Role of a glyocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments

VIRGINIA H. HUXLEY AND DONNA A. WILLIAMS
Department of Physiology, University of Missouri School of Medicine, Columbia, Missouri 65212

Huxley, Virginia H., and Donna A. Williams. Role of a glyocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments. Am J Physiol Heart Circ Physiol 278: H1177–H1185, 2000.—Whereas the glyocalyx of endothelial cells has been shown to influence solute flux from capillary microvessels, little is known about its contribution to the movement of macromolecules across the walls of other microvessels. We evaluated the hypothesis that a glyocalyx contributes resistance to protein flux measured in coronary arterioles. Apparent solute permeability (Pₜ) to two proteins of different size and similar charge, α-lactalbumin (α-lact) and porcine serum albumin (PSA), was determined in arterioles isolated from the hearts of 43 female Yucatan miniature swine. Pₜ was assessed in arterioles with an “intact” glyocalyx under control conditions and again after suffusion with adenosine (Ado, 10⁻³ M, n = 42 arterioles, N = 29 pigs). In a second set of experiments (n = 21 arterioles, N = 21 pigs) arteriolar Pₜ was determined before and after perfusion with enzyme (pronase or heparinase), which was used to digest the glyocalyx. Pₜ was assessed a third time on those microvessels after exposure to Ado. Consistent with the hypothesis, Pₜ for PSA (Pₜ⁰₅SA) and Pₜ for α-lact (Pₜα-lact) increased from basal levels following enzyme treatment. Subsequent suffusion with Ado, a significant metabolite known to alter coronary vascular smooth muscle tone and permeability, resulted in a significant reduction of basal Pₜα-lact in both untreated and enzyme-treated arterioles. Furthermore, in untreated arterioles, Pₜ⁰₅SA was unchanged by Ado suffusion, whereas Ado induced a pronounced reduction in Pₜ⁰₅SA of enzyme-treated vessels. These data demonstrate that in intact coronary arterioles an enzyme-sensitive layer, most likely at the endothelial cell surface, contributes significantly to net barrier resistance to solute flux.

porcine serum albumin; α-lactalbumin; microcirculation; endothelium; Yucatan miniature swine; protease; heparinase

AT THE LUMINAL SURFACE of intact microvessels is an electron lucent layer known as the glyocalyx. The presence of this layer was first demonstrated in the microcirculation by perfusing the vasculature with a cationic dye, ruthenium red, which bound to the glycated proteins on the surface endothelium (30). More recently, the presence of the layer has been identified in exchange microvessels using cationic ferritin (2, 38) or dye-labeled lectins (37). In the intact mammalian microvasculature, the presence of a glyocalyx has been demonstrated by examining the volume occupied by red blood cells flowing through a microvessel (14). More precise studies have identified a cylindrical sheath between the endothelial surface and the central core of red blood cells or macromolecular probes located within a given microvessel (39), whether arterioles, capillaries, or venules (18). As such, the glyocalyx has been implicated in oxygen delivery because it increases the diffusional distance between red blood cells and metabolizing tissue, and it may alter the distribution of red blood cells within the capillary network (14, 36, 39).

In addition to oxygen delivery, the glyocalyx also influences permeability to water and solutes, a function that appears to require plasma proteins to maintain its optimal structure. In whole organ preparations, absence of perfusate protein correlated with a rise in capillary filtration coefficient and permeability-to-surface area product (16, 31, 33); evidence to the contrary also exists (7, 41). In regions of the porcine aorta where Evans blue dye-labeled albumin permeated, the thickness of the glyocalyx, as revealed by ruthenium red binding, was one-third that of regions where Evans blue-albumin failed to permeate (15). In capillary preparations, glyocalyx structure has been manipulated by removing protein (19, 22, 32); by exposure to modified albumins and serum proteins (2, 16, 19, 20, 22); by use of charged probes (3, 38), electrical stimulation (8), or photo damage (39); and by enzyme perfusion (1, 14). All experimental maneuvers altered permeability to water, small dyes, and protein. Recently, Henry and Duling (18) have shown exclusion of FITC-labeled dextrans (70 kDa and larger) from a region extending almost 0.5 µm from the endothelial surface of arterioles, capillaries, and venules of the intact hamster microcirculation; again the rate of penetration was increased by enzyme treatment, specifically hyaluronidase.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
compared with venules isolated from the coronary microvasculature (23, 24, 42), and the permeability properties are more dynamic than anticipated. Two examples of the dynamic nature of barrier properties of coronary arterioles are that permeability to proteins was altered following chronic endurance exercise training (24) and was changed from control levels following exposure to adenosine (Ado) (23, 24, 29). The mechanisms and the structures responsible for these adaptations remain to be elucidated.

