Role of the endothelial glycocalyx in dromotrophic, inotropic, and arrhythmogenic effects of coronary flow

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Rubio, Rafael, and Guillermo Ceballos. Role of the endothelial glycocalyx in dromotropic, inotropic, and arrhythmogenic effects of coronary flow. Am. J. Physiol. Heart Circ. Physiol. 278: H106–H116, 2000.—Coronary flow regulates cardiac functions, and it has been suggested that endothelial membrane glycosylated proteins are the primary shear stress mechanosensors. Our hypothesis was that if these proteins are the sensors for flow, then intracoronary perfusion of lectins or specific antibodies should differentially depress coronary flow-enhanced responses of different parenchymal cell types such as auricular-ventricular (A-V) nodal cells (dromotropic effect), contractile myocytes (inotropic effect), and junctional Purkinje-muscle cells (spontaneous ventricular rhythm). The coronary flow stimulatory effects on A-V delay and spontaneous ventricular rhythm were selectively depressed by six of eight lectins. None of the lectins depressed the coronary flow inotropic effect. Antibodies against endothelial surface proteins, αβ3-integrin and sialyl-Lewisx, depressed the dromotropic but not the inotropic effects of coronary flow, whereas the vascular cell adhesion molecule 1 antibody had no effect on the dromotropic, but enhanced the inotropic effect. The fact that lectins and antibodies differentially depressed regional coronary flow effects suggests that there is a chemical distinctiveness in their intravascular endothelial cell surfaces. However, nonselective cross-linking of endothelial glycocalyx proteins with 2,000-kDa dextran aldehyde or vitronectin indistinctively depressed the dromotropic and inotropic effects of coronary flow. These results indicate that coronary flow-induced stress acts on specific structures located in the capillary intravascular membrane glycocalyx.

CORONARY HEMODYNAMIC FORCES, intravascular pressure, and flow are regulatory signals of cardiac metabolism and functions (4, 23, 29, 30, 38, 43, 49, 51, 53, 54). Gregg and Fisher (23) and other investigators (4, 17, 30, 52, 53) demonstrated that an increase in coronary flow stimulates ventricular contraction and oxygen consumption while ruling out the possibility of a compensatory ischemic correction response. Furthermore, Dijkman et al. (20) recently provided evidence that the inotropic response to flow results from changes in capillary perfusion. Coronary flow in addition to contraction stimulates other functions such as the release of the atrial natriuretic factor (38), the discharge of sinoatrial nodal cells (38), the coronary vascular tone (6, 37), the synthesis of protein (53), glycolytic flux (42, 49, 51), and the electrical propagation of auricular-ventricular (A-V) nodal cells (42, 43). Thus, is coronary flow a stimulus acting on the luminal surface of endothelial cells? To answer this question, we must reveal the endothelial mechanosensors to shear stress and characterize them.

Vascular endothelial cells have been shown to be responsive to frictional shear stress both in culture and in situ (9, 22, 25, 31). In cultured endothelial cells, increases in shear stress cause the release of a diversity of endothelial bioactive substances (9, 13, 17, 19, 22, 25, 29, 31) that, in vivo, could modulate the function of the parenchymal cells associated with the endothelium (15, 25, 40, 51). Endothelium-induced alterations of parenchymal functions have been demonstrated under coculture conditions using various parenchymal cell types (15, 32, 35, 50). Moreover, stripping isolated papillary muscle of its endocardial endothelial layer decreases its isometric tension (11).

Forcing a viscous fluid to flow parallel to the surface of anchored cells gives rise to stress that acts on luminal endothelial surface structures (10, 40, 49); these are balanced by reaction-tensile forces imposed in cytoskeletal elements that, in turn, transmit the stress to anchoring molecules in the abluminal side (16, 17). The abluminal endothelial β1- and β3-integrins are important in the signaling pathway of shear stress-induced tyrosine kinase activation (28, 37) and vasodilation (37), as demonstrated in cultured endothelial cells (28) and isolated perfused coronary arterioles (37), respectively. On the other hand, luminal endothelial glycosylated proteins have been shown to be important to shear stress-induced dilatation in isolated rabbit mesenteric arteries (40) and glycolytic flux in guinea pig hearts (50). Therefore, the application of stress forces may strain luminal, cytoskeletal, and abluminal structures, and these deformations alone or in combination may induce an array of intracellular second messenger pathways (9, 17, 27, 31, 40, 49).

