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Transcapillary adenosine transport and interstitial adenosine concentration in guinea pig hearts

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Abstract

We used the multiple-indicator-dilution technique to observe the capillary transport of adenosine in isolated Krebs-Henseleit-perfused guinea pig hearts. Tracer concentrations of radiolabeled albumin, sucrose, and adenosine were injected into the coronary inflow; outflow samples were collected for 10–25 s and analyzed by high-performance liquid chromatography (HPLC) and by γ - and β -counting. The albumin data define the intravascular transport characteristics; the sucrose data define permeation through interendothelial clefts and dilution in interstitial fluid (ISF). Parameters calculated from adenosine data include permeability-surface area products for endothelial cell uptake at the luminal and abluminal membranes and intraendothelial metabolism. We found that in situ endothelial cells avidly take up and metabolize adenosine. Tracer adenosine in the capillary lumen is twice as likely to enter an endothelial cell as it is to permeate the clefts. There was no adenosine in the arterial perfusate. Under control conditions, the steady-state venous adenosine concentration was 3.6 ± 0.8 nM, which from the flow and the parameters estimated from the tracer data gave a calculated ISF concentration of 6.8 ± 1.5 nM. During dipyridamole infusion ($10 \mu\text{M}$) at constant pressure, the cell permeabilities went essentially to zero, whereas the venous adenosine concentration increased to 44.0 ± 12.6 nM, giving an estimated ISF concentration of 191 ± 53 nM. With constant flow perfusion, venous concentration during dipyridamole infusion was 30.9 ± 6.3 nM, and estimated ISF concentration was 88 ± 20 nM. We conclude that in this preparation, at rest, the ISF adenosine concentration is about twice the venous concentration and the ISF adenosine concentration increases with dipyridamole administration.

Keywords

dipyridamole; coronary blood flow; endothelial cells; flow heterogeneity; myocardial metabolism; blood-tissue exchange models; interstitium

DIPYRIDAMOLE is a powerful coronary vasodilator that is thought to exert its actions by inhibiting adenosine uptake and raising extracellular adenosine concentration (21). In support of this, several groups have demonstrated that dipyridamole causes increased adenosine in tissue (19, 32), venous effluent (15, 51), and pericardial fluid (30). Also, dipyridamole causes an increased accumulation of adenosine in the media of isolated cardiac myocytes when they are stimulated to release adenosine by application of dinitrophenol (16). Finally,

aminophylline, theophylline, and adenosine deaminase inhibit the vasodilation caused by dipyridamole (1, 15).

At present there is no commonly accepted technique for directly measuring the concentration of interstitial adenosine. The adenosine concentration of coronary venous effluent is often used as an index of interstitial adenosine, but this approach is suspect because endothelial cells separating the interstitial and vascular compartments must influence the transcapillary flux of adenosine (49). Several studies suggest that the coronary endothelium may offer more than a passive barrier to the measurement of adenosine. Cultured aortic endothelial cells exhibit carrier-mediated transport of adenosine (42). In addition, indicator-dilution studies of rabbit lung (17), pig lung (27), and dog skeletal muscle (23) have demonstrated carrier-mediated uptake of adenosine by in situ endothelial cells. It is possible, therefore, that coronary microvascular endothelium modulates the relationship between concentrations of adenosine in the vascular and interstitial compartments by 1) acting as a metabolic sink for adenosine in each of the two compartments or by 2) providing a transcellular pathway for adenosine transport. Although we cannot directly measure interstitial adenosine, it should be possible to calculate the interstitial concentration of adenosine given the arterial and venous concentrations of adenosine, flow, and sufficient knowledge of transport parameters of adenosine via paracellular and cellular routes.

This is a report of experiments in which the multiple-indicator-dilution technique was used to measure transport parameters for adenosine in the isolated guinea pig heart. The data were analyzed by use of a model for capillary-tissue exchange that accounts for transport across the capillary wall and uptake by cells (11). This information was then used to calculate interstitial adenosine concentrations under basal conditions and during infusion of dipyridamole. The data and analyses affirm the hypothesis that dipyridamole infusion increases interstitial adenosine concentration, which may be the explanation for its action as a vasodilator.

METHODS

Preparation

Guinea pigs (male or female, 500–650 g) were stunned by a blow to the head, and their hearts were rapidly excised and immersed in ice-cold Krebs-Henseleit solution [(in mM) 117 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 21 NaHCO₃, 2.5 CaCl₂, 8 glucose, and 2 pyruvate]. The aorta was cannulated and the heart was retrogradely perfused with the Krebs-Henseleit solution. The perfusate was equilibrated with 95% O₂-5% CO₂; it was not recirculated, and it was maintained at 37°C and pH 7.4. A 5% solution of bovine serum albumin was infused into the aortic line to establish a 0.1% concentration in the coronary perfusate; this concentration of albumin prevents the development of a high capillary filtration coefficient in crystalloid-perfused tissue (36). The left ventricle was vented by placing a catheter through the mitral valve; this prevents fluctuations in ventricular pressure development that result from accumulation of thebesian drainage. The hearts were electrically paced at 300 beats/min. Perfusion pressure was measured with a transducer at a point in the arterial cannula about 2 cm above the heart. Coronary flow was measured by using a cannulating electromagnetic flow probe in the aortic line. The probe was periodically calibrated during each experiment by timed collection of the venous effluent with a graduated cylinder. Coronary flow is expressed as milliliters per minute per gram wet weight myocardium. The wet weight of the heart was measured at the conclusion of each experiment after trimming away the great vessels and fat and blot drying with filter paper. Perfusion pressure and coronary flow were monitored on a direct-writing oscillographic recorder. The pulmonary artery was cannulated for collection of the coronary venous

effluent. A blood gas analyzer was used to determine the oxygen tension (POT) of samples taken from the perfusate (arterial) and pulmonary artery. Myocardial oxygen consumption ($\dot{M}\dot{V}O_2$; $\mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was calculated according to the equation $\dot{M}\dot{V}O_2 = \text{CF} \cdot (\text{Pa}_{\text{O}_2} - \text{Pv}_{\text{O}_2}) \cdot (c/760)$ where CF is coronary flow, $\text{Pa}_{\text{O}_2} - \text{Pv}_{\text{O}_2}$ is perfusate minus coronary venous difference in P_{O_2} (mmHg), and c is Bunsen solubility coefficient of oxygen dissolved in perfusate at 37°C ($23 \mu\text{l O}_2 \cdot \text{atm}^{-1} \cdot \text{ml perfusate}^{-1}$).

Indicator-Dilution Technique

Capillary transport and metabolism of adenosine were measured by use of the multiple-indicator-dilution technique. The overall strategy is to observe the behavior of three radioactive substances, which are injected simultaneously into the arterial inflow, by measuring their relative concentrations over time in the venous effluent. An intravascular reference tracer gives the behavior of molecules caused by the delay and dispersion in the arterial, capillary, and venous portions of the vascular bed and the heterogeneity of flow. A second tracer, which is restricted to the extracellular compartment, gives the behavior of molecules caused by the above phenomena and also the permeation of the capillary wall via extracellular pathways and distribution in interstitial fluid volume. A third tracer gives the behavior of molecules caused by all of the above phenomena and also permeation of, metabolism by, and dilution in the volumes of cellular elements within the heart.

Tracer injections—A bolus (25 μl) containing three radioactive tracers was injected into the aortic cannula ~1 cm above the heart. The injections lasted <1 s. The injectant contained an intravascular reference substance, ^{125}I -labeled serum albumin (RISA), [^{14}C]sucrose, and [^3H]adenosine ([^3H]ADO). [^{14}C]sucrose served as a reference substance confined to the extracellular space, and [^3H]ADO served as the test substance entering the cells. The activities of the tracers were 0.2 μCi RISA; 0.5 μCi [^{14}C]sucrose; and 2 μCi [^3H]ADO. The radioactive tracers were diluted in normal saline.

Sampling—Coincident with injection of the isotopes, the coronary venous effluent was directed into a series of 20 tared tubes over a period of 10–25 s. Each tube contained 200 μl of a methanol-water solution (1:16) containing 3 μM erythro-9-(2-hydroxy-3-nonyl) adenine, an inhibitor of adenosine deaminase, and 32 μM dipyridamole, an adenosine transport inhibitor. These agents prevented metabolism of tracer adenosine by any adenosine deaminase in the venous effluent and uptake by the rare formed elements that continue to leave the heart during crystalloid perfusion.

Protocols

After a 45-min equilibration period, the perfusion system was switched to a constant flow mode using a roller pump, and coronary inflow was initially adjusted to achieve a coronary perfusion pressure of 46 mmHg. The P_{O_2} s of the arterial perfusate and coronary effluent were measured. Then, the first tracer injection was performed and venous samples were taken. Next, we began to infuse dipyridamole into the aortic line at a rate that established a concentration of $\sim 10^{-5}$ M in the arterial perfusate. Perfusion pressure declined and reached a lower steady-state value in the face of a constant flow. After 10 min, a second injection of radioactive tracers and sampling of venous effluent occurred. Finally, coronary flow was increased so as to return perfusion pressure to the control level (46 mmHg) while the infusion of dipyridamole continued. After 10 min, a third bolus of the three tracers was injected and the venous effluent sampled. In two experiments, the perfusion pressure conditions for the second and third injections were reversed. Samples of venous effluent were also taken before each injection for measurement of the concentration of unlabeled (endogenous) adenosine.

Sample Processing

Measurement of venous unlabeled adenosine concentration—Samples of coronary effluent were obtained from the pulmonary artery catheter; 4-ml aliquots were evaporated and resuspended in 400 μ l ultrapure distilled water. The undissolved salts were separated by centrifugation. The supernatant was assayed for adenosine by high-performance liquid chromatography (HPLC). Two pumps were programmed for gradient elution of 200- μ l samples injected directly onto a reverse-phase 5 μ m C-18 HPLC column. A linear gradient at a flow of 1.1 ml/min over 20 min was used, with initial conditions of 100% 4 mM KH_2PO_4 -4 mM K_2HPO_4 and final conditions of 60% 4 mM KH_2PO_4 -4 mM K_2HPO_4 and 40% 70:30 methanol-water. Final conditions were held for 4 min, then the system was returned to initial conditions for 15 min for reequilibration of the column. Absorbance of the column eluate was continuously monitored at 254 nm. The peaks were identified by comparison with retention times of external standards, and they were intermittently verified by comparison with duplicate samples that contained either a known concentration of adenosine or that were treated with adenosine deaminase. Adenosine was quantitated by determining the area under the optical density peak and comparing it with an adenosine standard. This procedure resulted in >90% recovery of adenosine added to coronary effluent. We have defined adenosine release as the product of coronary flow ($\text{ml}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$) and venous effluent concentration (pmol/ml). This is net production, representing the difference between total production and reuptake by cellular elements in the heart.