The present study was designed to extend investigations on the dynamic barrier properties of coronary arterioles, especially with reference to the glycocalyx and the nature of the changes in permeability elicited by exposure to Ado. We evaluated two hypotheses: 1) that the glycocalyx of arterioles contributes appreciable resistance to protein flux and 2) that Ado alters permeability by changing the structure of hydrophilic pathways between the cells of the microvessel wall and not the structure of the glycocalyx. Two proteins, α-lactalbumin and albumin, were used to probe the permeability barrier of isolated arterioles. In one experiment the glycocalyx was preserved and the arterioles were exposed to Ado. In the second experiment, the arterioles were perfused with either pronase, a mixture of proteolytic enzymes, or the specific enzyme, heparinase, to digest the glycocalyx (1, 13, 18). Relative to control, permeability properties to both proteins were elevated following enzyme treatment, and the magnitude of the response to Ado was greater in enzyme-treated arterioles compared with that observed in untreated arterioles. The basic findings of the study are consistent with a luminal structure, the glycocalyx, contributing significant resistance to protein flux in series with the other constituents of the walls of isolated coronary arterioles.

METHODS

General preparation. Adult, Yucatan miniature swine (N = 43 pigs; 9–12 mo of age, 35–55 kg, Charles River) were sedated with ketamine (25 mg/kg im) and rompun (2.25 mg/kg im) and anesthetized with pentobarbital sodium (20 mg/kg iv). The pigs were then intubated and ventilated with room air before administration of heparin (1,000 U/kg) into an ear vein. After completion of a left thoracotomy, the heart was removed and placed into cold (4°C) mammalian Krebs solution (23, 24).

Arteriolar plexus isolation and cannulation. The right ventricular wall (5–7 by 2–3 cm) was removed into fresh Krebs and prepared in a 1:100 dilution into PSA. The final concentration was 10 M Ado (23). The perfusion and suffusion solutions were prepared fresh daily. The Krebs solution consisted of (in mM) 132.8 NaCl, 4.7 KCl, 2.0 CaCl2, 1.2 MgSO4, 1.2 NaH2PO4, 2.0 pyruvate, 5.0 glucose, 6.0 NaHCO3, and 6.0 Na HEPES. The pH of the solution was 7.37 and 7.41 ± 0.01 at 4°C and 37°C, respectively. PSA was used as the colloid for the solutions. PSA was prepared from a 60 mg/ml stock solution that was dialyzed in 12,000–14,000 mol wt cut-off dialysis tubing (SpectroPor, Spectrum, Houston, TX) against 2 liters of Krebs solution in three sessions over 72 h. The dialysis procedure equilibrated the PSA solutions with the Krebs, removed small, water-soluble vasoactive contaminants, and set the pH of the protein solution (20). The protein content of the final dialysate was determined by absorbance spectroscopy. The final plasma protein concentration of the perfusion and suffusion solutions was 20 and 10 mg/ml, respectively.

Two globular proteins used to probe the permeability of the arteriolar wall were α-lactalbumin (14,000 mol wt, type III from bovine milk, Sigma) and PSA (65,000 mol wt). The proteins were present as an unlabeled test solute in one-half of the theta pipette and as a fluorescently labeled test solute (3, 21–24), tagged with either TRITC or Oregon Green 514, in one-half of the theta pipette and as a fluorescently labeled test solute (3, 21–24), tagged with either TRITC or Oregon Green 514, in one-half of the theta pipette. In general PSA was tagged with TRITC, and α-lactalbumin was tagged with Oregon Green 514. An 18 mg/ml stock solution of labeled α-lactalbumin was diluted with fresh PSA and filtered through a 0.45-µm cellulose filter cartridges (µStar LB, CoStar, Pleasanton, CA) to produce a 0.56 mM solution with 20 mg/ml PSA. Because TRITC-PSA was 0.17 mM, the total PSA concentration was 0.33 mM. The net oncotnic pressure of these protein solutions ranged between 6 and 8 cmH2O.