The luminal endothelial membrane is coated with a diversity of hydrated proteins glycosylated with a variety of glycans (5, 12, 18, 21, 33, 39, 41, 45, 47, 48), and it...
has been suggested that the primary shear stress transducers may be specific luminal surface proteoglycans (10). This hypothesis implies that selective chemical alteration of these proteins should differentially alter shear stress-induced responses. The selective chemical alteration can be achieved by exposing the endothelial lumen to either glycosidic hydrolyzing enzymes (12, 18, 33, 40, 47, 49) or to lectins (41, 45–49) or antibodies against defined luminal endothelial proteins (26).

Lectins are proteins with two to four high-affinity binding sites for specific saccharide residues (46). When intracoronarily infused, lectins discriminately bind with high affinity solely to structures localized in the luminal endothelial surface (41, 45, 47, 48). Also, a number of luminal endothelial proteins have been identified, and their antibodies are commercially available (26).

The purpose of this study was to test the hypothesis that luminal endothelial surface proteoglycans are transducers of the coronary flow-induced cardiac function modulation. We reasoned that intracoronary infusion of various lectins or antibodies against luminal endothelial proteins should depress coronary flow-enhanced responses of different parenchymal cell types such as A-V nodal cells (A-V delay) (1, 2, 7, 43), contractile myocytes (ventricular contraction), and junctional Purkinje-muscle cells (spontaneous ventricular rhythm) (8, 14, 44). These three different effects of coronary flow were differentially modulated by various lectins and antibodies.

METHODS

Isolated Saline-Perfused Hearts

Male guinea pigs (350–400 g) were anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg) and heparin (500 U). Later, the animals were artificially ventilated, the chests were opened, and a loose ligature was passed around the ascending aorta. The hearts were rapidly removed, immersed in ice-cold physiological saline, retrogradely perfused via a nonrecirculating perfusion system at constant flow, and trimmed of all noncardiac tissue. Coronary flow was adjusted by varying the output of a variable-speed peristaltic pump (Harvard Apparatus 1215). An initial perfusion rate of 25 ml/min for 5 min was followed by a 25-min equilibration period at a perfusion of 10 ml/min. All experimental measurements were initiated after this period of equilibration. The perfusion media was a Krebs-Henseleit (K-H) solution with the following composition (in mM): 117.8 NaCl, 6 KCl, 1.8 CaCl2, 1.2 MgSO4, 1.2 NaH2PO4, 24.2 NaHCO3, 0.027 NaEDTA, 5 glucose, and 5 pyruvate. All perfusion solutions were equilibrated with a gas mixture of 95% O2-5% CO2 at 37°C at pH 7.4. The coronary pressure was continuously recorded via a side arm of the perfusion cannula and, at a flow of 10 ml/min, was 46 ± 2.8 mmHg.

Electrical Stimulating and Recording Procedures

Small stainless steel wire vascular clamps (Fine Surgical Instruments) soldered to a thin flexible wire were used as recording and stimulating electrodes. The clamp was affixed to the myocardial surface so that it gently grasped the epicardial tissue layer.

A pair of stimulating electrodes was placed 2 mm apart in the apex of the right atrial appendage. Pacing was achieved by applying electrical square pulses of 2x threshold, 2-ms duration at a rate of 4.5 ± 0.1 Hz. To record the auricular and ventricular electrocardiograms, one electrode was placed in the left atrium and a second electrode on the apex of the left ventricle. These two electrodes were connected to an oscilloscope synchronized with the atrial pacing stimulator. The A-V interval (A-V delay, in ms) was continuously monitored and measured as the time interval between the application of the stimulus to the atrium and the rising phase of the ventricular electrical signal. The time interval between the application of the stimulus to the atrium and the rising phase of the atrial electrocardiogram was constant throughout all of the various manipulations utilized and had a value of 18 ± 0.8 ms. We (7, 43) and others (1–3) have shown that under a variety of experimental conditions, changes in the A-V delay are caused solely by changes in the delay in the A-V nodal region, as defined by the interval between the electrocardiogram of the atrium and the bundle of His (A-H interval). The A-V delay was at all times a constant 11.3 ± 0.2 ms longer than the A-H interval. In our studies the A-V delay reflects the transmission time across the A-V nodal region, a piece of tissue 1–2 mm long and 0.1 mm thick.