Fraction processing for ^3H , ^{14}C , and ^{125}I activity—The venous samples were centrifuged, reweighed, and counted for RISA using a gamma counter. Then, 100 μ l of each fraction was injected onto a reverse-phase 5 μ m C-18 HPLC column. An isocratic elution was used at a flow of 1.4 ml/min. Eluate composition was 88% 4 mM KH_2PO_4 -4 mM K_2HPO_4 and 12% 70:30 methanol-water (vol/vol). Under these conditions, the compounds elute in the order: RISA, [^{14}C]sucrose (coeluted), and [^3H]-ADO. Four-min HPLC eluate fractions were collected for RISA-[^{14}C]sucrose and [^3H]ADO. A 2-ml aliquot of each HPLC fraction was combined with 15 ml of scintillation fluid and was counted for either ^{14}C or ^3H , using a liquid scintillation counter. Counting rates were corrected for efficiency and, in the RISA-[^{14}C] fraction, for RISA and ^{14}C overlap.

Additional samples processed included triplicate injectant samples for calculation of total injected dose and coronary effluent samples spiked with 1:50 dilutions of injectant for calculation of recoveries. Analysis of recovery samples demonstrated a >90% recovery for these assays. RISA, [^{14}C] sucrose, and [^3H]ADO activities in each venous effluent sample were converted to relative concentrations by expressing them as percent of injected dose per milliliter of effluent. Relative concentrations are then plotted vs. time after tracer injection.

Heterogeneity of Coronary Flow

The regional heterogeneity of coronary perfusate flow was measured with radioactive microspheres (15 μ m) in nine preparations; this was done to account for heterogeneity of flow in the analysis of the data (see below). The preparation and protocol were identical to those described above, except that differently labeled batches (~1.25 μCi in 25 μ l) of microspheres (^{141}Ce , ^{51}Cr , and ^{85}Sr), instead of the radiolabeled tracers, were injected under each of the three experimental conditions (control, dipyridamole-low pressure, and dipyridamole-control pressure). At the conclusion of the experiment, the hearts were sectioned into 40 transmural pieces weighing 10–40 mg. The distribution of local myocardial flows was $w(f)$, the histogram or normalized probability of local flows relative to the mean flow, as described in detail by Bassingthwaite et al. (8). The f is flow relative to the heart mean, and $w(f)$ is the fraction of the heart with a given flow. They found that

microspheres give an adequate description of the flow distributions. We found that these distributions were close to Gaussian in shape and were therefore characterized by their relative dispersions (RD), which is the coefficient of variation, the standard deviation divided by the mean.

Methods of Analysis

The indicator-dilution curves were analyzed by making use of a mathematical model of tracer adenosine transport, which has been described in detail by Bassingthwaight et al. (12). The nomenclature follows that defined for such physiological studies by Bassingthwaight et al. (6). The model follows the principles and uses the same basic assumptions with respect to constancy of flow, uniformity of geometry, and such, as were given in detail by Bassingthwaight and Goresky (7). It is an extension to a four-region three-barrier model from the three-region two-barrier model with axial diffusion used by Kuikka et al. (33) for glucose studies and extends also the two-barrier model of Rose et al. (44) by the additions of endothelial cells, consumption in all four regions, and axial diffusion. Figure 1 shows a schematic representation of the capillary-tissue unit.

To account for flow heterogeneity, we used a multicapillary configuration consisting of five parallel capillary-tissue units that are identical except for flow. The distribution of the flows over the five pathways approximates the distribution of flows observed with microspheres, with the mean flow being that measured at the time the dilution curves were obtained. For each capillary-tissue unit, the model consists of a set of linear partial differential equations describing the change in mass of adenosine in plasma, endothelial cell cytosol, interstitial fluid, and parenchymal cell cytosol with respect to time and distance along the capillary-tissue unit. Because intercapillary radial diffusion distances are $<20\ \mu\text{m}$, radial diffusion was assumed to be instantaneous within compartments. Model solutions are obtained numerically, using a sliding-element algorithm described earlier (4), providing rapid and stable solutions. The overall multipath model accounting for flow heterogeneity includes the following. Capillary transit times (τ) in five parallel capillary-tissue units calculated by $\tau_i = V_p/F_i$ for the i th pathway. V_p is the intracapillary plasma space, taken from anatomic data of Bassingthwaight et al. (14) to be 0.035 ml/g. The parameter estimates are not significantly dependent on the value chosen (see APPENDIX B). F_i is the flow in a pathway where $F_i = f_i F$ and F is the measured mean flow of solute containing fluid ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$), and f_i , the relative flow, is given by the probability density function of microsphere depositions (no free parameters).

PS_g Capillary permeability-surface area product for extracellular paths between plasma and interstitium ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, from sucrose dilution curves)

PS_{ecl} Endothelial cell luminal permeability-surface area product between plasma and endothelial cell cytosol ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, free parameter no. 1)

PS_{eta} Endothelial cell abluminal permeability-surface area product between interstitial fluid and endothelial cell cytosol ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, free parameter no. 2)

G_{ec} Clearance for intracellular sequestration of consumption in endothelial cells ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, free parameter no. 3)

V'_{isf} Interstitial volume of distribution (ml/g, from sucrose dilution curves)

V'_{ec} Volume of distribution within endothelial cells (ml/g; V_{ec} fixed at 0.02 ml/g from anatomic data)

PS_{pc} Parenchymal cell permeability-surface area product ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$, free parameter no. 4)

V'_{pc} Volume of distribution within parenchymal cells (ml/g; insensitive parameter with values 0.5–0.7 ml/g, used $V_{pc} = 0.6$ –0.7 ml/g or left free)

G_{pc} Clearance rate constant for intracellular consumption in parenchymal cells (ml·g⁻¹·min⁻¹, free parameter no. 5)

The PS s, G s, and even the V s may be dependent on adenosine concentration, but for any given steady state, they are independent of the tracer concentration, and so, the use of linear equations is justified by the use of tracer.

The differential equations for each capillary-tissue unit are the following: in plasma

$$\frac{\partial C_p}{\partial t} = -\frac{F_p L}{V_p} \cdot \frac{\partial C_p}{\partial x} - \frac{PS_g}{V_p} (C_p - C_{isf}) - \frac{PS_{ecl}}{V_p} (C_p - C_{ec}) - \frac{G_p}{V_p} C_p + D_p \frac{\partial^2 C_p}{\partial x^2} \quad (1)$$

in endothelial cells

$$\frac{\partial C_{ec}}{\partial t} = -\frac{PS_{ecl}}{V'_{ec}} (C_{ec} - C_p) - \frac{PS_{eca}}{V'_{ec}} (C_{ec} - C_{isf}) - \frac{G_{ec}}{V'_{ec}} C_{ec} + D_{ec} \frac{\partial^2 C_{ec}}{\partial x^2} \quad (2)$$

in interstitial fluid

$$\frac{\partial C_{isf}}{\partial t} = -\frac{PS_g}{V'_{isf}} (C_{isf} - C_p) - \frac{PS_{eca}}{V'_{isf}} (C_{isf} - C_{ec}) - \frac{PS_{pc}}{V'_{isf}} (C_{isf} - C_{pc}) - \frac{G_{isf}}{V'_{isf}} C_{isf} + D_{isf} \frac{\partial^2 C_{isf}}{\partial x^2} \quad (3)$$

and in parenchymal cells

$$\frac{\partial C_{pc}}{\partial t} = -\frac{PS_{pc}}{V'_{pc}} (C_{pc} - C_{isf}) - \frac{G_{pc}}{V'_{pc}} C_{pc} + D_{pc} \frac{\partial^2 C_{pc}}{\partial x^2} \quad (4)$$

The subscripts are p for capillary lumen fluid, isf for interstitial fluid, ec for endothelial cell, and pc for parenchymal cell. C represents the concentration in a volume of distribution at position x and time t ; x is the distance along the capillary-tissue unit from the entrance at x equals 0 to the exit at x equals L . The rate constants for consumption in plasma (G_p) and in interstitium (G_{isf}) were set to zero, since the requisite enzymes were not contained in the artificial perfusate. The D_s are the axial diffusion coefficient. An analytic solution of these equations is given (APPENDIX A) for the case with diffusion coefficients of zero, but in practice, the numerical approach of Bassingthwaighe (4) and Bassingthwaighe et al. (12) is faster. The accuracy of the 1974 numerical approach with axial diffusion has been evaluated by Lenhoff and Lightfoot (34) in comparison to analytic solutions and is within 1%; the recent methods are better.

The unit impulse response from the i th capillary-tissue unit is $h_{C_i}(t)$. The aggregate of the parallel capillary-tissue units gives the overall transport function $h_C(t)$, which is given by $h_C(t)$ and the flow weighting obtained from the microsphere distribution ($w_i f_i \Delta f_i$)

$$h_C(t) = \sum_{i=1}^{i=5} w_i f_i \Delta f_i h_{C_i}(t) \quad (5)$$

For a given reference or test substance (i.e., RISA, sucrose, or ADO), all capillary-tissue units are assumed to have the same parameters, except for the relative flow (f_i). The number

of pathways used to represent the distribution was five. The five flow-weighting factors were calculated from the total flow at the time of tracer injection and the mean RD of flow for that experimental condition, assuming a Gaussian distribution and equal volumes for each pathway.

The capillary-tissue units are placed in series with arteries and veins, which also disperse and delay the tracers as distributed by a large vessel transport function $h_{LV}(t)$. We have assumed, following Levin et al. (35) and Kuikka et al. (33), that the dispersion occurring in large vessels (arteries and veins) is the same for all capillary-tissue units, which allows convolution of the set of capillary-tissue transport functions with the large vessel transport function to give the overall transport function for the entire system $[\hat{h}(t)]$

$$\hat{h}(t) = h_{LV}(t) * h_c(t) \quad (6)$$

where $*$ denotes the convolution operation. Arterial and venous dispersion can be represented by one transport function $[h_{LV}(t)]$ because for tracers this is a linear system, and the order of the operations on the injectant in a linear system does not influence the solution (29). Rose et al. (44) have proposed another model of heterogeneity in which there is a dispersionless set of large vessels linked one-on-one to capillaries. The two models may give different parameter estimates; this has not been explored.

Optimization of the Fit of Model Output to Data

The parameters listed above are optimized so as to give a good fit of the indicator-dilution data for each experiment. The method of optimization uses sensitivity functions, which are the partial derivatives of the model solutions with respect to each of the parameters, to determine the relative influence of each of the parameters on the goodness of fit at each iteration in the procedure. The details of this approach are reported elsewhere (5, 11, 33). The search for best fit ceases when the distance between solution and data no longer changes or when the coefficient of variation (CV) between the data curves at each time point and the model solution is $<1\%$. The sensitivity functions for each parameter change for each iteration in the optimization and are different for each curve at the final fit. It is critical that the sensitivity function for each parameter differs from all the others, as previously shown (11). They show that each parameter has a unique influence on the model solution because no two sensitivity functions are the same or are simple scalars or reciprocals of each other. The model solution is most sensitive to PS_g and PS_{ecl} , less to PS_{pc} and V_{isf} , and least sensitive to G_{pc} and V_{pc} .

