We frequently study processes that alter microvascular permeability in the heart. Myocardial microvascular permeability has been estimated by determining the filtration independent reflection coefficient for β-lipoprotein (σ_{pl,myo}). This technique requires the measurement of myocardial lymph flow rate and the lymph-to-plasma protein concentration ratio. Unfortunately, it is a nonsurvival procedure. An index of myocardial microvascular permeability was needed that could be determined in experimental preparations without sacrificing valuable chronically instrumented animals. We attempted to relate changes in myocardial microvascular permeability and myocardial edema formation to some index of myocardial performance.

In 33 acute, anesthetized dogs (with normal or disrupted myocardial microvasculatures), we measured systemic arterial pressure, systemic venous pressure, coronary sinus pressure, left ventricular pressure, the maximum rate of change in left ventricular pressure (dP/dt)_{max}, myocardial lymph flow rate, and myocardial extravascular fluid volume. Following an increase in coronary sinus pressure, the amount of edema fluid entering the myocardium varied as a function of myocardial microvascular permeability. Further, as the heart became edematous, (dP/dt)_{max} changed with respect to time [Δ(dP/dt)_{max}/Δt]. Finally, a significant relation was found between σ_{pl,myo} and Δ(dP/dt)_{max}/Δt. Since coronary sinus pressure can be elevated and Δ(dP/dt)_{max}/Δt can be measured in chronic animals, this technique may be useful for evaluating myocardial microvascular permeability on a long-term survival basis. (Circulation Research 1987;61:203–208)

From the Center for Microvascular and Lymphatic Studies and Department of Anesthesiology, University of Texas Medical School, Houston, TX 77030.

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Address for correspondence: Dr. Glen A. Laine, Department of Anesthesiology, University of Texas Medical School, 6431 Fannin, MSMB 5.020, Houston, TX 77030.

Change in (dP/dt)_{max} as an Index of Myocardial Microvascular Permeability

Glen A. Laine

The permeability of the myocardial microvasculature to plasma proteins is frequently evaluated by determining the filtration independent reflection coefficient (σ) of the microvascular exchange barrier. This technique requires the collection of myocardial lymph and is a nonsurvival procedure. When studying processes that alter microvascular permeability in the heart, such as myocardial ischemia or chronic arterial hypertension, it is imperative that expensive chronic arterial pressure transducers, and all data was recorded on a Grass (Quincy, Mass.) chart recorder. Systemic arterial
pressure (SAP) and systemic venous pressure (SVP) were recorded from the respective femoral catheters. CSP was obtained from the Swan-Ganz catheter within the coronary sinus. Left ventricular pressure (LVP) and the first derivative of LVP with respect to time (dP/dt) were obtained from the solid-state microtransducer in the left ventricle. Plasma protein concentration was determined, using a refractometer, on plasma derived from blood drawn from the femoral artery line. Protein concentration in cardiac lymph was determined in a similar manner. Both plasma and lymph samples were electrophoretically separated to obtain the ratio of the concentrations for individual protein fractions, such as β-lipoprotein.

Experimental animals were divided into 2 groups. The first group underwent the experimental protocol without insult to the myocardial microvasculature. In the second group, MMP was increased before initiating the experimental protocol. MNP was disrupted in the second group by one of several means, including direct injection of histamine, ethanol, or oleic acid into the coronary arteries. The intent was not to reproducibly or quantitatively increase MMP, but to increase permeability in a random fashion.

SAP, LVP, dP/dt, CSP, SVP, and myocardial lymph flow (Qj) were recorded for 30 minutes at the beginning of each experimental protocol. At the conclusion of the control period, CSP was elevated to approximately 50 mm Hg in each animal. In those animals with elevated CSP, σ for β-lipoprotein was determined as an index of MMP. A detailed description of this technique is outlined in “Data Interpretation and Analysis.” At the conclusion of each 3-hour experiment, the anesthetized animals were killed with KCl. The left ventricle from each heart was removed, and the amount of edema within the myocardium was quantitated by determining the ratio of extravascular fluid weight to blood-free dry weight. Control EVF values were determined on animals having no elevation in CSP.