Studies on Effects of Coronary Flow on A-V Transmission

In an initial set of measurements, coronary flow was increased stepwise and the A-V delay was measured at every level of coronary flow (control). After an experimental manipulation was performed, these measurements were repeated (experiment). The A-V delay was plotted for each coronary flow value. Each heart was its own control.

Measurements of Ventricular Contraction

Ventricular pressure development was measured in the same group of hearts used for A-V delay measurements. Via the left atrium, a fluid-filled latex balloon was introduced into the left ventricle. Diastolic pressure was adjusted to ~10 mmHg and the developed pressure continuously determined. The maximal pressure developed at the higher coronary flow under control conditions was defined as 100%, and all other amplitudes measured under control and experimental conditions were expressed as percentages.

Studies on Spontaneous Ventricular Rhythm

A separate group of hearts was used for these studies. Spontaneous ventricular rhythm was induced by destroying the A-V nodal area. For this purpose, an incision was made in the right atrium to clearly expose the coronary sinus ostium, the ventricular septum, and the tricuspid valve. Destruction of the A-V nodal area was achieved by crushing the surface tissue lying between the ostium and cardiac valves with small forceps. This manipulation resulted in a blockade of the impulses from atrium to ventricle and the appearance of a spontaneous ventricular rhythm expressed in beats per minute. In an initial set of measurements, coronary flow was increased stepwise and the spontaneous ventricular rhythm was measured in beats per minute at every coronary flow level (control). After an experimental manipulation was performed, these measurements were repeated (experiment). The spontaneous ventricular rhythm was plotted for each coronary flow value. Each heart served as its own control.

Intracoronary Infusion of Substances Capable of Binding to Molecules of Endothelial Cell Membrane Glycocalyx

Specific substances, lectins. As specific reactants to various glycosidic groups (46), eight different lectins (Sigma Chemical, St. Louis, MO; E-Y Laboratories, San Mateo, CA),
Table 1. Saccharide-binding specificities of lectins used and their interactions with cardiac endothelial plasmalemmal proteins

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Nominal Carbohydrate Specificity</th>
<th>Interacting Glycoproteins In Situ</th>
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<tbody>
<tr>
<td>Lycopersicon esculentum*</td>
<td>(β1,4GlcNAc)≠(β1,4GlcNAc)</td>
<td>gp100</td>
</tr>
<tr>
<td>Arachis hypogea</td>
<td>Galβ1GalNAc≠α and βGal</td>
<td>Unknown; lectin not tested</td>
</tr>
<tr>
<td>Concanavalia ensiformis</td>
<td>αMan≠βGlc</td>
<td>gp47, gp85/75, gp100, gp120, gp140</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>αMan≠αGlc</td>
<td>Unknown; lectin not tested</td>
</tr>
<tr>
<td>Griffonia simplicifolia*</td>
<td>αGalNAc≠αGal</td>
<td>gp47, gp100, gp120, gp140</td>
</tr>
<tr>
<td>Limulus polyphemus</td>
<td>NeuAc</td>
<td>Unknown; lectin not tested</td>
</tr>
<tr>
<td>Ricinus communis†</td>
<td>βGal≠αGal≠GalNAc</td>
<td>gp47, gp60, gp85/75, gp100, gp120, gp140</td>
</tr>
<tr>
<td>Tritium vulgaris</td>
<td>(β1,4GlcNAc)≠(β1GlcNAc)</td>
<td>gp47, gp60, gp85/75, gp100, gp120, gp140</td>
</tr>
</tbody>
</table>

*These lectins bind only to intravascular sites in rat myocardium (42). All other lectins showed binding at intra- and extravascular sites. †These lectins have no action despite binding intra- and extravascularly. Gal, galactosidase; Glc, glucosidase; Man, mannosidase; GalNAc, N-acetylgalactosidase; GlcNAc, N-acetylgalactosidase; NeuAc, N-acetylneuraminic acid.

