of GLP-1 on vascular endothelium has also been suggested. Ban et.al. [3] exhibited GLP-1 receptor dependent vasodilatory actions of GLP-1. Green et.al. [19] demonstrated the involvement of the GLP-1 receptor and cAMP pathways in GLP-1 induced relaxation of rat aorta.

By using a specific antagonist to the GLP-1 receptor, we demonstrated that the action of GLP-1 in attenuating microvascular permeability is at least partially mediated by a direct effect on the GLP-1 receptor. Both GLP-1 alone and GLP-1 plus the GLP-1 receptor antagonist reduce LPS-induced microvascular permeability. However, the GLP-1 plus the GLP-1 receptor antagonist had less of an effect than that of GLP-1 alone. GLP-1 receptor antagonism reduced the effects of GLP-1 on the LPS-induced increase in microvascular permeability by 60%. The partial effect of the GLP-1 receptor antagonist suggests that GLP-1 may also affect microvascular permeability independent of the GLP-1 receptor.

One explanation for the partial effect of the GLP-1 receptor antagonist is the existence of another GLP-1 receptor, unaffected by exendin (9–39) [6]. Others have demonstrated GLP-1 activity independent of the GLP-1 receptor. For instance, the vasodilatory effects of GLP-1 are in part due to the NO/cGMP-dependent actions of GLP-1 (9–36), a GLP-1 metabolite [3]. These actions of GLP-1 (9–36) occur independent of the GLP-1 receptor [3]. Because nitric

oxide and cGMP are important second messengers involved in regulation of microvascular permeability [9,20,32,37], it is plausible that the effect of GLP-1 on microvascular permeability may be partially mediated through a GLP-1(9–36)-NO/cGMP pathway.

The GLP-1 receptor is a transmembrane G-protein coupled receptor. A common factor among several vasoactive mediators of endothelial permeability is a G-protein coupled endothelial cell surface receptor that interacts with adenylate cyclase to manipulate intracellular cAMP levels [9]. We found that GLP-1 also decreases microvascular permeability through cAMP signaling pathways. Both GLP-1 alone and GLP-1 plus ddA reduced LPS-induced microvascular permeability. However, the GLP-1 plus ddA had less of an effect than that of GLP-1 alone. Inhibition of cAMP synthesis with ddA reduced the effects of GLP-1 on the LPS-induced increase in microvascular permeability by 60%. One explanation for the partial effect of ddA may be the ability of the cell to overcome adenylate cyclase inhibition over time by other cellular processes. Inhibition of cAMP degradation with Rolipram enhanced the ability of GLP-1 to attenuate the LPS-induced increase in microvascular permeability. Inhibition of PKA with H-89 completely blocked the effect of GLP-1 on the LPS-induced increase in microvascular permeability.

The role of cAMP in the regulation of microvascular permeability has been well established [21]. Cyclic AMP regulates inter-endothelial cell gap formation via PKA which in turn stimulates actin/myosin cytoskeleton interaction [1,21]. Others have documented that the GLP-1 transmembrane G-protein coupled receptor manipulates intracellular cAMP levels with subsequent PKA activation [15,22,24]. Furthermore, we and others have documented the ability of cAMP and PKA signal transduction pathway manipulation to affect microvascular permeability [8,9,21]. Taken together, these data suggest that GLP-1 decreases microvascular permeability through the cAMP-PKA pathway.

A limitation of this study is the lack of quantifying cAMP levels. The microvessels that are used in this study are each approximately 400 micrometers in length and 30 micrometers in diameter. Isolation and measurement of intracellular cAMP levels from this minute cell mass would be difficult. However, our data demonstrates that the CAMP-PKA pathway clearly has a role in the ability of GLP-1 to affect microvascular permeability.

5. Conclusions

To our knowledge, this is the first study to explore the role of GLP-1 as a modulator of microvascular permeability. In a resting post-capillary venule GLP-1 does not affect microvascular permeability. After basal state microvascular physiology is altered by exposure to an inflammatory mediator, GLP-1 decreases microvascular permeability. The effects of GLP-1 partially involve the GLP-1 receptor and a cAMP second messenger pathway. GLP-1 may protect mesenteric endothelium after inflammatory injury and thereby decrease third-space fluid loss. The pharmacologic manipulation of this function of GLP-1 may be beneficial in shock states to reduce intravascular fluid loss and the clinical complications of endothelial dysfunction.

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FIG 1.

Schematic of Experimental Protocols. Protocols were comprised of pretreatment and measurement periods. Pretreatment involved perfusion with the test mediator for 30 minutes (gray bar) prior to systemic injection of LPS (arrow). Microvascular Permeability (Lp) measurements (black bar) began 15 minutes after LPS injection.

Dose Response of GLP-1 on Basal Microvascular Permeability

FIG 2.

