SHEDDING OF THE ENDOTHELIAL GLYCOCALYX IN RESPONSE TO ISCHEMIA AND INFLAMMATION

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INTRODUCTION

Recent studies have highlighted the active role that the endothelial cell glycocalyx plays in many physiological processes. This molecular coating on the surface of the endothelial cell has been implicated as a barrier to transvascular exchange, blood cell to endothelium adhesion, and capillary perfusion; and has been estimated to vary in thickness from 0.1 to as much as 1 μm (1). It has also been established that its physical structure results from an intricate combination of protein and carbohydrate synthesis that produce an array of proteoglycans on the cell surface consisting of a protein core decorated with branches of glycosaminoglycans (GAGs) (2). This structure is actively maintained by a material balance among synthesis of constituents and transport to the cell surface, their enzymatic degradation with either shedding to the lumen of the blood vessel or reinternalization into the cellular cytosol. Evidence suggests that the concentration of GAGs on the cell surface may be shear dependent (3). The present studies were undertaken to examine the net balance of these processes in so far as they affect the major GAG, heparan sulfate (HS), in response to shear dependent and inflammatory stimuli that affect perfusion of the microvascular network. To this end, post-capillary venules of the intestinal mesentery were observed by intravital microscopy in anesthetized rats and HS concentration on the endothelial cell (EC) surface was measured by quantitative fluorescence microscopy following either simulation of inflammation by the topical application of fMLP, or ischemia.

METHODS

Rats (250-350 g) were anesthetized with Inactin (125 mg/kg) and the intestinal mesentery exteriorized via a mid-line abdominal incision. The mesentery was draped over an optically clear pedestal, irrigated with heated Ringer's solution (37 deg °C), and viewed under either brightfield transmitted light or epifluorescence microscopy. Post-capillary venules, ranging in width from 30 – 50 μm were singled out for observation and measurement of red cell velocities (V_{RBC}) using the two-slit photometric technique. Wall shear rates were estimated by empirical correlations between V_{RBC}, vessel diameter (D) and mean velocity, $V_{MEAN} = V_{RBC}/1.6$, and an index of shear rate was estimated from the Newtonian value, $\dot{g} = 8V_{MEAN}/D$. Wall shear stress, τ, was calculated as the product of $\dot{g}$ and apparent viscosity, where the latter was assumed to be equal to about 2.5 cP.

The amount of HS on the EC surface was quantified by systemic infusion of the fluorescently labeled lectin, FITC-BS1, which is specific for heparan sulfate. Lectin conjugated HS was measured by fluorescence densitometry calibrated against predetermined concentrations of FITC-BS1. Fluorescence intensity was measured following fixation of the tissue by superfusion with 5% glutaraldehyde and viewing the tissue by laser-scanning confocal microscopy. The FITC-BS1 was infused prior to the experimental manipulations.

Inflammation was simulated by superfusion of the tissue with the chemoattractant f-met-leu-phe (fMLP) at a concentration of 10^{-7}M to obtain HS concentration on the EC prior to and following fMLP exposure. The effects of ischemia were studied by gently occluding the proximal arteriole for 60 min with a blunted microprobe held in a micromanipulator. The occlusion was then removed and flow was reestablished to within 5% of its pre-occlusion value, as verified by measurements of red cell velocity. The responses to the fMLP and ischemia were observed with and without inhibition of G-protein signaling which was inhibited by superfusion of the tissue with Pertussis toxin (PTX).

RESULTS

Presented in Fig. 1 is the concentration of HS on the EC prior to (control) and following 10 min superfusion with fMLP, with and without PTX. The concentration of FITC-BS1 was taken as the peak value at the endothelial cell surface that was obtained along a radial direction across the width of the venule. Peak values were averaged along a 100 μm length of the venule. Superfusion with fMLP caused a

65% reduction in HS concentration on the EC, that was limited to a 15% reduction with PTX.