Ado (10–3 M stock, Sigma) for suffusion was dissolved in Krebs and prepared in a 1:100 dilution into PSA. The final concentration was 10–8 M Ado in 10 mg/ml protein. Pronase
Measurement of arteriole protein flux. The method for assessing apparent permeability to proteins in isolated arterioles, modified from that developed for in situ perfused capillaries (3, 22), has been published (23). Briefly, perfusion pressure controlled flow into the arteriole from the two sides of the theta pipette and was set to ensure exclusive perfusion with either dye-free (washout) or dye-labeled solution. When the dye-labeled protein solution perfused the vessel, fluorescence intensity emanated from the perfused arteriole. A rectangular measurement window (at least 3 vessel diameters wide), located in the light path between the vessel and the photometer, was used to limit and define the area of the solute permeability (P_{\alpha}) measurement along the vessel. The photometer output was recorded on a strip-chart recorder (Hewlett-Packard) for calculation of J_{\alpha} (3, 21, 23, 24). After a measure of flux, perfusion with the nonfluorescent washout solution was resumed. Repeat measures of flux were made when fluorescence intensity returned to within 1% of the baseline level. Consequently, for a 40-µm diameter arteriole with a permeability of \(-3 \times 10^{-7}\) cm/s, a minimum of 30 min was required to obtain five measures of J_{\alpha}. All experimental protocols were performed at 15°C to minimize changes in diameter (26).

Experimental protocols. Permeability of arterioles to \alpha-lactalbumin and PSA, proteins of similar charge, shape, and dissimilar size (3, 21), was measured in two sets of arterioles.

In one set of arterioles, basal flux was measured during perfusion and suffusion with PSA in Krebs. Flux was measured again after a minimum exposure of 5 min suffusion with 10^{-5} M Ado added to PSA in Krebs.

In the second set of isolated arterioles, basal flux was measured. The arteriole was then recannulated with a single-barreled pipette containing PSA and 0.1 mg/ml pronase. After 5 min of perfusion with the enzyme at 30 cmH2O, the pipette was removed and replaced with the original theta pipette. Flux measurements were repeated. As the final step in this protocol, measurement of J_{\alpha} was made a third time following a 5-min suffusion with 10^{-5} M Ado. As a control for the action of pronase, the microvessel was suffused rather than perfused with PSA and 0.1 mg/ml pronase following measurement of basal flux. As above, the isolated arteriole was exposed to enzyme for 5 min before suffusion with Krebs-PSA was resumed. Flux measurements were repeated before and after suffusion with 10^{-5} M Ado in four additional arterioles, heparinase (100 U/ml) was used instead of pronase, and the perfusion period was reduced to 2 min. Ado was also tested on the heparinase-treated arterioles.

In all cases apparent basal P_{\alpha} was calculated from the measures of flux (21, 22). Arteriolar diameter was assessed during basal conditions and again after perfusion with enzyme and/or suffusion with Ado.

Statistical analyses. A minimum of five measures of the surface area and concentration gradient (J_{\alpha}/SAC) at a single ΔP was averaged to represent P_{\alpha} for an individual arteriole during a given treatment. Data from individual arterioles (n) were averaged to represent a single value for each pig (N). Values for P_{\alpha}, especially \alpha-lactalbumin, were skewed positively (35); therefore, medians ± absolute deviation (MAD) are reported and used in the statistical comparisons (StatView, SAS Institute, Cary, NC). A power analysis (35) indicated that seven animals were required per solute to minimize both type I and type II errors. The Wilcoxon signed rank test was used to determine differences between permeability to PSA and \alpha-lactalbumin(13). The Kolmogorov-Smirnov two-sample test was used to determine differences in the shape of the distribution of basal measures of flux between groups and between treatments. The null hypothesis was that two independent samples came from identical populations with respect to location and dispersion (13). Skewness values (13) are reported in which a value -0 represents a distribution where the mean equals median. For skewness values -0 and a mean of the population > the median, the distribution are positively skewed (13, 35). In the first set of arterioles, the response of P_{\alpha} to Ado was normalized to control, and treatments were compared using paired Student's t-test. In the second set of arterioles, the response of P_{\alpha} to enzyme or Ado after enzyme was calculated as the ratio of the treatment relative to P_{\alpha} under control suffusion. Comparisons among treatments were made using ANOVA. A significance level of P < 0.05 was set before the power was calculated and before the experiments were performed.