Confidence in parameter estimation is greatest when the degrees of freedom in fitting the data are minimized. We have reduced the degrees of freedom in fitting the data with the following approach. PS_g and PS_{ecl} for albumin are assumed to be zero, which is appropriate for short capillary residence time, such as is true in these studies. The albumin curve, the measured mean flow, the measured heterogeneity of flow, and a chosen, fixed capillary volume (V_p) completely define the large vessel transport function $h_{LV}(t)$. $h_{LV}(t)$ can be obtained by deconvolution, as is indicated by Eq. 6, in which $\hat{h}(t)$ is given by the observed albumin curve and $h_c(t)$ is given by heterogeneity of regional flows around the mean flow (F) and the capillary space (V_p). The $h_{LV}(t)$ obtained in this way is then used as the input function for each of the three tracers in each set of indicator-dilution curves. Because all tracers were injected simultaneously, they were subjected to the same large vessel dispersion, as well as the same capillary flow and volume. The albumin curve is fitted nearly exactly because it was used to get $h_{LV}(t)$. There are no free parameters in defining $h_{LV}(t)$ and in fitting the outflow albumin curve.

To fit the sucrose data, two parameters are estimated, PS_g and V_{isf} , using $h_{LV}(t)$ and everything else as for the albumin curves.

To fit the adenosine data, the value for $PS_g(ADO)$ was taken to be 1.128 times $PS_g(\text{sucrose})$, in which 1.128 is $\sqrt{342/269}$, the square root of the reciprocal of the ratio of molecular weights for adenosine and sucrose and, for adenosine relative to sucrose, represents the expected diffusion coefficient and permeability through the aqueous channel, the interendothelial clefts. The remaining parameters, PS_{ec1} , PS_{eca} , PS_{pc} , G_{ec} , and G_{pc} , were either adjusted or constrained in a logical fashion (APPENDIX B). V_{ec} and V_{pc} showed little sensitivity and furthermore are constrained by knowing tissue interstitial fluid (ISF) volume, total water content, and literature values of capillary endothelial volume, so it is the “clearance parameters,” the PS s and G s, that are free to be varied. Sensitivity analysis shows that the adenosine outflow curves are mainly sensitive to PS_{ec1} and less to G_{ec} (11). There would be sensitivity also to PS_g if it were free, but it is not, because it is calculated from PS_g for sucrose. Because PS_g and PS_{ec1} have only moderately different sensitivity functions, the separate determinations of PS_g from the extracellular reference curve for sucrose is vital to the analysis.

Calculation of Endogenous Interstitial Fluid Adenosine

The adenosine concentration of the ISF can be calculated once the parameters for adenosine transport and metabolism have been obtained. This is done by using a set of equations similar to Eqs. 1–4, except that in the steady state, the derivatives with respect to time are zero. In addition, unlike the case for tracer, these equations must include a term representing the source of endogenous adenosine within the capillary-tissue unit. The details of the solution to these equations are in APPENDIX C. The use of the parameter values determined for tracer is correct, since they are applied under the same conditions as those for which they were derived, i.e., the PS s and G s for tracer are the appropriate ones for nontracer adenosine at the concentrations at which they were determined.

Measurement of [^3H]ADO Release From In Situ Endothelial Cells

The following experiments were performed to establish the origin of adenosine released from the heart in response to dipyridamole infusion (e.g., endothelium vs. myocytes).

An isolated heart preparation, like that described above, was used in these experiments ($n = 4$). After perfusion was switched to constant flow, tritiated adenosine ($[^3\text{H}]\text{ADO}$) was infused for 30 min at a delivered concentration of 10^{-8} M (sp act = 40 Ci/mmol). After infusion of $[^3\text{H}]\text{ADO}$, 30 min were allowed for washout of the tracer adenosine. This procedure has been shown to result in the selective uptake of the $[^3\text{H}]\text{ADO}$ by coronary endothelium, as opposed to myocytes (3, 31, 39). After the washout period, dipyridamole was infused for 20 min at a coronary concentration of 10^{-5} M. Samples of the venous effluent were taken before, during, and after dipyridamole infusion.

The venous concentration of adenosine was determined by HPLC analysis as described above. The radioactivity associated with adenosine was determined from the HPLC eluate fraction corresponding to the spectrophotometrically determined adenosine peak. The eluate fraction was evaporated, resuspended in 500 μl ultrapure water, and a 400- μl aliquot was combined with 10 ml of scintillation fluid and counted for ^3H in a liquid scintillation counter. The specific activity of adenosine was calculated by dividing the radioactivity by the mass (counts $\cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$). Relative specific activity was calculated by dividing the specific activity in the venous effluent by the specific activity of the $[^3\text{H}]\text{ADO}$ that was infused into the heart.

Statistical Analysis

A one-way within-subjects analysis of variance and Student-Neuman-Keuls test for significance were used. Differences between means were considered significant at $P < 0.05$.

RESULTS

Hemodynamic Data

Initially, all hearts ($n = 7$) were perfused under constant flow conditions at a flow that gave a perfusion pressure of 46 mmHg. During control conditions, CF was $4.8 \pm 0.2 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, $\text{M}\dot{\text{V}}\text{O}_2$ was $58 \pm 1 \mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, and coronary vascular resistance was $9.4 \pm 0.3 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{g} \cdot \text{min}$. Dipyridamole infusion (perfusate concentration $\approx 10^{-5} \text{ M}$) under constant flow conditions significantly reduced perfusion pressure to $24 \pm 1.0 \text{ mmHg}$ and coronary vascular resistance to $5.0 \pm 0.2 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{g} \cdot \text{min}$. When perfusion pressure was returned to the control level of 46 mmHg during dipyridamole infusion, CF increased significantly to $11.2 \pm 0.5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and coronary vascular resistance decreased further ($P < 0.05$ to $4.0 \pm 0.2 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{g} \cdot \text{min}$).

Multiple-Indicator-Dilution Data and Parameter Optimization

Figure 2 shows an example of the multiple-tracer-dilution data. The venous concentration-time plots for the three tracers are shown for the control situation (Fig. 2A), for dipyridamole infusion at control flow (Fig. 2B), and for dipyridamole infusion at high flow (control perfusion pressure; Fig. 2C). The lines through the points are those predicted by the model. The fitted lines are superimposed on the experimental data points; this shows that the parameter optimizing routine does an excellent job of fitting the experimental data. At any given time, under control conditions, the relative venous concentration of adenosine is less than the extracellular tracer, sucrose. During dipyridamole infusion, the adenosine curves become almost superimposed on the sucrose curves, differing only in having slightly higher extractions and earlier back flux. These results suggest that dipyridamole blocks a carrier-mediated uptake of adenosine into cells. In the control studies, the adenosine curve does not cross the sucrose or RISA curves, indicating that there is very little back diffusion of adenosine and implying that adenosine is being sequestered intracellularly.

Fitting model solutions to the observed indicator-dilution data generated values for the model parameters. The values from individual experiments are shown in Table 1. Under control conditions, PS_g was $1.88 \pm 0.5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ and PS_{ecI} was $4.57 \pm 1.05 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. A comparison of PS_g and PS_{ecI} ($P < 0.05$) indicates that about two-thirds of the tracer adenosine leaving the vascular compartment crossed the luminal surface of the endothelial cell, whereas about one-third passed through the interendothelial gaps. In APPENDIX B, we provide the results of alternative sets of analyses of the parameters under either no constraints or other constraints. Table 1 summarizes the most reasonable set of results.

These analyses, using equations with constant coefficients, do not and should not account for the concentration dependency of the rate constants of the carrier transport process for adenosine (43). Because the concentrations of nontracer adenosine are higher in ISF than plasma and, in this preparation, higher at the downstream end of the capillary-tissue unit than at the upstream end, the PS estimates are spatial averages representing a nonlinear distribution and so contain some inaccuracy, but little enough that none of the concepts and conclusions are affected.

Because of the short periods of data acquisition and the near absence of return flux of unmetabolized tracer from the cells, the values of G_{ec} and G_{pc} are not accurately determined

other than to be certain that they are quite high; their sums are given in Table 1, since their influences on the early curve shape are somewhat overlapping. Values of G_{ec} and G_{pc} over $40 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ are at a maximal influence, so 40 was set as an upper bound in the optimization. Because there is so little reflux of adenosine from ISF to plasma, PS_{pc} plus PS_{eca} must be greater than PS_g , although their individual values are imprecise. In the optimization, to minimize free parameters and to account for the intraendothelial accumulation observed by Nees et al. (39) and Bardenheuer et al. (3), PS_{pc} was free to vary, but PS_{eca}/PS_{pc} was set at 10 (see APPENDIX B for discussion of this assumption). To obtain good estimates of PS_{ec1} and PS_g , we do not need exact determination of the individual PS s facing the interstitium (PS_{eca} and PS_{pc}) or the intracellular consumptions, the G s. The background for this statement is provided in APPENDIX B. The sums ($G_{ec} + G_{pc}$) were always higher than PS_{eca} plus PS_{pc} , and G_{ec} was always higher than PS_{ec1} , indicating that tracer which entered the cell was more likely to undergo reaction than to escape from the cell.

The unidirectional extraction from the perfusate into the tissue is governed by the sum of the luminal endothelial and cleft permeabilities divided by the flow. The fraction of tracer leaving the lumen is given by $E = 1 - \exp[-(PS_{ec1} + PS_g)/F_p]$. The values are listed in the third column of Table 1; in the control state, 74% of the tracer adenosine escaped in the single pass. This was reduced to 34 and 22% in the presence of dipyridamole at low and high flows. The higher values of PS_g with dipyridamole at high flow do not affect the reduction in extraction by raising the flow; the increased values do suggest recruitment compared with the low-flow dipyridamole state and compared with the control state.

These unidirectional extractions were only slightly higher than the observed maximal extractions (E_{max}) calculated as the maximum smoothed value of $1 - h_{ADO}/h_{Alb}$. The closeness of E_{max} to E occurs because the retention of adenosine by the tissue is considerable. In the control state, the retention is very high by virtue of high cellular uptake and transformation. With dipyridamole blocking the transporter, retention is only by dilution in the ISF, and E_{max} is closest to E at the higher flows in which reflux is less.

In the presence of dipyridamole, PS_{ec1} and PS_{eca} were reduced to zero; for the analyses reported in Table 1, these values were set to zero rather than found by the curve-fitting routine, but values obtained by unconstrained optimization were mostly close to zero, without any improvement in the goodness of fit. When perfusion pressure was allowed to fall during dipyridamole infusion, PS_g was $2.05 \pm 0.40 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. Vasodilation at high flow (constant perfusion pressure) gave PS_g of $2.79 \pm 0.73 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ($P < 0.05$ vs. control), suggesting that the vasodilating action of dipyridamole increased capillary surface area. Values of G_{pc} and G_{ec} cannot be determined during dipyridamole infusion because PS_{ec1} , PS_{eca} , and PS_{pc} are either zero or close to it, and no adenosine reaches the cytosol to be metabolized.