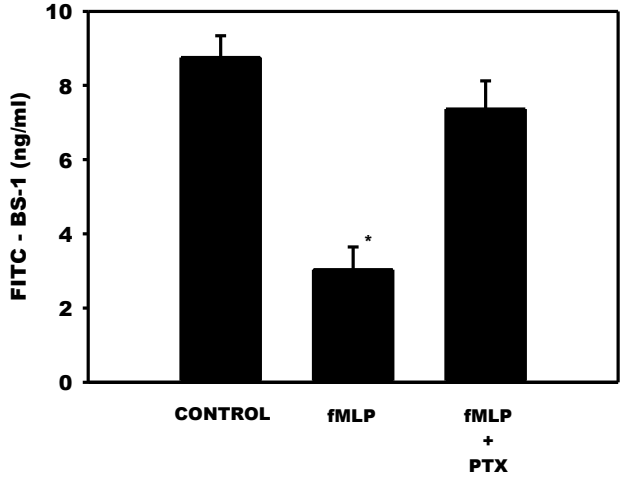

Figure 1. Concentration of FITC conjugated lectin (BS-1) measured on the endothelial cell by confocal fluorescence microscopy in post-capillary venules under control conditions and following 10 min of superfusion of the tissue with either fMLP or fMLP plus Pertussis toxin (to inhibit G-protein signaling).

The response to ischemia/reperfusion is illustrated in Fig. 2. Shown are values of FITC-BS1 concentration immediately following 60 min of ischemia (time = 0) and 10 min following reperfusion. The concentration of FITC-BS1 has been normalized by taking the ratio of average values in the wall of occluded venules to that in the walls of non-occluded venules in the same sector of tissue. It should be noted that the occlusion itself did not cause hypoxia since the tissue was continuously irrigated with Ringer's solution and exposed to ambient air.

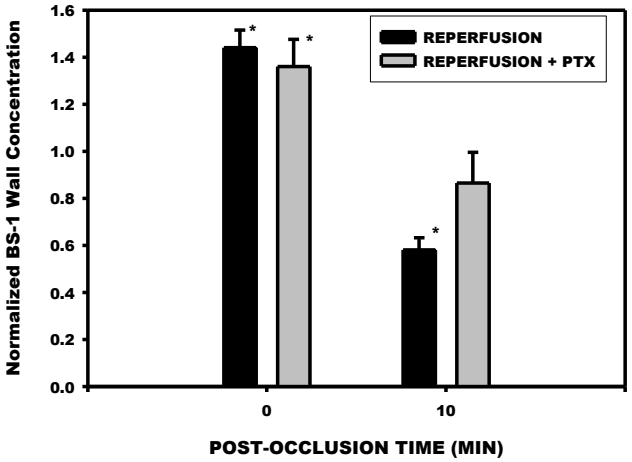

Figure 2. Shown are the concentrations of FITC-BS1 along the endothelium during the initial (time = 0) reperfusion following 60 min of ischemia, and 10 min following reestablishment of flow. The concentrations have been normalized by taking their ratio from occluded to non-occluded vessels in the same tissue field. Shown are the results for untreated vessels and those treated by superfusion of the tissue with PTX.

It is evident that during the occlusion, the continuous production of HS resulted in a 40% increase in HS during the ischemic period, both with and without superfusion of the tissue with PTX. Pertussis toxin did not affect the accumulation of HS during the ischemic period. Following 10 min of reperfusion, FITC-BS1 fell 60% without PTX and only 35% with superfusion of the mesentery with PTX

DISCUSSION

The present results are consistent with prior observations on the lability of the glycocalyx during stimulated adhesion of leukocytes (WBCs) to the venous endothelium (4). As shown therein, enzymatic removal of the glycocalyx enhanced exposure of EC bound adhesion molecules (namely ICAM-1). The reduction in HS levels in response to fMLP suggest that the HS chains present a substantial barrier to WBC-EC contact. Since it is known that the fMLP response of the WBC is G-protein mediated it is not surprising to find that the EC response is also dependent upon G-protein signaling (Fig. 1). Inasmuch as many flow dependent responses of the EC are also G-protein dependent, it is interesting to find that loss of the glycocalyx following reperfusion (Fig. 2) may be significantly mitigated by inhibition of G-protein signaling. The production of HS during the ischemic episode does not appear to be G-protein dependent (Fig 2, time = 0). However, its subsequent shedding or enzymatic degradation is significantly diminished by PTX. These results raise the question of how much of this reduction is due to shear stress effects or enzymatic removal of HS by either direct cleavage of HS chains or its supporting proteoglycan core protein. Several candidates for enzymatic cleavage of HS chains are apparent, such as membrane bound metalloproteases on the EC. It remains to be determined if these are shear dependent and upregulated during periods of ischemia, thus resulting in the rapid loss of HS during reperfusion. Exploration of factors that affect the balance of production and shedding of GAGs from the EC appears essential in order to understand the physiological role of the glycocalyx during pathological conditions.

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