Data Interpretation and Analysis

Use of the technique outlined in this manuscript depends on an understanding of two important concepts. The first is the determination of the current permeability status of the myocardial microvascular exchange barrier; the second is the method of obtaining an index of myocardial performance, which varies as a function of ventricular edema formation.

We chose to use σ as an index of MMP. The σ for any given molecule varies as a function of MMP, having a value of zero when a barrier is permeable to a given molecule and a value of 1 when the barrier is impermeable. A fundamental law of physics dictates that when a protein solution (Cj) is forced across a semipermeable membrane, the concentration of protein on the effluent side of the membrane (Cj) will decrease until a constant ratio between Cj and Cj is reached (Cj/Cj). This is called filtration independence and will not vary even when the rate of filtration across the barrier is increased. Protein solutions can be sampled from both sides of the microvascular exchange barrier. Blood plasma represents the protein solution within the microvasculature, and lymph represents the protein solution on the interstitial side of the microvascular barrier. Myocardial microvascular pressure can be elevated by increasing CSP, thus increasing fluid flow across the myocardial microvascular exchange barrier. When the proper molecule is chosen, rapid movement of the protein solution across the exchange barrier will lead to filtration independence for that molecule, and an index of microvascular permeability may be calculated. Simply stated, this relation can be written:

\[ σ = 1 - \frac{C_j}{C_p} \]

We chose to use β-lipoprotein for our σ determinations for two reasons. First, it is an endogenous plasma protein that can be easily quantitated in both plasma and lymph, eliminating the need to introduce foreign substances, such as markers, into the animal. Any decrease in σ for the large β-lipoprotein molecule would also signal a change in the reflection coefficient for molecules of low molecular weight. Second, due to its large molecular weight, β-lipoprotein reaches filtration independence quickly with a Peclet number well above the theoretical minimum necessary to use this technique.

The second quantity that we required was an index of myocardial performance that varies as fluid accumulates within the myocardium. We chose to use the beat-to-beat change in (dP/dt)\textsubscript{max}, as the index for the reasons that follow. dP/dt and (dP/dt)\textsubscript{max} are single-beat indicators of myocardial performance although they have not been demonstrated to be the best indicators of myocardial contractility. We found that although dP/dt and (dP/dt)\textsubscript{max} may vary greatly from animal to animal or from day to day, the beat-to-beat decreases in (dP/dt)\textsubscript{max} following the elevation in CSP in damaged hearts is reproducible. If MMP is in a normal healthy state, elevating CSP will cause little change in the index since the defense mechanisms against myocardial edema formation (i.e., elevation of myocardial interstitial pressure and an increased Qj) can limit excess interstitial fluid accumulation, thus preventing a change in dP/dt\textsubscript{max}. The fact that this technique was independent of the initial value for contractility or (dP/dt)\textsubscript{max} is very attractive. Since (dP/dt)\textsubscript{max} exists only one time for each heart beat, it forms a discontinuous function with respect to time. Because of this discontinuity, we chose to express it in the form \( Δ(dP/dt)\textsubscript{max}/Δt \) and not in the differential format.

All data analysis was carried out on a VAX 8600 computer. Regression lines were generated from data points using the SAS program \texttt{STEPWISE}. Means were compared with the two-sample \texttt{t} test.

Results

Figure 1 depicts the characteristic decrease in the maximum rate of change in left ventricular pressure, \( Δ(dP/dt)\textsubscript{max} \), following elevation of CSP in a heart with increased MMP. Only the contractile portion of the dP/dt curve (greater than 0) is shown. Elevations of
CSP above approximately 50 mm Hg are not possible due to the redirection of venous blood through low resistance pathways such as the Thebesian veins. Following an initial increase, \((dP/dt)_{max}\) normally decreases following elevation of CSP at a constant rate until a level of approximately 500 mm Hg sec\(^{-2}\) is reached. At lower levels, animals frequently develop both heart rate and myocardial contractility instabilities. The time for \((dP/dt)_{max}\) to fall, as well as the slope of the fall, to this level of instability varies as a function of the damage to the myocardial microvasculature.