Calculations of Interstitial Adenosine Concentrations

Table 2 shows the individual values for the measured adenosine concentrations in the venous effluent and those calculated via the equations of APPENDIX C for ISF, capillary fluid, and endothelial cells. Arterial adenosine was not detectable, so arterial concentration (C_a) equals zero.

Regional adenosine concentrations were calculated via the same model (Fig. 1) under steady-state conditions as was used for the data analysis of the tracer transients, using the parameters listed in Table 1. As described in APPENDIX C, we considered a net production of adenosine into the interstitial space, calculating $\bar{C}_{isf}$, the mean interstitial concentration along the capillary-tissue unit, from the observed venous concentration (C_v) and the

predetermined endothelial parameter values. The estimates of $\bar{C}_{\text{isf}}$ reported in Table 2 require only the assumption that adenosine is produced into or in the ISF. [Although this could be from the abluminal surface of endothelial cells or from interstitial AMP hydrolysis, it is likely that the parenchymal cells are the source, an assumption strongly supported by Deussen et al. (20), Kroll et al. (31), and Bardenheuer et al. (3) and our own data (see below).] Table 2 includes only five of the seven experiments from Table 1 because the venous concentration of adenosine, which is required for the calculation of adenosine concentration, was not obtained in *experiments 16* and *17*. C_{ec} cannot be determined for the dipyridamole interventions because PS_{ecI} and PS_{eca} are fixed at zero or found to be very small. Under control conditions, the calculated concentration of adenosine in the interstitial fluid space ($\bar{C}_{\text{isf}}$) is 6.8 ± 1.5 (SE) nM ($n = 5$), which is almost twice the venous perfusate concentration ($C_v = 3.6 \pm 0.8$ nM; $P < 0.05$). $\bar{C}_{\text{isf}}$ was greatly increased to 88.1 ± 19.9 nM ($P < 0.05$) by dipyridamole infusion when flow was held constant, and when flow was increased to normalize perfusion pressure, $\bar{C}_{\text{isf}}$ increased to 191 ± 52 nM ($P < 0.05$). These values for $\bar{C}_{\text{isf}}$ were three and four times the values for venous adenosine during constant-flow (30.9 ± 6.3 nM; $P < 0.05$) and high-flow conditions (44.0 ± 12.6 nM; $P < 0.05$), respectively.

Note that $\bar{C}_{\text{isf}}$ values are higher with high flow, with dipyridamole, and not lower as one might expect if wash-out by flow were to dominate the situation. In fact, in three of the five cases, the C_v of adenosine is higher at the high flow. In all cases, the product $F_p \cdot C_v$ is higher at high flow. Why this occurs is not known but may relate to the Gregg effect of higher O_2 consumption at higher flows. In the last two studies, O_2 consumption was measured during dipyridamole infusion at constant pressure and high flow, and in both studies, the O_2 consumption increased from 61 to 96 $\mu\text{l } O_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ at high flow in *experiment 34* and from 60 to 97 $\mu\text{l } O_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ in *experiment 35*.

Figure 3 provides information on the effect of the parameters for capillary transport and metabolism of adenosine on the calculated C_{isf} . The effect of these parameters depends to some extent on the value of G_{ec} . In Fig. 3 we therefore calculated $\bar{C}_{\text{isf}}/C_v$ at a high G_{ec} ($G_{\text{ec}} = 27.5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, the average value from Table 1) and at an arbitrarily chosen low value of G_{ec} ($1.0 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$). In these calculations, the C_v was kept constant at the average control value (3.6 nM). All parameters other than the one being varied were held constant at the average value from Table 1. At high G_{ec} , PS_g is the most influential parameter, especially in the range $< 5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ as it was in our experiments. At low G_{ec} , PS_g is less influential than PS_{ecI} and PS_{eca} , and the overall $\bar{C}_{\text{isf}}/C_v$ ratio is lower in this case.

Because the parameter optimizing routine has little sensitivity to either PS_{eca} or PS_{pc} and there is some trade-off between their values, since we do not have the tails of the curves in these experiments, we report only their sum in Table 1. Neither of these parameters is critical to the estimate of $\bar{C}_{\text{isf}}$ (see APPENDIX B).

[^3H]ADO Release From In Situ Endothelial Cells

When 10^{-5} M dipyridamole was infused into hearts after prelabeling the endothelium with [^3H]ADO via a 30-min infusion, the relative specific activity of [^3H]ADO in the venous effluent fell ($P < 0.05$) from a predipyridamole value of $360 \pm 130 \times 10^{-5}$ to $8.2 \pm 2.3 \times 10^{-5}$ and $5.2 \pm 1.3 \times 10^{-5}$ at 10 and 20 min of dipyridamole infusion, respectively. Despite the decreased relative specific activity of [^3H]ADO, dipyridamole increased ($P < 0.05$) the venous concentration of total adenosine (labeled + unlabeled) from 4 ± 1 to 35 ± 18 and 71 ± 48 nM at 10 and 20 min, respectively. Because specific activity decreased 70-fold and adenosine production increased 18-fold, these results imply that the additional adenosine released in response to dipyridamole originated from a nonlabeled adenosine source, most likely myocytes and not endothelial cells.

DISCUSSION

The results of the study may be summarized as follows. 1) PS_{ec1} is about two to three times PS_g , meaning that a molecule of tracer adenosine is three times as likely to enter the luminal surface of coronary endothelial cells as to diffuse into the interstitium through paracellular gaps. 2) Values for G_{ec} were always higher than for PS_{ec1} and usually higher than for PS_{eca} , indicating that once inside an endothelial cell, the tracer adenosine is more likely to be metabolized than to escape. 3) When the transport parameters obtained from indicator-dilution experiments are used with measurements of endogenous adenosine in venous effluent to calculate ISF adenosine concentrations, we find that C_{isf} is two to four times the venous concentration. 4) Dipyridamole elevates the adenosine concentration of the venous effluent by 8- to 12-fold, and the concentration of the ISF by 6- to 30-fold. 5) At high flow, the effect of dipyridamole on adenosine production (P_m) and interstitial concentration (C_{isf}) is about twice that at control flow and low perfusion pressure.

Linearized Equations for Tracer

Because the concentrations of nontracer adenosine are presumably constant for each experiment, the rate coefficients and volumes of distribution can both be considered constant as far as tracer is concerned (7). The estimated PS s, G s, and V s therefore represent the values applying at the ambient concentration of nontracer adenosine. If first-order saturation kinetics apply to the transporters, the enzymatic reactions, and any adsorption or binding sites, then all of these parameter values would be higher at lower concentrations. No evidence concerning this point is revealed by these experiments. In the control series, the range of venous concentrations is too small to have a discernible relationship to the parameter estimates. In the dipyridamole series, the transporters are so completely blocked that nothing inside the cells can be revealed concerning concentration dependence of the parameters.

Justification of Preparation and Techniques

We used isolated crystalloid-perfused guinea pig hearts in these experiments for a number of reasons. The study of adenosine capillary permeation is made easier by the use of a crystalloid perfusate that eliminates production or removal of adenosine by formed elements or plasma enzymes. In this circumstance, adenosine in venous effluent must come from, and uptake of tracer must be the result of, processes in one or more cell types in the heart. Furthermore, the crystalloid perfusate simplifies the chemical measurements, making the analysis of large numbers of samples feasible. Finally, the isolated perfused heart exhibits the two salient physiological characteristics necessary for the purposes of this study. It releases adenosine in response to a wide variety of stimuli, and it exhibits dilation in response to dipyridamole.

One purpose of these studies was to estimate changes in interstitial adenosine concentration after dipyridamole administration. We also wanted to know how dipyridamole affected capillary transport and metabolism of adenosine to reconcile some puzzling points in the literature (see below). The indicator-dilution technique is the only available method to obtain information about in situ capillary adenosine transport and metabolism.

Other investigators have used measures of adenosine in whole heart tissue or pericardial fluid to estimate interstitial adenosine concentration. The advantages and disadvantages of these techniques have been reviewed elsewhere (30, 48). Although it is probable that these measurements give directionally correct estimates of changes in adenosine in the ISF space, neither is quantitative. The combination of indicator-dilution experiments to define capillary transport parameters and measurements of endogenous adenosine in venous effluent gives us

a quantitative estimate of interstitial adenosine and the fluxes on which it depends and begins to distinguish events in parenchymal cells from those in endothelial cells.

Recently, several investigators have used the concentration of adenosine in the transudate appearing on the surface of the rodent heart as a measure of interstitial adenosine (18, 22, 25, 26). These data, showing transudate concentrations several times higher than those in the venous effluent, are similar to those obtained from dog studies (24, 30). To date, there have been no rigorous tests of the assumption that transudates are precisely representative of ISF, but this is a promising approach. In general agreement with our results, the published transudate concentrations are higher than the venous concentrations, but their experimental conditions were not the same as ours, so rigorous comparisons with our values would not be justified.

We used a mathematical model of minimal, but still complex, form to estimate parameters of capillary transport. We chose this approach (7) because such a model allows us to fit all the available data with one integrated analysis rather than just fitting the first few points, as is the case when simple models and the early extraction of solute are used. The processes determining capillary-tissue transport of adenosine are multiple and complex. They include heterogeneity of flow, the capacity of endothelial cells to take up and metabolize adenosine, diffusion between endothelial cells, and parenchymal cell uptake and metabolism. These factors are adequately taken into account in our analysis but not by any simpler models of capillary transport. The model development paralleled the experimental work.

Endothelial Cell Uptake of Adenosine. Implications for Capillary Transport

Cultured endothelial cells from porcine aorta (42) and guinea pig hearts (39) take up adenosine with a Michaelis constant (K_m) of $\sim 3 \mu\text{M}$. Porcine aortic endothelial cells also transport adenosine via a low-affinity carrier (42). Furthermore, studies on crystalloid-perfused porcine (27), laparine (17), and rat (2) lung show that adenosine is removed from the pulmonary vascular compartments by endothelial cells. We previously used these data to calculate C_{isf} in dog hearts, with the major unproven assumption that coronary endothelial cell behavior could be predicted from isolated cells (50). Although the above studies strongly suggest that capillary endothelia of the heart take up adenosine, they did not provide any direct indication of the relative importance of endothelial uptake vs. paracellular diffusion through water-filled channels. Furthermore, they do not tell us the ultimate fate of adenosine taken up by in situ endothelial cells. It could pass through the cells to the interstitium, be phosphorylated to AMP, or be deaminated to inosine. We used the triple-indicator-dilution technique to answer these questions.