Interactions of lectins with cardiac endothelial plasmalemmal proteins were previously determined by Schnitzer et al. (45). Using intravascular radiodination of rat heart endothelium in situ, they separated by gel SDS electrophoresis seven 125I-labeled protein bands. To characterize their glycan composition, each band (glycoprotein [gp]) was subjected to affinity chromatography using various lectins as ligands. These gp were identified by their apparent molecular weight (gp47 through gp140). Species from which lectins were derived are listed in order of the lectin’s auricular-ventricular effect. These lectins bind only to intravascular sites in rat myocardium (42). All other lectins showed binding at intra- and extravascular sites. These lectins have no action despite binding intra- and extravascularly. Gal, galactosidase; Glc, glucosidase; Man, mannosidase; GalNAc, N-acetylgalactosidase; GlcNAc, N-acetylgalactosidase; NeuAc, N-acetylneuraminic acid.

Covalently coupled to either 50-nm gold particles or to ferritin (molecular mass 500 kDa), were used. These large particle sizes were chosen to slow the diffusion of lectins through the blood vessel wall. The eight lectins are listed in Table 1. These large particle sizes were chosen to slow the diffusion of lectins through the blood vessel wall.

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Table 2. Monoclonal antibodies utilized against luminal endothelial glycocalyx proteins

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Intracoronary Concentration</th>
</tr>
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<tbody>
<tr>
<td>Mouse anti-human VCAM-1</td>
<td>0.5 µg/ml</td>
</tr>
<tr>
<td>Mouse anti-human Lewisb</td>
<td>1.500 dilution</td>
</tr>
<tr>
<td>Mouse anti-human αββ-integrin</td>
<td>0.5 µg/ml</td>
</tr>
<tr>
<td>Rabbit anti-human vitronectin receptor (αβββ-integrin)</td>
<td>0.4 µg/ml</td>
</tr>
<tr>
<td>Mouse anti-human ββ-integrin</td>
<td>0.5 µg/ml</td>
</tr>
<tr>
<td>Mouse anti-human Lewisb</td>
<td>1.500 dilution</td>
</tr>
<tr>
<td>Mouse anti-human ICAM-1</td>
<td>0.7 µg/ml</td>
</tr>
<tr>
<td>Mouse anti-human PECAM</td>
<td>0.8 µg/ml</td>
</tr>
<tr>
<td>Mouse anti-human E-selectin</td>
<td>0.8 µg/ml</td>
</tr>
<tr>
<td>Mouse anti-human LFA</td>
<td>0.7 µg/ml</td>
</tr>
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</table>

Only vascular cell adhesion molecule 1 (VCAM-1) affected coronary flow effects on cardiac function. ICAM-1, intercellular adhesion molecule 1; PECAM, platelet endothelial adhesion molecule; LFA, leukocyte function-associated antigen.

Electron Microscopy Studies of A-V Nodal Area

After the initial equilibration period and at a coronary flow of 8 ml/min, the lectin derived from Concanavalia ensiformis,
coupled to ferritin (concanavalin A-ferritin), and the lectin derived from Arachis hypogea, coupled to colloidal gold (Arachis hypogea-gold; 50-nm diameter) were intracoronarily infused at a final concentration of 10 µg/ml for a period of 10 min, followed by a 10-min period of washout with K-H. Thereafter, the heart was perfused with a fixative solution of 2.0% glutaraldehyde in phosphate buffer (pH 7.4) for 10 min. The hearts were then removed, and the A-V nodal area was removed (~0.5 mm thick and 2 mm long) and divided into two 1-mm-long tissue pieces using a fine razor blade. These tissue pieces were placed in fixative solution overnight at 4°C, and the next morning the tissue pieces were rinsed in phosphate buffer, postfixed in buffered osmium tetroxide, and processed for transmission electron microscopy (47, 48). Before they were viewed, the tissues perfused with the Arachis hypogea-gold were stained with uranyl acetate and lead nitrate because the gold particles are highly electron opaque and can be clearly seen against the darkly stained background. Conversely, it was not necessary to stain the tissues perfused with concanavalin A-ferritin because these particles are less electron opaque.