In Figure 2, the relation between myocardial edema and coronary sinus pressure are plotted for both normal hearts (dashed lines) and for hearts with a disruption in the myocardial microvasculature (solid lines). It is not surprising that for any given CSP, hearts that have received injuries to their microvascular exchange vessels contain a significantly greater volume of edema fluid than normal hearts.

The amount of myocardial edema formation plotted as a function of the \(\sigma\) for \(\beta\)-lipoprotein (\(\sigma_{\beta\text{-LIP}}\)) is shown in Figure 3. It is clear that over the 3-hour course of these experiments the amount of myocardial edema formation increased as MMP increased. As explained in "Materials and Methods," MMP increases as the values for \(\sigma_{\beta\text{-LIP}}\) decrease below 1. It is important to remember that in each animal, CSP was elevated for two reasons: to obtain the filtration independent reflection coefficients and to force fluid to filter into the myocardial interstitial matrix in an attempt to compromise myocardial performance. Elevation of microvascular pressure by venous occlusion, within the physiologic range, has not been shown to significantly alter microvascular permeability. If, as pointed out in "Materials and Methods," the lymph-to-plasma protein concentration ratio for any given organ \(C_L/C_P\) is plotted as a function of transmicrovascular fluid flux or lymph flow rate, \(C_L/C_P\) falls as transmicrovascular fluid flux increases until a minimum (filtration independent) value for \(C_L/C_P\) is attained. If, at this point, the microvasculature is disrupted or if further elevations in microvascular pressure were to cause an increase in exchange vessel permeability, the filtration independent value for \(C_L/C_P\) would increase, resulting in a U-shaped curve. Although this characteristic pattern may be seen following chemical disruption of the microvasculature, we know of no organ in which this finding has been documented following elevation of venous pressure.

Figure 4 plots the index of myocardial microvascular permeability, \(\sigma_{\beta\text{-LIP}}\), as a function of the beat-to-beat change in \((dP/dt)_{max}\) \(\Delta(dP/dt)_{max}/\Delta t\). Although a linear regression line provided a good fit to this experimental data, we utilized the sigmoidal regression to increase our \(r^2\) values. This was done since we were interested...
in using this relation to predict our index of MMP when \( \Delta(dP/dt)_{max}/\Delta t \) was to be measured in future experiments. The equation for the regression line in Figure 4 is \( y = 1.004 - 2.12x + 7.19x^2 - 9.38x^3 \) with an \( r \) value equal to 0.93. It should be noted that, due to the compliance characteristics of the myocardial interstitium, a small increase in EVF causes a relatively large rise in myocardial interstitial fluid pressure. Consequently, in Figures 3 and 4, a 20% change in \( \sigma_{\beta-LIP} \) 0.6 to 0.8, causes only a small increase in EVF, although the increase in EVF resulted in a significant change in \( \Delta(dP/dt)/\Delta t \). Open triangles in Figure 4 were obtained during a test of the predictive value of this technique in chronically hypertensive dogs. Data are described in "Discussion.”

**Discussion**

The purpose of this study was to develop a technique for the evaluation of myocardial microvascular permeability that could be used on a long-term basis in survival animals. It was hypothesized that as edema fluid entered the myocardial interstitium, myocardial function, represented by \( (dP/dt)_{max} \), would decrease. It was also our contention that if MMP increased, fluid would enter the myocardial interstitium more rapidly and \( (dP/dt)_{max} \) would decrease at an accelerated rate.