During the dilution curve, the relative outflow concentration of tracer adenosine is below that of the extracellular tracer, sucrose (Fig. 2). This difference exists throughout the upslope portion of the curves when the interstitial concentrations of the tracers are close to zero. The higher extraction of adenosine compared with sucrose early in the curves indicates that adenosine permeated the capillary endothelial barrier more readily than did sucrose. Because the difference between sucrose and adenosine extraction is nearly abolished by dipyridamole, we can attribute the higher early extraction of adenosine to uptake by endothelial cells. This qualitative analysis is buttressed by the quantitative approach in which the parameters describing adenosine transport in our model are optimized to give the best fit of the concentration curves. PS_{ec1} is $4.57 \pm 1.05 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ compared with a PS_g of $1.88 \pm 0.50 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ (Table 1). We conclude that of the tracer adenosine leaving the vascular compartment, $\sim 70\%$ directly enters endothelial cells, whereas $\sim 30\%$ diffuses through water-filled channels between endothelial cells.

Another difference in the relative concentration curves occurs after the peak. The sucrose concentration declines less rapidly than albumin so that the curves cross (Fig. 2). This is because once the injectant bolus has left, the concentration in ISF exceeds the concentration in plasma, and the sucrose diffuses back into the vascular compartment. The adenosine curve does not cross either the RISA or the sucrose curve. This suggests that adenosine does not return from endothelial or parenchymal cells once it leaves the vascular compartment. Again, the qualitative analysis is supported by the parameter optimization, with G_{ec} , the metabolic clearance from the cytosol of endothelial cells, being higher than PS_{ec} , and reflux is small.

As we described in METHODS, for the control studies, the parameter optimization gives explicit values for PS_{pc} and G_{pc} . We prefer not to emphasize these parameters because we cannot report their values accurately from these brief runs, and they have 100- to 1,000-fold less effect on the model dilution-curve shape than do PS_g and PS_{ec} . In any case, they need not be known accurately to calculate C_{isf} when myocytes are the source of venous adenosine, as we assume in defining a net production term (P_m), representing adenosine released into the ISF (See APPENDIX C).

The summed PS s, PS_{eca} and PS_{pc} , represent total conductance from ISF into cells. Parameter optimization is not effective at distinguishing between these two parameters over the time course of our indicator-dilution curves. We therefore made the assumption that PS_{eca}/PS_{pc} equals 10 on the basis of the observation that infused tracer adenosine is highly concentrated in endothelial cells (3, 38, 39). This assumption is certainly unproven and somewhat questionable (see APPENDIX B), but using much lower ratios of PS_{eca}/PS_{pc} has little effect on estimates of $\bar{C}_{isf}$ (APPENDIX B). We conclude, therefore, that although the available short time-course data do not allow us to determine PS_{eca} and PS_{pc} separately, these parameters are minor determinants of calculated $\bar{C}_{isf}$, and in any case, PS_{pc} is not used in the calculation.

Calculation of Interstitial Adenosine Concentration

APPENDIX C gives steady-state equations for calculating C_{isf} when there is net efflux of adenosine from myocytes. The values required for the calculations are the parameters obtained from fitting the indicator-dilution curves, perfusate flow, and the concentration of adenosine in the arterial and venous perfusate. The final piece of information is the source of the venous adenosine, endothelial cell or cardiac myocyte. At present there are no experiments that provide unequivocal evidence concerning the source of adenosine under control conditions; however, Kroll et al. (31) have data that indicate that endothelial cells supply only 14% of venous adenosine under resting conditions. Given the assumption that adenosine comes from cardiac myocytes, we calculate a control C_{isf} of 6.8 ± 1.5 nM. We are on safer ground in calculating C_{isf} during dipyridamole infusion, since the endothelial cell-labeling experiments referred to above suggest that almost none of the venous adenosine comes from endothelial cells during dipyridamole.

There are two parameters involved in the calculation of C_{isf} under control conditions that the indicator-dilution curves do not determine conclusively, G_{ec} and PS_{eca} . By letting these two parameters assume values producing the widest variations in C_{isf} , we can put limits on our estimates. The $\bar{C}_{isf}/C_v$ ranges from 1.4 ($G_{ec} = 1.0$; $PS_{eca} = 29.1$) to 3.3 ($G_{ec} = 27.5$; $PS_{eca} = PS_{ec} = 4.6$). With C_v equals 3.6 nM, this corresponds to bounding the estimates of average $\bar{C}_{isf}$ to between 5 and 12 nM under control conditions.

Mechanism of Action of Dipyridamole

Dipyridamole causes coronary vasodilation and increases the adenosine concentration of the coronary venous effluent (Table 2). These findings substantiate those of previous investigators (15, 51). The increased release of adenosine is difficult to reconcile with growing evidence that adenosine is formed in the cytosol (37, 46, 47) and exits the cell via the nucleoside carrier, which should be blocked by dipyridamole (19, 32, 37). However, studies of isolated myocytes confirm that dipyridamole raises net adenosine release (16). It appears that despite any fall in exit of adenosine from myocytes, appearance in venous effluent increases because cellular uptake of adenosine is prevented by dipyridamole. Why is release from myocytes less inhibited than cellular uptake? Measurements of tissue content of adenosine during dipyridamole administration indicate that myocyte cytosol levels can be very high (19, 37). The high concentration of cytosolic adenosine may be able to overcome the dipyridamole blockage enough to allow a small amount of adenosine to exit the cells. The lower extracellular concentration would not be expected to compete with dipyridamole for carrier sites and so would be cleared from the interstitial space solely by washout into venous effluent. In this way, release from the heart can be increased despite a fall in exit of adenosine from myocytes. This analysis is in line with the calculations of Newby (40), who reached the same conclusion.

Imai et al. (28) reported recently that dipyridamole in the presence of a blocker of AMP hydrolysis [α,β -methyleneadenosine 5'-diphosphate (AOPCP)] causes the release of as much AMP as adenosine. The implication is that in the presence of dipyridamole a large fraction of the adenosine comes from AMP by extracellular hydrolysis. Bukoski and Sparks' (16) observation that dinitrophenol-stimulated adenosine release from dispersed cardiomyocytes is more inhibited by AOPCP than by a transport blocker {nitrobenzylthioinosine 6-[(4-nitrobenzyl)thio]-9- β -D-ribofuranosylpurine} also supports the possibility that AMP release is significant. Our results do not distinguish between AMP and adenosine release into the interstitium, and for the calculation of ISF adenosine concentration, it makes no difference. This is an area requiring deeper study.

The final question is how to relate the dipyridamole-induced increases in interstitial adenosine with the observed vasodilation. Dipyridamole increases C_{isf} ~27-fold, at constant pressure, from 7 to 191 nM. CF increased 2.4-fold from 4.8 to 11.7 ml·g⁻¹·min⁻¹. Becker et al. (15) have demonstrated in a similar preparation that dipyridamole's vasodilator effect is markedly reduced (abolished) by theophylline and adenosine deaminase. It is possible that we have two points on the adenosine C_{isf} vs. coronary resistance concentration-response curve and that interstitial adenosine is indeed a powerful vasodilator in submicromolar concentrations.

In summary, we have employed the indicator dilution method to estimate quantitatively the transport parameters governing capillary-tissue exchange of adenosine. These parameters, plus measurements of the venous outflow of endogenous adenosine, have allowed us to calculate interstitial fluid adenosine concentration. During dipyridamole infusion, interstitial adenosine concentration increases as does release into venous effluent. We conclude that dipyridamole exerts a vasodilator effect by raising the interstitial adenosine concentration by a factor of 10 or more.

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APPENDIX A

Solution to Four-Region Capillary-Endothelium-Interstitial-Parenchymal Cell Model

The solutions to Eqs. 1–4 of the text have been obtained through Laplace transforms (12). The four-region solution for the outflow response at $x = L$ to a Dirac delta function input $\delta(t)$ of mass or tracer activity, q_0 , with the capillary transit time $T = V_p/F_p$ is

$$\begin{aligned}
 h(t) = \frac{F_p}{V_p} \frac{C_p(L, t)}{q_0} = e^{-\mu_0} \left\{ \delta(T) + S(T) \left\{ \sum_{i=1}^3 e^{s_i T} \sqrt{\frac{\mu_i}{T}} I_1(2\sqrt{\mu_i T}) \right. \right. \\
 + \int_0^T e^{s_1(T-r)} \sqrt{\frac{\mu_1}{T-r}} I_1[2\sqrt{\mu_1(T-r)}] e^{s_2 r} \sqrt{\frac{\mu_2}{r}} I_1(2\sqrt{\mu_2 r}) dr \\
 + \int_0^T e^{s_2(T-r)} \sqrt{\frac{\mu_2}{T-r}} I_1[2\sqrt{\mu_2(T-r)}] e^{s_3 r} \sqrt{\frac{\mu_3}{r}} I_1(2\sqrt{\mu_3 r}) dr \\
 + \int_0^T e^{s_3(T-r)} \sqrt{\frac{\mu_3}{T-r}} I_1[2\sqrt{\mu_3(T-r)}] e^{s_1 r} \sqrt{\frac{\mu_1}{r}} I_1(2\sqrt{\mu_1 r}) dr \\
 \left. \left. + \int_0^T e^{s_1(T-p)} \sqrt{\frac{\mu_1}{T-p}} I_1[2\sqrt{\mu_1(T-p)}] \int_0^p e^{s_2(p-r)} \sqrt{\frac{\mu_2}{p-r}} I_1[2\sqrt{\mu_2(p-r)}] e^{s_3 r} \sqrt{\frac{\mu_3}{r}} I_1(2\sqrt{\mu_3 r}) dr dp \right\} \right\} \quad (A1)
 \end{aligned}$$

The solution, Eq. A1, is exact; the terms are defined below. At the outflow ($x = L$), the solution is composed of a leading spike occurring at one capillary transit time, $t = T$, the first term on the right in Eq. A1, followed by a tail function described by the terms windowed by $S(T)$. The spike is the transmitted or nonextracted part of the injected tracer, being scaled down by the losses of tracer by permeation through clefts and across the luminal surface of the endothelial cell and by intracapillary reaction

$$e^{-\mu_0} = e^{-(k_1+k_2+k_3)V_p/F_p} = e^{-(PS_g+PS_{ec1}+G_p)/F_p}$$

When PS_{ec1} and G_p are zero (no endothelial luminal surface permeation and no consumption in the plasma region), this is identical to the spike term given by Sangren and Sheppard (45) and Rose et al. (44).