Statistics

Values are expressed as means ± SE. In all experiments each heart was its own control; responses under control conditions and during specific manipulations were compared in the same heart. Statistical significance was determined using a paired t-test with a Bonferroni correction factor for multiple comparisons. A P value ≤0.05 was considered to be statistically significant.

RESULTS

Lectin Binding to Intravascular Glycocalyx of Capillaries in A-V Nodal Region

The hearts perfused with concanavalin A-ferritin and Arachis hypogea-gold show that these lectins were bound in the A-V nodal region and remained confined to the intravascular glycocalyx coat of capillary endothelial cells (Fig. 1). Concanavalin A-ferritin (Fig. 1A) and Arachis hypogea-gold particles (Fig. 1B) were bound to the intravascular endothelial basement membrane. As described by others (3, 34), the myocytes of this tissue, in contrast to the contractile ventricular and atrial myocytes, show a lesser organization and lower density of contractile filaments and a lower mitochondrial density (Fig. 1). The myocyte basement membrane did not show the presence of lectin particles (Fig. 1). Our results on the intravascular distribution of lectin binding on the A-V nodal region agree with the results by other investigators who studied the distribution and sites of binding of different lectins on the coronary capillary blood vessel at the level of ventricular contractile myocytes (41, 45, 47, 48).

Effect of Lectins on Positive Dromotropic Action of Coronary Flow

We previously established (43) that increases in coronary flow reduce the A-V delay as a result of a hydraulic stimulatory effect on the transmission of electrical impulses in the A-V nodal region. We showed that in the isolated, perfused guinea pig heart, as coronary flow increases within a range of 4–20 ml/min, the A-V delay decreases rapidly and later plateaus. Control A-V delay-coronary flow curves are shown in Fig. 2. This curve remained unchanged during the experiment and was the same at 40 and 60 min after initiation of perfusion. The vertical differences between these two curves were not statistically different from zero (Fig. 3).

The infusion of most lectins caused a shift upward and to the right of the A-V delay-coronary flow curve. Examples of individual experiments are given for the effects of lectins derived from Arachis hypogea (Fig. 2A) and Lycopersicum sculentum (Fig. 2B). In every experiment the corresponding A-V delay control value at the various coronary flow rates was subtracted from the A-V delay value after lectin treatment. These differences were taken for all lectins, and the mean ± SE (n = 5 hearts per lectin) were plotted against coronary flow rates. These plots are shown in Fig. 3. The different lectins caused variable effects on the A-V delay-coronary flow curves. Lycopersicum sculentum lectin was the most effective, followed by Arachis hypogea = concanavalin A = Lens culinaris lectins, then Griffonia simplificolia lectin, and, last, Limulus polyphemus lectin. Each one of these curves was statistically different from the time control curve. However, two of the tested lectins, those derived from Ricinus communis and Triticum vulgaris, were without effect (Fig. 3).

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Fig. 1. Electron micrographs of auricular-ventricular (A-V) nodal region showing electron opaque lectin particles (indicated by short lines) confined to intravascular space and resting against the endothelial (E) wall of capillary adjacent to myocyte (M). A: lectin derived from Concanavalia ensiformis (concanavalin A) was coupled to ferritin and infused. B: lectin derived from Arachis hypogea was coupled to 50- to 60-nm gold particles and infused. Calibration bar, 1 µm.
Effect of Lectins on Coronary Flow Stimulation of Spontaneous Ventricular Rhythm and Ventricular Contraction

It is well known that coronary flow stimulates ventricular contraction amplitude (4, 52, 54) as well as ventricular utilization of glucose and protein synthesis (49, 51, 53). We decided to determine whether coronary flow also stimulates spontaneous ventricular rhythm. We observed that as coronary flow increases, the frequency of the spontaneous ventricular rhythm rises and reaches a plateau (Fig. 4, control). Infusion of concanavalin A or *Griffonia simplificolia* lectin caused a downward displacement and a flattening of the control curve, clearly inhibiting the arrhythmogenic positive effects of coronary flow (Fig. 4). Similar effects were obtained with the *Lycopersicum esculentum* and *Limulus polyphemus* lectins (not shown).