RESULTS

Data were collected from 65 coronary arterioles of 39.8 ± 2.1 µm in average internal diameter (range 13–101 µm) and thus represented A1 through A5 order vessels as defined by Kassab et al. (25). The arterioles (~1,000 µm long) branched from vessels 100–250 µm diameter, which had originated ultimately from the right coronary artery or the left anterior descending artery. Hydrostatic pressure averaged 15.3 ± 0.3 cmH2O during all measurements of P_{\alpha}.

Solute permeability. For all arterioles in both experiments, basal P_{\alpha} to PSA (P_{\alpha}^{PSA}) was 4.0 ± 2.2 × 10^{-7} cm/s (MAD, solid arrow in Fig. 1A, skewness = 0.2; P = 0.29...
indicating that the data are derived from a normal distribution, N = 25) and \( P_s^{\alpha}\)-lactalbumin (\( P_s^{\alpha}\)-lactalbumin) was \( 6.6 \pm 3.6 \times 10^{-7} \text{ cm/s} \) (solid arrow in Fig. 1B, mean = \( 11.9 \times 10^{-7} \text{ cm/s} \), skewness = 2.6; the data are not derived from a normal distribution, \( P = 0.03, N = 40 \)). Distributions of the values for individual vessel permeabilities to the two solutes are illustrated in Fig. 1. On both a per animal and per vessel basis, \( P_s^{\alpha}\)-lactalbumin was less (\( P < 0.05 \)) than \( P_s^{\alpha}\)-lactalbumin. The diameters did not differ for the arterioles in which \( P_s^{\alpha}\)-lactalbumin and \( P_s^{\alpha}\)-lactalbumin were determined (35 ± 3, means ± SE vs. 43 ± 3 \( \mu \text{m} \), respectively).

Permeability response of untreated arterioles to Ado (experiment 1). In untreated arterioles, \( P_s^{\alpha}\)-lactalbumin remained unchanged from a median basal level of \( 4.8 \pm 1.9 \times 10^{-7} \text{ cm/s} \) (N = 18, Fig. 2) following suffusion with Ado. For the smaller protein, Ado suffusion induced a 20% decrease (\( P < 0.001 \)) in \( P_s^{\alpha}\)-lactalbumin (N = 24, Fig. 2). The difference in permeability responses to Ado between the two solutes was also significant (\( P < 0.05 \)).

Influence of proteolytic enzyme treatment on \( P_s^{\alpha}\)-lactalbumin response to Ado (experiment 2). In arterioles exposed to pronase perfusion, \( P_s^{\alpha}\)-lactalbumin increased (\( P = 0.01, N = 7 \)) by \( 2.3 \pm 0.4\)-fold (Fig. 3). Similarly, perfusion with pronase increased \( P_s^{\alpha}\)-lactalbumin by \( 3.2 \pm 0.5\)-fold (\( P < 0.01, N = 10 \)) from control levels. The difference in the response to pronase perfusion did not differ with solute (\( P = 0.09 \)). In four arterioles perfused with heparinase in place of pronase, \( P_s^{\alpha}\)-lactalbumin rose (\( P < 0.01 \)) by \( 4.8 \pm 1.1\)-fold. The magnitude of the increase in \( P_s^{\alpha}\)-lactalbumin did not differ with the enzyme solutions used to perfuse the vessels (\( P = 0.29 \)); thus the pooled result was a 3.6 ± 0.5-fold increase (\( P < 0.001 \)) from basal levels (Fig. 3). The magnitude of the change in permeability following pronase treatment did not differ with solute (\( P = 0.13 \)). Additionally, suffusion rather than perfusion with pronase was without effect on \( P_s^{\alpha}\)-lactalbumin (1.14 ± 0.09-fold, N = 2).

Microvessel diameter increased significantly after pronase perfusion (\( D_{\text{pronase}}/D_{\text{control}} = 1.12 \pm 0.04, P < 0.05 \)), suffusion with pronase did not alter diameter (1.02 ± 0.03). The increase in permeability following pronase perfusion did not correlate with either diameter or change in diameter (\( r = 0.19 \) and 0.30, respectively). This outcome was the same for the vessels exposed to heparinase (\( r = 0.43 \) and 0.58, respectively).

A plot (not shown) of permeability after pronase relative to that before treatment for the two solutes fit a linear model (\( R^2 = 0.7 \)) with an average \( 2.5 \pm 0.4\)-fold increase in permeability following enzyme digestion. Finally, the magnitude of the change in permeability was independent of the control level before enzyme treatment.