The $\delta(T)$ and $S(T)$ are the unit impulse and unit step functions at $t = V_p/F_p = T$. The volume ratios are $\gamma_{ec} = V'_{ec}/V_p$, $\gamma_{isf} = V'_{isf}/V_p$ and $\gamma_{pc} = V'_{pc}/V_p$. Rate constants for exchanges and transformations are $PS_g/V_p \equiv k_1$, $PS_{ec1}/V_p \equiv k_2$, $PS_{eca}/V_p \equiv k_4$, $PS_{pc}/V_p \equiv k_6$, $G_p/V_p \equiv k_3$, $G_{ec}/V_p \equiv k_5$, $G_{isf}/V_p \equiv k_7$, and $G_{pc}/V_p \equiv k_8$. These give three functions of the Laplace frequency, s , $B_1 \equiv \gamma_{ec}s + k_2 + k_4 + k_5$, $B_2 \equiv \gamma_{pc}s + k_6 + k_8$, and $B_3 \equiv \gamma_{isf}s + k_1 + k_4 + k_6 + k_7$ and the composite cubic (D) whose roots are s_1 , s_2 and s_3 : $D \equiv B_1(B_2B_3 - k_6^2) - B_2k_4^2$. From these one obtains the μ_i s appearing in the first-order modified Bessel functions denoted in Eq. A1

$$\mu_i = \lim_{s \rightarrow s_i} (s - s_i) \cdot \frac{V_p}{F_p} \cdot \frac{B_1 B_2 k_1^2 + 2 B_2 k_1 k_2 k_4 + k_2^2 B_2 B_3 - k_2^2 k_6^2}{D} \quad \text{for } i=1, 2, 3$$

and $\mu_0 = (PS_g + PS_{ec1} + G_p)/F_p$. The r and p in Eq. A1 are dummy variables used for the integrations.

The awkward features of Eq. A1 are the need to find the roots of a cubic equation, the s , and the one double and three single convolution integrals. Because the use of this expression would require an additional convolution with an input function obtained from the data, the process of iterative parameter adjustment for curve fitting is too unwieldy for practical use. Consequently, we used a fast yet accurate numerical approach based on a Lagrangian sliding fluid element algorithm (4, 12).

APPENDIX B

Adenosine Permeabilities: Tests of the Parameter Estimation

A realistic model useful in analysis should be able to at least describe the data from several sources, thereby providing the first stage of a comprehensive explanation. The particular experiments reported here provide outflow tracer-dilution data on three solutes simultaneously, giving evidence of the time course of their exchanges, retention in tissue, and volumes of distribution. Data from other experiments are used to infer that albumin is intravascular and washes out quickly (e.g., Ref. 7) and that sucrose remains extracellular. With respect to adenosine, the presence of transporters on cell membranes is well documented, as are the intracellular processes of deamination to inosine and intracellular sequestration as nucleotides or S -adenosyl-L-homocysteine. Of great relevance to our studies are the observations of Nees et al. (39), and Bardenheuer et al. (3) that show that tracer adenosine, infused over a 30-min period, results in an endothelial adenine nucleotide specific activity much higher than in myocytes. For this to occur, any adenosine that enters the ISF must be taken up more rapidly into endothelial cells than into myocytes, implying that PS_{eca} must be much higher than PS_{pc} (10). These observations can be used in a logical approach to estimating parameters that are poorly constrained by our data.

Parameter optimization without constraints

The simplest approach to parameter estimation is simply to let an optimizing parameter adjustment routine find good fits of the model solutions to the data. This cannot give very good results when the data do not provide information on the “late” parameters, V'_{pc} , PS_{pc} , and G_{pc} , the ones in which sensitivity functions peak later and in which estimation requires long tails on the data. There is difficulty, too, when one undeterminable late parameter has an influence overlapping, even if not the same as another, e.g., PS_{psc} and PS_{eca} , since both are conductances between cells and the ISF. Completely unconstrained optimization therefore seems useless, but we approached it in the interest of providing some evidence on the point. The analyses summarized in Table 3 follow the strategy of using albumin to define intravascular transport and sucrose extracellular transport and to assume that the ratio of metabolic rates G_{ec}/G_{pc} equals 5 in accord with the strong evidence that the bulk of tracer adenosine metabolites are retained by endothelial cells. As anticipated, the estimates of late parameters are very scattered; even the sums PS_{eca} plus PS_{pc} and G_{ec} plus G_{pc} do not have any real consistency. At the same time it should be remarked that values of PS s and G s >40 “high,” meaning that membrane resistance ($1/PS$) is minimal or that consumption (G) is so high that tracer entering the cell is virtually all transformed.

In the presence of dipyrindamole, the estimates of the transmembrane permeabilities, PS_{ecl} , PS_{eca} , and PS_{pc} , diminished to small values as befits processes known to be blocked by dipyrindamole. However, unconstrained estimates of the rate constants for consumption, G_{ec} and G_{pc} , also diminished. This is inappropriate, since so far as is known dipyrindamole has no such direct effect, and is explained by the behavior of any optimizing routine. When starting values of PS s and G s are initially high, then the model solution gives too little reflux of untransformed tracer, and the next iterations reduce the PS s and G s to improve the fit. As

the model's cellular PS s become small, the sensitivity to the G s approaches zero and no further adjustment is made. It is obvious that when cellular PS s are zero the G s have no influence and may take any value from zero to infinity; the practical situation is that as PS s are iteratively reduced, estimates of G s cannot be made.

These inevitable limitations to any automated parameter adjustment routine are quite independent of the nature of the routine chosen. This limitation is reflected by the four high values of PS_{eca} plus PS_{pc} and five high values of PS_{ec} seen in the two dipyridamole groups in Table 3. Although the PS estimates are much less than in the control state, the results of unconstrained optimization fulfill expectations for neither the PS s nor the G s.

Optimization under constraints

The best constraints to impose are those that are based on observed data. Results of imposing a specific set of constraints are reported in Table 4. The estimation procedure was allowed only one degree of freedom. Because dipyridamole does not affect adenosine deamination or phosphorylation, the logical step is to constrain the values of the G s to the control values so that the iterative parameter adjustment routine cannot go astray for the reasons given above. G_{pc} was kept at $4 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, a moderate value but at the minimum found for the control studies (reported in Table 3, control group). The value of G_{ec} was set at a relatively high value of $40 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$, higher than that required to explain entry of tracer adenosine into the nucleotide pool in endothelial cells observed by Nees et al. (39), Kroll et al. (31), and Bardenheuer et al. (3) with 30-min infusions followed by 30-min tracer-free perfusion. Values $\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ are not meaningful in the context of these experiments and models and simply imply that substrate reaching the enzyme is consumed. The logic of using this high value rather than a low one is that the retention on entry is guaranteed by the higher number.

The other constraint imposed for the analyses reported in Table 4 is on the relative values of PS_{eca} and PS_{pc} . The finding that infused tracer adenosine metabolites are highly concentrated in endothelial cells (3, 38, 39) suggests that tracer adenosine reaching the ISF preferentially enters endothelial cells rather than parenchymal cells. Accordingly, PS_{eca}/PS_{pc} was fixed at 10. For testing of contrary constraints, see below. An additional constraint, in accord with their analyses and those of Bassingthwaite et al. (13) and suggested also by the unconstrained analyses reported in Tables 1 and 3, was to put PS_{pc} equal to PS_{ec} , reducing the degrees of freedom by one.

The combination of constraints on PS s and G s should be regarded as a specific hypothesis. The test of the hypothesis is: can the data be fitted as well with these logical constraints imposed as they can be when there are no constraints, which in this case means four greater degrees of freedom? (Instead of G_{ec} , G_{pc} , PS_{ec} , and PS_{pc} being free, only one, PS_{ec} , is freely adjustable.) Thus the degree of freedom for the fitting of the adenosine curves in

Table 3 is really 1, since PS_g and V'_{isf} are determined by the albumin and sucrose curves. V'_{pc} has no impact at all when PS_{pc} approaches zero (as with dipyridamole) and is unimportant even in the control state and could have been fixed at the value for intracellular water space of $\sim 0.6 \text{ ml/g}$.

The other constraints imposed for this test are less consequential, e.g., the fixing of estimates of volumes V'_{ec} and V_p and a description for the flow heterogeneity. First, none of the parameters are strikingly dependent on the volume of the capillary plasma space (V_p) as is shown in Fig. 4. Most importantly, the values of PS_g and PS_{ec} are little dependent. The values of V_p are constrained by experimental data from microvascular casting and transcoronary blood volumes; the approximate range of $0.035\text{--}0.055 \text{ ml/g}$ is suggested by

the shaded region. The overall sum of the tubing volumes, the arterial and venous volumes, and the capillary volume are defined for each curve by the mean transit time volume for albumin. Capillary volume, from anatomic data on capillary dimensions, is ~ 0.04 ml/g (14) at normal pressures or not more than one-third of the total vascular space, the value used here for the analysis of the control studies. At the low pressures occurring with dipyridamole infusion at control flows (*group 2*), the volume was considered to be reduced to 0.03 ml/g and at high flows increased to 0.05 ml/g on the basis of derecruitment at the low pressure and recruitment with vasodilation at constant pressure. On the basis that changes in capillary volume are caused by recruitment or derecruitment, the estimates of V'_{ec} were changed in proportion, holding V_p/V'_{ec} constant at 2.0. The estimates of V'_{ec} in the vasodilated recruited state were made by calculation of capillary surface area times cell thickness: $500 \text{ cm}^2/\text{g}$ times $0.45 \text{ } \mu\text{m} = 0.025 \text{ ml/g}$ for the fully vasodilated and recruited state of the *group 3* experiments. (A comparable volume calculation for V_p is taken from the surface-to-volume ratio $S/V_p = 2\pi r/\pi r^2 = 2/r$ so that $V_p = rS/2$; with $r = 2 \text{ } \mu\text{m}$, $V_p = 500 \times 10^{-4} = 0.05 \text{ ml/g}$). This is equivalent to 4,200 capillaries/ mm^2 , a little higher than usual estimates of capillary density. The heterogeneities used were those found in the specific circumstances under study; for controls, the microsphere distributions gave relative dispersions (RD, the coefficient of variation) of 50%, with vasodilation at low pressure of 63%, and with vasodilation and high flow of 30%. The results are shown in Table 4.

The result of the test is that the highly constrained fits (Table 4) are not significantly worse than the unconstrained results (Table 3) as assessed by the coefficients of variation for the adenosine curve fitting (last column, Table 4). Consequently, the hypothesis that the constrained fitting is realistic cannot be rejected. For the controls, the average coefficient of variation was 9.2% compared with the 8.5% for unconstrained fitting. For dipyridamole at low flow, the constrained fits are actually better, constrained coefficient of variation is 6.2% compared with 8.3% unconstrained. For dipyridamole at high flows, the coefficients of variation are slightly worse but still represent good fits to the data.

Testing of contrary constraints

The next question to raise is whether or not this demonstration of good fits on constraint “proves” that these constraints are demanded by our data. We used them because they were compatible with the experiments of others, those of Bardenheuer et al. (3) and Nees et al. (39). Can the data in the control studies be fitted as well with alternative constraints that ignore the intraendothelial accumulation? We therefore imposed a contrasting set of constraints that provide for preferential retention in cardiac myocytes. The surface area of myocytes we take to be about eight times the luminal capillary surface area, i.e., $4,000 \text{ cm}^2/\text{g}$, including T-tubular system surface (9, 41). We made two assumptions. 1) The densities of the transporters, numbers per unit surface area, were the same on endothelial cells as myocytes, so $PS_{ec1} = PS_{eca} = PS_{pc}/8$. Under this assumption, PS_{pc}/PS_{eca} is ~ 60 times what is obtained with unconstrained optimization. 2) The concentrations of cytosolic enzymes were the same in the two cell types, and the rate constants, G/V were also the same; thus $G_{pc} = G_{ec}$. $G_{pc} = G_{ec} \cdot V'_{pc}/V'_{ec}$. Because V'_{pc}/V'_{ec} turns out to be ~ 33 , the values strongly favor retention inside myocytes. The free parameters are PS_{ec1} and G_{ec} , and very secondarily, with little sensitivity, V'_{pc} . This test was applied to the data for the control state.