In contrast, in the case of the positive inotropic effects of coronary flow, none of the eight lectins tested showed an inhibitory effect on the coronary flow-induced inotropism, as shown for *Lens culinaris* lectin (Fig. 5A) and concanavalin A (Fig. 5B). Thus lectins affected the dromotropic and ventricular arrhythmogenic actions of coronary flow but did not change its ventricular inotropic actions.

Effects of Antibodies on Dromotropic and Inotropic Actions of Coronary Flow

Ten antibodies against different intraluminal endothelial membrane proteins were infused intracoro-

![Fig. 2. Dromotropic effect of coronary flow and inhibitory modulation of this effect by lectins derived from *Arachis hypogaea* (Arach. Hyp.) (A) and *Lycopersicum esculentum* (Lyc. Soul.) (B). Results are from 2 separate individual experiments. As coronary flow increases, A-V delay decreases, and after infusion of lectin, the curve was displaced upward and rightward.](image)

![Fig. 3. Difference between A-V delay after lectin treatment and corresponding A-V delay control at various coronary flows. Results from 8 lectins are presented on separate graphs A and B to avoid crowding; n = 5 hearts for each lectin. Curves corresponding to 6 of 8 lectins were active and statistically different from time control curve (40 & 60 min). Lectins derived from *Triticum vulgaris* (Trit. vulg.) (A) and *Ricinus communis* (Ric. comm.) (B) had no effect. *Griff. simp.*, lectin derived from *Griffonia simplificolia*; *Con A*, concanavalin A; *Limul. pol.*, lectin derived from *Limulus polyphemus*; *Lens cul.*, lectin derived from *Lens culinaris*.](image)
narily at the final concentrations indicated in Table 2. Three of these ten were found to produce an effect. Anti-αvβ5-integrin (Fig. 6) and anti-sialyl-Lewis b (Fig. 7) caused displacement upward and to the right of the control A-V delay-coronary flow curve and were without effect on the coronary flow ventricular inotropic action. In contrast, anti-vascular cell adhesion molecule (VCAM)-1 (Fig. 8) produced no change in the dromotropic-coronary flow effect but did have a small yet significant enhancing effect on the coronary flow ventricular inotropic action.

Dextran-Aldehyde and Vitronectin Inhibit Both Positive Dromotropic and Ventricular Inotropic Actions of Coronary Flow

Both the coronary flow-induced dromotropic and ventricular inotropic actions were importantly reduced after infusion of either dextran-aldehyde (Fig. 9) or vitronectin (Fig. 10). The A-V delay-coronary flow curves after coronary infusion of either dextran-aldehyde (Fig. 9A) or vitronectin (Fig. 10A) were displaced upwardly and to the right, and the respective curves of ventricular contraction amplitude-coronary flow were displaced downwardly (Figs. 9B and 10B). Thus neither the effects of dextran-aldehyde nor those of vitronectin were function selective.

DISCUSSION

Our results show that coronary flow affects spontaneous ventricular rhythm in addition to its well-known metabolic (4, 23, 49, 51–54), inotropic (4, 20, 30, 52, 54), dromotropic (43), and secretory effects (38). In addition, the results indicated that the luminal surface of the endothelium is a key element involved in the modulatory effects of coronary flow. Our electron microscopy studies show that in the A-V nodal region lectins remain confined to the intravascular endothelial glycoalkyx and that the coronary flow effects on A-V delay were selectively depressed by lectins. Lectins also depressed the coronary flow stimulation of spontaneous ventricular rhythm. However, none of the lectins tested affected the coronary flow-induced ventricular inotropism despite the fact that lectins bind with high affinity to the capillary endothelial intravascular glycoalkyx (Table 1) (41, 45, 47, 48). In addition, antibodies against defined endothelial surface proteins also, differentially, affected the dromotropic and inotropic effects of coronary flow. Nevertheless, 2,000-kDa dextran-aldehyde and vitronectin, which would be expected to cause
nonselective cross-linking of endothelial glyocalyx proteins, indistinctively affected the dromotropic and inotropic actions of coronary flow. Dextran-aldehyde reacts with every primary and secondary amino group of surface proteins, whereas vitronectin binds with high affinity to most integrins and heparinic groups (26). These results support the proposed hypothesis that shearing forces associated with coronary flow, most likely at the capillary endothelial cell (20, 30, 52, 54), act on the luminal surface proteoglycans and that the subsequent deformation of some specific molecule(s) is transduced by the endothelium into function modulation of neighboring parenchymal cardiac cells.