For pronase-treated arterioles, permeability to both solutes decreased (\( P < 0.01 \) for each solute) after exposure to Ado (Fig. 3). \( P_s^{\alpha}\)-lactalbumin decreased 38 ± 9% from the level determined following treatment with pronase; in the case of \( \alpha\)-lactalbumin the decrease was 56 ± 7%. The magnitude of the decrease in response to Ado did not differ between probes (\( P > 0.3 \)). In arterioles treated with heparinase, \( P_s^{\alpha}\)-lactalbumin dropped by 48 ± 12% after addition of Ado to the suffusion, a response not different from that induced by Ado in arterioles exposed to pronase perfusion. The magnitude of the reduction in permeability induced by Ado did not differ between suffusion and perfusion of pronase (\( P = 0.71 \)). Ado also increased the diameter of enzyme-treated arterioles by
and then suffusion with 102 M (A) activity decreased consistently and significantly for both arterioles exposed to pronase, permeability to albumin (Fig. 2, experiment 1). Furthermore, permeability responses to Ado suffusion were magnified when the layer was disrupted. The important findings of the study are that protein permeability of coronary arterioles was influenced by the glycocalyx and that the resistance to protein movement through the arteriolar wall that is sensitive to pronase and heparinase digestion. Furthermore, these data provide additional evidence that the decrease in permeability induced by Ado results from changes in the cell structure, likely endothelial cells, in the arteriolar wall, a response that was blunted and masked when the glycocalyx was intact.

Models of permeability. A working model of the arteriolar permeability barrier is one of resistances in series, e.g., the glycocalyx, the endothelium, plus the other cells making up the arteriolar wall, with the primary pathway for proteins being through the junctions between endothelia (4, 11, 22). Because junctional width, by ultrastructural studies (4, 9–11, 15, 43), has been shown to be quite uniform in noninflamed vessels, the primary determinants for variable resistance for more than one vessel are area of junction available for solute permeation (A_A) and junctional depth (Δx). Two studies (4, 11) have shown that A_A exceeds that required to account for water and small solute Δs. Thus it appears that, whereas A_A could be a variable, its contribution to basal hydraulic conductivity, an index of permeability to water, remains relatively constant. Furthermore, in a study by Clough and Michel (11), the correlation between hydraulic conductivity and Δx was diminished when A_A was included in the analysis. Thus it is believed that Δx is of greater importance to the movement of water and hydrophilic solutes across the barrier. Ultrasound data from capillaries in amphibia and rat (4, 9–11) suggest that Δx is not a constant but varies by over 20-fold from 0.1 to 2.0 µm. Structure and functional measurements from Clough and Michel (11) demonstrated a strong correlation between junctional depth and hydraulic conductivity. The study also demonstrated that values for junctional depth are not distributed normally, but instead are skewed positively (12). It has also been shown in frog capillaries that basal hydraulic conductivity and P_s are skewed positively (17, 23, 32, 34). In the present study, Fig. 1B illustrates the distribution for permeability to α-lactalbumin (a water-soluble solute), which was also skewed similarly. Thus, by inference, if water-soluble solutes travel predominately via the junctions between endothelia, then values of permeability should correlate directly with junctional depth.

A simple model of permeability in coronary arterioles is illustrated in Fig. 5. Here the pathways for J_s across the barrier are represented as resistors (R) in series such that R_total = R_glycocalyx + R_wall. As such, permeability measured in the untreated vessels would equal (P_total)−1 = (P_glycocalyx)−1 + (P_wall)−1 (left-hand side of Fig. 5A). In a model such as this, it can be argued that enzyme treatment eliminates R_glycocalyx and the mea-