The results in Table 5 show that estimates of PS_{ec1} obtained under these restraints are higher than in Table 1 by $\sim 40\%$. The overall transendothelial conductance is $1/PS_{EC} = 1/PS_{ec1} + 1/PS_{eca}$; PS_{EC} is, however, $\sim 20\%$ less, since values of PS_{eca} were higher in Table 1. For Table 1 control experiments, $1/PS_{EC}$ averages 0.256 or $PS_{EC} = 3.9 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$; from Table 5, the overall PS_{EC} is $6.2/2 = 3.1 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. The reason that these constraints do not influence

the estimate of PS_{ec1} very much is that the PS_{ec1} is directly and strongly influenced by the upslope and peak of the data curves. However, the values of G_{ec} are moderately high, from 1 to 20, indicating that intraendothelial consumption is indeed important, even though the analysis is biased against revealing it by putting G_{pc}/G_{ec} to ~330 times higher for Table 5 than for Table 1. When we calculate C_{isf} under control conditions using the parameters in Table 5, we get an average value of 7.8 nM compared with 6.8 nM using the values in Table 1. The conclusions from this test are 1) the values of PS_{ec1} are slightly higher but the total transendothelial conductances less, and the endothelial consumption is important; 2) despite setting up the “rules” to favor intramyocyte retention, strong endothelial retention is demonstrated by the optimized parameter values; 3) accurate determination of parameters influencing the shape of the tail of the curves is not possible when retention by the cells is high and the curves are recorded for a short time; 4) biased high uptake and consumption by myocytes is not equivalent to or a substitute for endothelial uptake and consumption in fitting model to data; and 5) the calculation of C_{isf} is only slightly influenced by the choice of these alternative parameters.

Reconciliation of Possible Parameter Sets with Tracer Deposition Experiments

The parameter optimization routine by itself does not allow us to separate PS_{eca} and PS_{pc} , because their influences become separated only later in the tails of the indicator-dilution curves beyond the samples we collected. In Tables 1 and 5, we imposed the constraint that $PS_{eca}/PS_{pc} = 10$ based on studies indicating that infusions of tracer adenosine lead to preferential accumulation of tracer in endothelial cells (ECs) (3, 38, 39). The equations for tracer adenosine injection (Eqs. 1–4 in text) allow us to model the case of a steady-state tracer adenosine infusion by setting the partial derivatives of concentration with respect to time (dc/dt) equal to zero in each compartment. The accumulation ratios are, in this simplification, based on the premise that G_{ec} and G_{pc} represent sequestration processes without loss of metabolites from the cytosol. We also set the axial diffusion terms equal to zero. Solving these four simultaneous equations, we can get a steady-state ratio of average EC concentration to parenchymal cell concentration

$$\frac{\bar{C}_{ec}}{\bar{C}_{pc}} = \frac{k_2 D + (k_1 B_1 + k_2 k_4) B_2 k_4}{B_1 k_6 (k_1 B_1 + k_2 k_4)}$$

where $k_1 = PS_g$; $k_2 = PS_{ec1}$; $k_4 = PS_{eca}$; $k_6 = PS_{pc}$; $B_1 = PS_{ec1} + PS_{eca} + G_{ec}$; $B_2 = PS_{pc} + G_{pc}$; $B_3 = PS_g + PS_{eca} + PS_{pc}$; and $D = B_1 (B_2 B_3 - k_6^2) - k_4^2 B_2$.

The rate of tracer deposition per unit volume in compartment x can be estimated as $C_x \cdot G_x / V_x$. This is actually the rate of tracer metabolism, but at low adenosine concentrations, the predominant fate is phosphorylation by adenosine kinase and subsequent retention in the adenine nucleotide pool (2, 43). Obviously not all metabolized tracer adenosine stays intracellular, since tracer-labeled metabolites, inosine, hypoxanthine, and uric acid, appear in the venous effluent (23), accounting for one-sixth to one-quarter of the tracer. If we assume that the fractional uptake into nucleotide is the same in ECs as in parenchymal cells, then the deposition rates and the accumulation rates will be proportional to the metabolic rate constants G_{ec} and G_{pc} . After a 30-min tracer adenosine infusion and washout period, Bardenheuer et al. (3) measured the specific activity of adenine nucleotides in guinea pig coronary ECs and in total myocardium. Using these specific activity ratios (ECs/total myocardium) and an ATP/ADP/AMP ratio in myocardium of 27:6.5:1 (38), we determined

the nucleotide specific activity ratio of ECs/myocardium to be 43.3. To convert this to a counts-to-volume ratio (as is calculated by our tracer equations), we must multiply by the ratio of “cold” adenosine concentration in ECs to that in myocardium. Nees et al. (38) have reported this to be 2.6 based on cultured ECs, making the counts-to-volume ratio of ECs/myocardium from Bardenheuer et al. (3) equal to 112. We can make a similar calculation from the tracer deposition rate ratio equation above and the parameters obtained from the indicator-dilution curves. We used the average parameters from Table 1 or 5 and relative volumes of $V'_{pc}=33V'_{ec}$. The tracer equations with parameters from Table 1 give a ratio of 30, whereas the parameters from Table 5 give a ratio of 15. Both are below the experimental value, but the parameters in Table 1 come closest. When the value of PS_{eca} is varied over a very wide range, however, it has little influence on the calculated ratio (because so much tracer enters ECs from the luminal side). We conclude that although the parameters from Tables 1 and 5 fit the indicator-dilution data equally well, the Table 1 values are preferable. The value of PS_{eca} is not well defined but has little influence on the calculation of C_{isf} .

APPENDIX C

Estimation of Interstitial Adenosine Concentrations

Calculation of interstitial fluid adenosine concentration can be made by combining the data in the tracer-dilution curves with data on the nontracer venous adenosine concentrations that were obtained in five hearts. The capillary transport parameters (Fig. 1, Table 1) determined from the tracer-dilution curves are used in these calculations. We make the following assumptions: 1) endogenous adenosine fluxes are in steady state; 2) adenosine transport across plasma membranes is directionally symmetrical; 3) adenosine is generated in myocytes, not endothelial cells (endothelial cytosolic adenosine must have come from either interstitial fluid or plasma); 4) adenosine removal occurs only by washout with the flow and by consumption inside endothelial cells (transformation or uptake along the interendothelial cells is considered negligible, Ref. 50); and 5) parameters obtained for tracer via Eqs. 1–3 are applicable to nontracer adenosine at the same concentrations under which the tracer experiments were performed. This is a restatement of the usual definition of tracer, combined with the understanding that the experiments were done under steady-state conditions for all adenosine concentrations so that concentration-dependent parameters are not changing.

With these assumptions, the steady-state expressions can be reduced to those for three regions, capillary, endothelium, and interstitium, since only the net production (P_m , mol/min) of parenchymal cells into ISF is relevant. Note that P_m includes adenosine entering the ISF from all cell types, as well as any adenosine generated in the ISF from extracellular nucleotides. Because P_m is net production, reuptake by myocytes is accounted for. Apart from P_m , all terms are as defined in the main text. In the absence of consumption in plasma and ISF, the production P_m (mol·g⁻¹·min⁻¹) into the ISF is balanced by endothelial consumption and washout in the venous effluent

$$P_m = F_p \cdot (C_v - C_a) + G_{ec} \cdot C_{ec}$$

which simplifies to $P_m = F_p \cdot [C_v - C_a]$ when dipyridamole blocks uptake by endothelial cells. The steady-state equations omit time derivatives but contain the derivatives with respect to position x . We omit the axial diffusion terms for convenience and simplicity, since their effect is a modest one, raising the intravascular concentration slightly at points upstream from the capillary end at $x = L$. Eqs. 1–3 were correctly written using constant coefficients

for tracer exchanges. In the steady state, these reduce to ordinary 9 not partial, differential equations in the three spatially extended regions plasma

$$0 = -F_p L \cdot \frac{dC_p}{dx} - PS_g (C_p - C_{isf}) - PS_{ecl} (C_p - C_{ec}) \quad (C1)$$

endothelial cell

$$0 = -PS_{ecl} (C_{ec} - C_p) - PS_{eca} (C_{ec} - C_{isf}) - G_{ec} \cdot C_{ec} \quad (C2)$$

interstitial fluid

$$0 = PS_g (C_{isf} - C_p) - PS_{eca} (C_{isf} - C_{ec}) + P_m \quad (C3)$$

From Eqs. C2 and C3, we solve the linear system of equations for C_{ec} and C_{isf} in terms of C_p , PS_g , G_{ec} , and P_m

$$C_{ec} = \frac{C_p (\theta_1 + \theta_2 - G_{ec} \cdot PS_g - G_{ec} \cdot PS_{eca}) + P_m \cdot PS_{eca}}{\theta_1 + \theta_2} \quad (C4)$$

$$C_{isf} = \frac{C_p (\theta_1 + PS_{eca} \cdot PS_{ecl}) + P_m (PS_{ecl} + PS_{eca} + G_{ec})}{\theta_1 + \theta_2} \quad (C5)$$

where

$$\begin{aligned} \theta_1 &= PS_g (PS_{ecl} + PS_{eca} + G_{ec}) \\ \theta_2 &= PS_{eca} (PS_{ecl} + G_{ec}) \end{aligned} \quad (C6)$$

Substituting Eqs. C4 and C5 into Eq. C1, we have the first-order differential equation

$$L \frac{dC_p}{dx} + A C_p = B \quad (C7)$$

where

$$\begin{aligned} A &= \frac{G_{ec} (PS_{ecl} \cdot PS_g + PS_{eca} \cdot PS_g + PS_{eca} \cdot PS_{ecl})}{F_p (\theta_1 + \theta_2)} \\ B &= \frac{(\theta_1 + PS_{eca} \cdot PS_{ecl}) P_m}{F_p (\theta_1 + \theta_2)} \end{aligned} \quad (C8)$$

The solution to Eq. C7, using the boundary condition $C_p(0) = C_a$ = arterial concentration, is

$$C_p(x) = \frac{B}{A} + \left(C_a - \frac{B}{A} \right) \exp(-Ax/L) \quad (C9)$$

With the outlet boundary condition $C_p(L) = C_v$, this gives

$$C_v = \frac{B}{A} + \left(C_a - \frac{B}{A} \right) \exp(-A) \quad (C10)$$

From this and Eq. C8, we obtain the only yet unknown parameter, the production

$$P_m = \frac{F_p A (\theta_1 + \theta_2) [C_v - C_a \exp(-A)]}{(\theta_1 + PS_{eca} PS_{ecl}) [1 - \exp(-A)]} \quad (C11)$$

The average concentration in any region ($\bar{C}$) is obtained by integrating from $x = 0$ to $x = L$ and dividing by L

$$\bar{C} = \frac{1}{L} \int_0^L C(x) dx \quad (C12)$$

Thus, from Eq. C9, we obtain

$$\bar{C}_p = \frac{B}{A} + \frac{[C_a - (B/A)]}{A} [1 - \exp(-A)] \quad (C13)$$

From Eq. C5, the average interstitial concentration ($\bar{C}_{isf}$) is obtained

$$\bar{C}_{isf} = \left[(\theta_1 + PS_{eca} PS_{ecl}) \bar{C}_p + P_m (PS_{ecl} + PS_{eca} + G_{ec}) \right] / (\theta_1 + \theta_2) \quad (C14)$$

Notice that the capillary length (L) is not needed for the computations of average concentrations.