We studied three different cardiac functions responsive to flow: A-V delay, ventricular contraction, and spontaneous ventricular rhythm. These functions take place in different parts of the heart, each containing myocytes with distinct functional and structural properties (34). Approximately 90% of the A-V delay takes place in the A-V nodal region (1–3, 7, 43), which is ~2 mm long and 0.1 mm thick and consists of A-V nodal cell types (3). Left ventricular contraction takes place in the muscle mass, which is made up of contractile myocytes (34). Finally, spontaneous ventricular rhythm is principally the discharge of a dominant pacemaker in Purkinje fibers within the Purkinje-myocyte region (8, 14, 44). In each of these three functional and anatomic regions, the inherent parenchymal cells are closely apposed to capillary endothelial cells, forming a parenchymal-endothelial functional junction with distinct responses to coronary flow. The distinctiveness of each region could be explained if their intravascular luminal endothelial surfaces were chemically and structurally different. This conclusion is derived from the observa-

Fig. 6. Pretreatment with antibody against $\alpha_\beta_\gamma$-integrin inhibits dromotropic effect of coronary flow (A; n = 4 hearts) but has no action on inotropic effect (B; n = 4 hearts).

Fig. 7. Pretreatment with monoclonal antibody against sialyl-Lewis/b (MAb sialyl b) inhibits dromotropic effect of coronary flow (A; n = 4 hearts) but has no action on inotropic effect (B; n = 4 hearts).
tion that the coronary flow actions on these three functional regions were affected differentially by lectins and antibodies.

Possible Mechanism of Action of Lectins Involved in Coronary Flow Effects on A-V Delay and Spontaneous Ventricular Rhythm

Schnitzer et al. (45) isolated a group of coronary intravascular endothelial proteins and found that each protein possesses affinity for several lectins (Table 1). All lectins that we utilized, when intra-arterially infused for periods of <1 h, bind with high affinity solely to glycosylated proteins localized in the luminal endothelial surface (12, 41, 45, 47, 48). This indicates that lectins affect coronary flow-modulated responses by binding solely to intraluminal endothelial proteins. However, although lectin binding to specific glycosidic groups is a necessary step for the coronary flow response modulation, it is not sufficient because the lectin must bind to a distinctive site within the protein. This is suggested because lectins such as the Ricinus communis and Triticum vulgaris lectins do not exert a coronary flow modulatory effect, yet they bind to most intravascular coronary endothelial proteins, as characterized by Schnitzer et al. (45) (Table 1). Ricinus communis lectin shares a similar glycosidic group recognition with Arachis hypogea and Griffonia simplifolia lectins (both active) and Triticum vulgaris lectin affinity compares with that of Limulus polyphemus and Lycopersicum esculentum lectins (also both active;
Thus it appears that for a lectin to be an effective coronary flow modulator, it must bind to intravascular protein(s) by fulfilling two conditions, chemical recognition of 1) the glycosidic group and 2) the distinctive site within the protein.

It remains to be explained why the coronary flow-induced inotropism is not affected by any of the eight lectins tested, despite the fact that the presence of intravascular lectin binding sites in capillaries feeding ventricular contractile myocytes have been well established (41, 45, 47, 48). This suggests that in these capillaries the glycoproteins responsive to shearing stimuli have different site distribution of their glycosidic groups compared with that of similar glycoproteins from other regions of the coronary vasculature. The implication of this reasoning is that a proper lectin treatment should inhibit the coronary flow-induced inotropism. We found that simultaneous intracoronary infusion of two or three lectins caused this effect even though individually each lectin had no action (data not shown).

Lectins may cause cross-linking between neighboring intraluminal glycoproteins by binding to specific glycosidic groups. When infused intravascularly, each of the two to four subunits of a lectin (46) can bind to a specific glycosidic group. These glycosidic groups can be located in the same glycoprotein or in neighboring glycoproteins. This cross-linking could make the glycoproteins less susceptible to deformation by shearing stress, resulting in inhibition of the coronary flow-induced response.