DISCUSSION

The data presented in this study are consistent with a glycocalyx in coronary arterioles that offers significant resistance to the flux of anionic proteins. Permeability of the arterioles more than doubled after enzymatic treatment used to digest the glycocalyx from the lumen of perfused microvessels (Figs. 3 and 4). Furthermore, permeability responses to Ado suffusion were magnified when the layer was disrupted. The important findings of the study are that protein permeability of coronary arterioles was influenced by the glycocalyx and that the resistance to protein movement through the layer was sufficient to mask changes in permeability induced by cellularly mediated changes in barrier function of the basal permeability (P_control). Each circle and triangle pair represents responses from one coronary arteriole. Permeability of arterioles suffused with pronase (squares) and then suffused with 5 M Ado (triangles) and plotted as a function of (P_control)PSA x 10⁻⁷ cm/s. I n B dark gray symbols indicate pronase-treated arterioles and light gray symbols are heparinase treatment.
sured permeability is that which remains, \( P_{\text{wall}} \) (right-hand side of Fig. 5A). Thus knowledge of permeability before \( (P_{\text{total}}) \) and after enzyme treatment \( (P_{\text{wall}}) \) allows \( P_{\text{glycocalyx}} \) to be estimated. From the data in this study, a 2.4-fold increase in permeability to protein after pronase treatment is consistent with the glycocalyx accounting for \(~60\%\) of the resistance to macromolecule flux across the walls of coronary arterioles.

The estimate that \( 60\% \) of the resistance to large molecule flux resides in the enzyme-sensitive layer raises some interesting questions. Permeability equals the free diffusion coefficient \( (D_f) \) divided by the mean...
path length ($\Delta x$). Thus if one assumes that solute $D_p$ is the same in the glycocalyx as it is in the aqueous pathways traversing the arteriolar wall, then glycocalyx thickness ($\Delta x_{glycocalyx}$) would be expected to approximate the mean thickness of the vessel wall ($\Delta x_{wall}$). The mean wall thickness of the arterioles was 6 ± 3 µm, resulting in a mean glycocalyx depth on the order of 7 µm, a value well in excess of previous estimates. If, instead, the flux we measure occurs across the glycocalyx and endothelial layer and if the mean endothelial junctional depth of rat coronary microvessels approximates that of pig arterioles (0.5 µm (9)), then the expected mean depth of the glycocalyx would be on the order of 0.6 µm, a conclusion similar to that derived by both Vink and Duling (39) in hamster capillaries and by Henry and Duling (18) in hamster cremasteric capillaries, arterioles, and venules.

Although it is reasonable to view the arteriolar wall as a multicomponent barrier consisting of the glycocalyx, endothelium, and vascular smooth muscle, the logical predictions of a series resistance model fail to account for the data from the present study. First, the data show that following perfusion of enzyme, treatment of coronary arterioles elevated $P_a$ independent of basal permeability (Fig. 4). If the glycocalyx is of uniform depth and junctional depth is variable, as suggested by histological studies (9, 11), then removing the glycocalyx should have increased permeability by a variable amount. Furthermore, if permeation through the glycocalyx is the primary determinant of intact microvascular permeability, then we would have predicted that enzyme treatment would produce the greatest increase for the largest solute, albumin, less for $\alpha$-lactalbumin, and the least for water. Instead, irrespective of the protein probe, enzyme treatment resulted in a uniform, not graded, 2.4-fold elevation of $P_a$. Similarly, in pronase-treated frog mesenteric capillaries, Adamson (1) found an approximate 2.5-fold increase in hydraulic conductivity without a change in effective oncotic pressure or change in endothelial junctional width. We could account for the data of the present study 1) if the glycocalyx depth varied as a function of junctional depth, 2) if the enzyme only partially digested the matrix as a function of endothelial junctional depth, or 3) if our picture of the structures at the microvascular wall is not represented adequately by the simple series resistor model.

The second point of departure from the series resistor, static barrier model relates to the first and to the relationship between microvascular permeability to water and solutes with junctional depth. If the protein probes travel via the junctions between the cells of the vessel wall, whether endothelium alone or endothelium and vascular smooth muscle, then the shape of the distribution of $P_a$ values should not differ with the size of the probe. Whereas the distributions for values of basal hydraulic conductivity (11, 17, 34) and $P_a$ to $\alpha$-lactalbumin (Fig. 1B; 22, 23) are skewed positively, the same cannot be said for the distribution of $P_a$ to albumin in untreated arterioles (Fig. 1A). Furthermore, after enzyme treatment the shape of the distributions remained skewed positively for $\alpha$-lactalbumin, (skewness = 1.49) and normally distributed for albumin (skewness = −0.03). The fact that the distributions did not change with enzyme treatment argues that permeation of the proteins through the glycocalyx does not determine this feature. In summary, the homogenous response to enzyme perfusion (Figs. 3 and 4) in addition to the distributional characteristics of the data are not consistent with the basal permeation of $\alpha$-lactalbumin and albumin across the intact arteriolar wall being simply a consequence of the differences in the diffusion coefficients of the probes.