The dose response of GLP-1 on basal L_p . Illustrated is the continuous effect of perfusion with GLP-1 at 50 $\mu\text{mol/L}$, 5 $\mu\text{mol/L}$, and 0.5 $\mu\text{mol/L}$ on basal L_p . After 5 minutes of infusion, GLP-1 did not increase baseline L_p at any dose. *Significant difference in L_p between GLP-1 dose and basal L_p . Data is shown as mean $L_p \pm \text{SEM} \times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$.

FIG 3.

FIG 3A. The effect of GLP-1 on the increase in L_p induced by LPS. Illustrated are the continuous effects of perfusion with GLP-1 (solid line) compared to the effect of LPS alone (large dashed line) and LPS plus GLP-1 (small dashed line). GLP-1 attenuated the increase in microvascular permeability induced by LPS. *Significant difference in L_p between LPS alone and LPS plus GLP-1 ($p < 0.05$). Data is shown as mean $L_p \pm \text{SEM} \times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$. FIG 3B. The effect of GLP-1 on the increase in L_p induced by LPS. Data are represented as the area under the curve (AUC) of L_p over time. LPS alone increased L_p 2-fold over baseline. Subsequent perfusion with GLP-1 reduced this increase in L_p by 75%. *Significant difference

from baseline ($p < 0.0001$). #Significant difference from LPS alone ($p < 0.0001$). Data is shown as mean AUC $\pm$ SEM.

FIG 4.

FIG 4A. The involvement of the GLP-1 receptor in the effect of GLP-1 on L_p . Illustrated are the continuous effects of perfusion with LPS (large dashed line) compared to the effect of LPS +GLP-1 (small dashed line) and LPS+GLP-1+ a GLP-1 receptor inhibitor (solid line). Perfusion of a GLP-1 receptor inhibitor reduced the effect of GLP-1 on the LPS-induced increase in L_p . *Significant difference in L_p between LPS+GLP-1 and LPS+GLP-1+GLP-1 Receptor Inhibitor ($p < 0.05$). Data is shown as mean $L_p \pm \text{SEM} \times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$.

FIG 4B. The involvement of the GLP-1 receptor in the effect of GLP-1 on L_p . Data are represented as the area under the curve (AUC) of L_p over time. Perfusion of a specific GLP-1 receptor antagonist attenuated the effect of GLP-1 on the LPS-induced increase in L_p by 60%.

*Significant difference from LPS alone ($p < 0.001$). #Significant difference from LPS plus GLP-1 ($p < 0.0001$). Data is shown as mean AUC $\pm$ SEM.

FIG 5.

FIG 5A. Inhibition of cAMP synthesis reduces the effect of GLP-1 on L_p . Illustrated are the continuous effects of perfusion with LPS (large dashed line) compared to the effect of LPS +GLP-1 (small dashed line) and LPS+GLP-1+ a cAMP synthesis inhibitor (solid line). Perfusion of a cAMP synthesis inhibitor reduced the effect of GLP-1 on the LPS-induced increase in L_p . *Significant difference in L_p between LPS+GLP-1 and LPS+GLP-1+cAMP synthesis inhibitor ($p < 0.05$). Data is shown as mean $L_p \pm \text{SEM} \times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$.

FIG 5B. Inhibition of cAMP degradation enhances the effect of GLP-1 on L_p . Illustrated are the continuous effects of perfusion with LPS (large dashed line) compared to the effect of LPS +GLP-1 (small dashed line) and LPS+GLP-1+ a cAMP degradation inhibitor (solid line). Perfusion of a cAMP degradation inhibitor enhanced the effect of GLP-1 on the LPS-induced increase in L_p . *Significant difference in L_p between LPS+GLP-1 and LPS+GLP-1+cAMP degradation Inhibitor ($p < 0.05$). Data is shown as mean $L_p \pm \text{SEM} \times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$.

FIG 5C. Inhibition of PKA eliminates the effect of GLP-1 on L_p . Illustrated are the continuous effects of perfusion with LPS (large dashed line) compared to the effect of LPS+GLP-1 (small dashed line) and LPS+GLP-1+ a PKA inhibitor (solid line). Perfusion of a PKA inhibitor completely blocked the effect of GLP-1 on the LPS-induced increase in L_p . *Significant difference in L_p between LPS+GLP-1 and LPS+GLP-1+PKA inhibitor ($p < 0.05$). Data is shown as mean $L_p \pm \text{SEM} \times 10^{-7} \text{ cm/s}^{-1}/\text{cmH}_2\text{O}^{-1}$.

FIG 5D. The involvement of the cAMP/PKA Pathway in the effect of GLP-1 on L_p . Data are represented as the area under the curve (AUC) of L_p over time. Perfusion of a cAMP synthesis

inhibitor and a PKA inhibitor blocked the effect of GLP-1 on the LPS-induced increase in L_p by 60% and 100%, respectively. *Significant difference from LPS alone ($p < 0.001$). #Significant difference from LPS plus GLP-1 ($p < 0.0001$). Data is shown as mean AUC $\pm$ SEM.