We found that it was to our benefit to acutely elevate CSP in our experimental animals to a fixed level for several reasons. First, myocardial microvascular pressure was elevated, causing edema fluid to enter the interstitium much more rapidly. This allowed the time...
for microvascular permeability estimations to be shortened. Second, when CSP is elevated, microvascular surface area is maximized and myocardial lymph can be collected for the determination of a microvascular permeability index ($\sigma_{A P/LV}$). In addition, chronic Swan-Ganz catheters can be placed into the coronary sinus through the jugular vein using fluoroscopy. This allowed us to elevate CSP in chronic animals at any time for the evaluation of MMP. For the purposes of this study, a 3-hour elevation in coronary sinus pressure was chosen to demonstrate the accumulation of extravascular fluid. When microvascular pressure is elevated and fluid begins to enter the interstitium, the interstitial fluid pressure begins to increase. Due to the tight compliance characteristics of the myocardium, as fluid begins to accumulate within the interstitial spaces, smaller and smaller volumes are necessary to generate larger and larger increases in interstitial fluid pressure. We believe that this increase in interstitial fluid pressure and relative stiffness of the myocardium results in our observations of a decreased $dP/dt_{max}$. Although we are capable of measuring small increases in myocardial interstitial fluid pressure, we cannot, using current technology, accurately quantify the smallest volume changes associated with these pressure increases. For the present, we must rely on longer-term collections to document that edema formation is in fact taking place.

We first attempted to relate changes in MMP and edema formation following CSP elevation to changes in $dP/dt$. We found this approach to be unacceptable since the absolute value for $dP/dt$ tended to vary from dog to dog and from day to day in the same dog. $dP/dt$ also changes as a function of other factors such as ventricular hypertrophy, calcium and catecholamine levels, and circulatory fluid volume. We were able to free ourselves from this variability in the absolute value for $dP/dt$ by measuring beat-to-beat changes in $(dP/dt)_{max}$. As with CSP, $(dP/dt)_{max}$ is easily obtained in chronic animals by permanent placement of a solid-state microtransducer within the left ventricle.

One of the most attractive features of this technique is that although the absolute value of $(dP/dt)_{max}$ may vary considerably between animals, the rate at which $(dP/dt)_{max}$ changes with respect to time following elevation of CSP to a set pressure is very predictable for a given $\sigma$. The increase in $(dP/dt)_{max}$ seen immediately following the elevation in CSP (Figure 1) is not clearly understood. It has been proposed that elevation of CSP may result in the opening of additional myocardial microvascular exchange vessels, thus increasing the oxygen supply to the myocardium. It has also been suggested that myocardial fibril stretch may result from venous engorgement, leading to enhanced end-diastolic loading and function of the left ventricle. This improvement in myocardial performance will last for varying lengths of time depending on the magnitude of injury to the myocardial microvascular exchange vessels. In any case, our determinations of the change in $(dP/dt)_{max}$ do not begin until a constant rate of decrease has been established.

The usefulness of this technique is illustrated in Figure 4. The solid dots and sigmoidal regression line represent the data obtained in acute dogs. This curve defines the relation between MMP as determined by $(\sigma_{A P/LV})$ and the beat-to-beat change in $(dP/dt)_{max}$ with respect to time. We believe the use of a sigmoidal curve in Figure 4 is justified for two reasons. First, a better regression coefficient was obtained than for a linear fit, thus improving the predictive value of the curve. Second, there may be a physiologic reason for the curve to have this form. At low values for $\Delta(dP/dt)_{max}/\Delta t$, the myocardial microvasculature is intact, and the intrinsic defense mechanisms of the heart against edema formation protect against changes in myocardial performance. Once MMP has increased, as indicated by the decrease in $\sigma_{A P/LV}$ in Figure 4, myocardial interstitial fluid pressure increases at a rapid rate causing $\Delta(dP/dt-\Delta t)$ to change more quickly. Once MMP has been significantly disrupted, and relatively large volumes of edema have accumulated, the experimental animals become unstable and may die at any time. This may account for the relative insensitivity of $(dP/dt)_{max}$ to changes in $\sigma_{A P/LV}$ at the far right side of Figure 4.

With the establishment of this relation, it is possible to determine myocardial microvascular permeability without terminating the experimental animals. The 3 hypertensive animals, represented by open triangles in Figure 4, had their coronary sinus pressures elevated and their $\Delta(dP/dt)_{max}/\Delta t$ determined to test the predictive value of our curve. From this information, $\sigma_{A P/LV}$ and MMP could be estimated. The animals were then subjected to the terminal procedure for the direct evaluation of MMP by $\sigma$. The predictive value of the regression relation in Figure 4 was excellent.

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**Key Words**: myocardial microvascular permeability • myocardial lymph flow • myocardial contractility • myocardial edema
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G A Laine

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