Finally, the magnitudes of the differences in permeability under basal and Ado-stimulated untreated and enzyme-treated arterioles are not consistent with the predictions of a simple series resistor model. To account for these responses, additional pathways for proteins in parallel with the endothelial junction are required. One suggestion, to account for the apparent lack of change in permeation of albumin following Ado stimulation when $\alpha$-lactalbumin flux decreases (Fig. 2), is the existence of separate pathways for the two proteins. This would be observed if a major component of albumin flux occurred via vesicular rather than via junctional complexes (diffusive and convective mechanisms). These notions require further rigorous investigation.

The present study also demonstrates that prior perfusion of the microvessel lumen with pronase to remove the glycocalyx would be a useful method for revealing the cellularly based mechanisms that regulate macromolecular flux in intact microvessels. It also remains to be determined how macromolecules permeate the glycocalyx itself, whether pathways, such as vesicles, in addition to the intraendothelial junctions are accessed by the probe proteins, and whether the permeation pathway in arterioles is that of the anatomically shortest path or follows a more tortuous route (4, 9, 11, 15, 43). One depiction of a more complicated model of permeability in arterioles is illustrated in Fig. 5B.

Reconciling data of the present study with previously published work. In our initial studies of isolated arteriolar permeability to macromolecules, we (23) published protocols for isolating coronary microvessels (27, 28, 42). In that procedure a mixture of porcine gelatin and india ink was injected into the coronary vasculature. After immersion of the hearts into cold Krebs-Ringer solution, the gelatin solidified (23), highlighted the microvasculature, and facilitated microvessel isolation. The expectation was that this treatment would not change microvascular function. One index of function, coronary microvascular tone, was unaltered by the presence of gelatin and india ink (27, 28, 42). Additionally, isolated coronary venule permeability to macromolecules was reported to be unaffected by the mixture lending further support this expectation (42).

In our initial study (23), characterizing the permeability properties of isolated coronary arterioles, median for $P_s^{\alpha-la}$ was 14.4 ± 5.1 × 10⁻⁷ cm/s at 14 cmH₂O and for $P_s^{PSA}$ was 6.8 ± 3.4 × 10⁻⁷ cm/s at 16 cmH₂O. In the present study, when the arterioles were not exposed...
Indeed, given that albumin and the magnitude of the response to Ado. These outcomes also argue for the ability and no alteration in the response to the application of superfusate Ado. These outcomes also argue for perfusate pronase acting on a structure that hinders the movement of proteins accessible from the luminal compartment. Heparinase (14, 36), an enzyme specific for the glycoprotein heparin sulfate, also elicited responses that were comparable to pronase resulting in an increase in permeability from basal levels (Fig. 4). In heparinase-treated arterioles, Ado suffusion also decreased permeability from postenzyme treatment levels (Fig. 4).

In conclusion and summary, the data from the present study are consistent with the presence of a glycoprotein layer on the inner surface of coronary arterioles that offers appreciable resistance to the passage of proteins of different size and similar charge in coronary arterioles. Functionally, the glycocalyx has been associated with water, respiratory gas, water-soluble solute, and protein flux in aorta, venules, and capillaries. This study is the first to demonstrate the functional presence of such a layer in coronary arterioles. Permeability responses to Ado suffusion were magnified when the layer was disrupted enzymatically or after removal of a gelatin plug. The data further suggest that disruption of the glycocalyx could result in altered permeation of solutes into the arteriolar wall as well as a more narrow boundary layer between bulk flow and the endothelial surface.

We thank Li Ping Ji, Pam Thorne, Chris Samples Aaker, Rachel Johnson, Jim Vutchetich, Tammy Strawn, and Denise Stowers for technical assistance in all aspects of these studies and Drs. M. Harold Laughlin, Leona J. Ruben, Allan W. J. ones, and Rolando E. Rumbaut for discussions on the content.

This work has been supported by National Heart, Lung, and Blood Institute Grants P01-HL-52490, R01-HL-34872, R01-HL-36088, R01-HL-42528, and T32-HL-07094.

Address for reprint requests and other correspondence: V. H. Huxley, Dept. of Physiology, Univ. of Missouri School of Medicine, Columbia, MO 65212 (E-mail: HuxleyV@health.missouri.edu).

Received 19 January 1999; accepted in final form 14 October 1999.

REFERENCES