Identification With Antibodies of Intraluminal Molecular Structures Involved in Coronary Flow-Modulated Responses

We hypothesized that if a protein of the intravascular endothelial cell membrane glyocalyx is a primary sensor to a coronary flow-induced effect, then by binding to its antibody its role in this flow effect should be discovered. We used 10 monoclonal antibodies against 10 different luminal endothelial proteins, three of which caused modulation of coronary flow-induced effects. Two of the active monoclonal antibodies, anti-αvβ5-integrin and anti-sialyl-Lewisx glycan, affected the coronary flow-induced dromotropic effect without affecting the inotropic response. The third antibody, anti-VCAM-1, enhanced slightly the inotropic coronary flow-induced response without affecting the dromotropic response. We have no explanation for the potentiating effect of anti-VCAM-1. These results indicate that at each region of the coronary capillary vasculature, the molecular sensors of flow vary, and that there is more than one sensor per region and/or function; i.e., there is heterogeneity of the chemical composition and function of the intravascular surface of the endothelium along the coronary vasculature.

Further support for the concept that the intravascular surface of the endothelium along the coronary vasculature is chemically and functionally heterogeneous comes from studies perfusing hydrolyzing enzymes with specificities toward different specific glycosidic groups. Intracoronary perfusion of these enzymes differentially modulates coronary flow-induced responses. In the isolated, perfused guinea pig heart, Suarez and Rubio (49) showed that the coronary flow stimulation of glycolytic flux was inhibited by the infusion of heparinase, whereas the infusion of hyaluronidase was without effect. In contrast, the inotropic effect of coronary flow was inhibited by hyaluronidase but not by heparinase (42). Heparin and hyaluronidate residues are known to be present in high concentrations at the endothelial surface of the glyocalyx (12, 18, 33, 39). Similarly, Pohl et al. (40) in small rabbit mesenteric arteries showed that flow-induced dilation was completely inhibited by infusion of neuraminidase and suggested that removal of sialic acid moieties from...
membrane-anchored glycoproteins, the mechano-sensors, renders them flow insensitive. Bevan and Siegel (10) also suggested that endothelial membrane proteins with electrically charged glycosidic groups are the primary sensors of flow in the flow-induced vasodilation response.

Effects of Nonspecific Cross-Linking Agents on Dromotropic and Inotropic Actions of Coronary Flow

If cross-linking between distinctive anchoring sites of intravascular endothelial-specific glycoproteins made them less susceptible to deformation by shearing stress and this was the cause of a reduced coronary flow-induced specific response, it would imply that nonspecific cross-linking between intravascular endothelial glycoproteins would affect all coronary flow-modulated responses. Thus we decided to use two nonspecific cross-linking agents, 2,000-kDa dextran-aldehyde and vitronectin. The 2,000-kDa dextran-aldehyde contains multiple aldehyde groups (2 groups per glucose moiety) that react with the terminal amino groups of all the luminal endothelial proteins, causing their polymerization. Similarly, Melkumyants et al. (36), by infusing 0.02% glutaraldehyde for 30 s to isolated, perfused feline arteries, blocked the flow-induced dilation. A vitronectin molecule has several binding sites for most integrin molecules and also has affinity for the heparinic groups (26), which are abundant in the intravascular lumen (18, 33, 39). These two agents, when intracoronarily perfused, in contrast to lectins and antibodies, did not discriminate between the effects of coronary flow on A-V delay and myocardial contraction, suggesting that they could equally affect all vascular territories.

In summary, our results support the hypothesis that coronary flow-induced stress acts on specific structures located on the capillary intravascular membrane glyocalyx, causing modulation of specific functions of the subjacent parenchymal cell. This is indicated by the fact that the coronary flow-modulated responses are inhibited by agents that bind with high specificity to intravascular structures. Furthermore, agents that cause indiscriminate cross-linking of endothelial intravascular membrane proteins nonspecifically depress the coronary flow modulation of functions. The fact that lectins and antibodies discriminate between actions of coronary flow on different regions of the coronary vasculature indicates that there is chemical and functional heterogeneity of the intraluminal endothelial glyocalyx.

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