When the plasmalemmal carriers are blocked so that PS_{ecl} and PS_{eca} are zero, $\theta = G_{ec} \cdot PS_g$, $\theta_2 = 0$, the terms containing G_{ec} cancel out in Eqs. C8, C11, C13, and C14, and $A = 0$ and $B = P_m/F$, giving

$$P_m = F_p (C_v - C_a) \quad \text{and} \quad C_v = C_a + \frac{P_m}{F_p} \quad (C15)$$

$$\bar{C}_p = C_a + \frac{P_m}{2 \cdot F_p} \quad (C16)$$

$$\bar{C}_{isf} = \bar{C}_p + \frac{P_m}{PS_g} \quad (C17)$$

When $C_a = 0$, then $\bar{C}_p = C_v/2$, and

$$\bar{C}_{isf} = C_v (0.5 + F_p/PS_g) \quad (C18)$$

The gradient in C_{isf} along the capillary-tissue unit is always linear when PS_g and PS_{ecl} are constant along the length of the capillary. The maximum C_{isf} is at the downstream end and is always equal to or higher than C_v .

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FIG. 1.

Diagram of a capillary-tissue unit showing parameters used to characterize adenosine transport and metabolism. F_p , mean flow of solute containing fluid ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$); PS , permeability-surface area product ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$); G , clearance rate constant for intracellular sequestration and/or consumption ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$); V , volume of tracer distribution (ml/g); C , tracer concentration (radioactivity/vol); P_m , production (mol/min). Subscripts: g, interendothelial gaps; ecl, endothelial cell luminal surface; eta, endothelial cell abluminal surface; pc, parenchymal cell; p, perfusate; isf, interstitial fluid.

FIG. 2.

Example of appearance of radioiodinated serum albumin (RISA), sucrose, and adenosine (ADO) in coronary venous effluent after multiple-tracer injection during control conditions (A) and dipyridamole infusion (perfusate concentration $\approx 10^{-5}$ M) at control coronary flow (B) and at high flow (C). In control curves, relative amount of adenosine is less than sucrose, even in earliest samples. In presence of dipyridamole, adenosine curve is virtually same as sucrose curve. Also, under control conditions, adenosine curve does not cross over sucrose curve. Lines through points are those predicted by model with optimum parameter selection.

FIG. 3.

Ratio of myocardial interstitial adenosine concentration ($\bar{C}_{isf}$) to venous concentration (C_v) plotted as a function of parameters that determine transcapillary transport of adenosine. $\bar{C}_{isf}/C_v$ was calculated over a wide range of values for each parameter while holding all other parameters constant at average value from Table 1. C_v was held at 3.6 nM, the average control value. PS and G ($\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) are as defined in Fig. 1. *A*: calculations with $G_{ec} = 27.5$ (Table 1 average). *B*: calculations with G_{ec} set arbitrarily low at $1.0 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. See text for discussion.

FIG. 4.

Effect of estimate of capillary volume (V_p) on estimates of other parameters of endothelial transport of adenosine (*expt 35*, control). Parameters to be evaluated in this study (PS_g , PS_{eccl} , and V'_{isf}) varied by <10% over expected potential range for V_p from 0.035 to 0.055 ml/g. Less well-determined parameters governing uptake from interstitial fluid (ISF) and intracellular consumption showed greater sensitivity to variation in V_p as can be expected with these short-duration dilution curves. Shading covers expected physiological range. Definitions are as in Fig. 1.

Estimates of parameters

Expt No.	Flow, ml·g ⁻¹ ·min ⁻¹	Extr, %	Duration, s	PS _{ge} , ml·g ⁻¹ ·min ⁻¹	V' _{iss} , ml/g	PS _{ec} , ml·g ⁻¹ ·min ⁻¹	PS _{ge} +PS _{ec} , ml·g ⁻¹ ·min ⁻¹	G _{pc} +G _{ec} , ml·g ⁻¹ ·min ⁻¹	CV(alb), %	CV(suc), %	CV(ADO), %
<i>Control (V_p = 0.04 ml/g, V'_{ec} = 0.02 ml/g; RD = 50%)</i>											
16c	5.28	70	11	1.32	0.095	5.02	31.5	80.0	4.1	5.0	9.5
17c	4.69	78	11	2.18	0.098	4.85	5.7	80.0	9.2	7.3	11.1
19c	4.55	62	12	1.73	0.110	2.67	40.4	59.0	5.7	6.1	7.5
20c	3.61	76	12	1.15	0.097	4.07	45.8	51.0	3.8	10.9	8.2
21c	4.25	79	15	2.11	0.168	4.52	48.0	80.0	5.7	7.5	10.9
34c	5.33	76	23	2.21	0.210	6.12	16.4	19.0	6.1	13.0	7.2
35c	5.15	73	23	2.48	0.169	4.77	36.1	16.0	5.1	5.2	6.3
Means ± SD	4.70±0.63	73.5±5.9	15.3±5.4	1.88±0.50	0.135±0.046	4.57±1.05	32.0±15.6	55.0±28.0	5.7±1.8	7.8±3.0	8.7±1.9
<i>Dipyridamole, constant flow and low pressure (V_p = 0.04 ml/g, V'_{ec} = 0.02 ml/g; RD = 63%)</i>											
16d1	5.28	30	11	1.86	0.120	0	0		7.9	8.1	7.4
17d1	4.69	35	8	2.01	0.110	0	0		6.7	11.6	13.2
19d1	4.55	31	12	1.66	0.170	0	0		5.2	5.6	4.5
20d1	4.63	46	15	2.83	0.130	0	0		4.6	7.3	5.2
21d1	4.34	32	11	1.68	0.100	0	0		5.4	4.8	4.8
34d2	5.33	32	22	2.07	0.220	0	0		4.6	13.5	9.7
35d2	5.15	35	25	2.22	0.240	0	0		5.6	8.3	3.9
Means ± SD	4.85±0.39	34.4±5.4	14.9±6.3	2.05±0.40	0.156±0.056	0±0	0±0		5.7±1.2	8.5±3.1	7.0±3.4
<i>Dipyridamole, constant pressure and high flow (V_p = 0.04 ml/g, V'_{ec} = 0.02 ml/g; RD = 30%)</i>											
16d2	9.89	17	13	1.90	0.240	0	0		5.2	8.2	8.2
17d2	10.62	21	11	2.49	0.250	0	0		9.5	10.8	11.5
19d2	13.84	23	7	3.63	0.240	0	0		8.0	6.4	8.5
20d2	10.74	20	8	2.39	0.160	0	0		4.7	4.8	5.2
21d2	10.32	20	9	2.26	0.250	0	0		7.4	7.3	9.0
34d1	12.00	22	18	3.03	0.210	0	0		2.3	11.1	12.6
35d1	11.53	28	19	3.84	0.210	0	0		2.5	4.8	8.1
Means ± SD	11.28±1.34	22±3	12.1±4.8	2.79±0.73	0.223±0.033	0±0	0±0		5.7±2.8	7.6±2.6	9.0±2.4

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ec, endothelial cell; p, perfusate. RD, relative dispersion of perfusate flow; Extr, instantaneous extraction of adenosine, $E(t) = 1 - h(\text{adenosine}, t)/h(\text{albumin}, t)$ at time that $h_R(t)$ peaked. Constraints were: $PS_{\text{eca}} = 10$ PS_{pc} ; $G_{\text{ec}} = G_{\text{pc}}$, each 40.

TABLE 2

Estimates of interstitial and other concentrations

Expt No.	Control				Dipyridanole (10^{-5} M)					
	Control				Control flow, low pressure			Control pressure, high flow		
	$\bar{C}_{\text{isf}}$	$\bar{C}_{\text{ec}}$	$\bar{C}_p$	$\bar{C}_v$	$\bar{C}_{\text{isf}}$	$\bar{C}_p$	$\bar{C}_v$	$\bar{C}_{\text{isf}}$	$\bar{C}_p$	$\bar{C}_v$
19	5.77	3.1	1.5	2.6	35	5.4	10.9	62	7.2	14.4
20	7.47	4.5	2.4	4.0	68	15.9	31.9	133	13.4	26.7
21	12.2	6.2	4.0	6.5	156	25.3	50.5	172	17.0	34.0
34	5.2	2.9	1.7	2.9	97	15.8	31.5	376	42.2	84.3
35	3.3	2.5	1.2	2.2	84	14.9	29.8	212	30.3	60.5
Means $\pm$ SE	6.8 $\pm$ 1.5	3.8 $\pm$ 0.7	2.2 $\pm$ 0.5	3.6 $\pm$ 0.8	88 $\pm$ 20	15.5 $\pm$ 3.2	30.9 $\pm$ 6.2	191 $\pm$ 52	22.0 $\pm$ 6.3	44.0 $\pm$ 12.6

All values are given in nanomolar. $\bar{C}$, mean concentration. Subscripts are as in Table 1.

TABLE 3

Tests of parameter optimization

Expt No.	PS_g , $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	PS_{ec} , $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	V'_{is} , ml/g	$PS_{pc} + PS_{ec}$, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	$G_{pc} + G_{ec}$, $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	CV, %
<i>Control ($V_p = 0.035$ ml/g, $V'_{ec} = 0.0175$ ml/g; $RD = 50\%$)</i>						
16c	1.340	4.300	0.092	700.0	2,200.0	7.9
17c	2.100	4.500	0.110	220.0	36.0	10.6
19c	1.680	2.230	0.120	225.0	480.0	6.6
20c	1.400	4.050	0.100	21.0	24.0	11.4
21c	2.090	3.890	0.170	960.0	1,900.0	9.5
34c	2.230	5.890	0.210	99.0	21.0	8.0
35c	2.510	4.610	0.170	112.0	17.0	5.3
Means $\pm$ SD	1.907 $\pm$ 0.441	4.210 $\pm$ 1.090	0.139 $\pm$ 0.045	334.0 $\pm$ 354.0	668.0 $\pm$ 962.0	8.5 $\pm$ 2.2
<i>Dipyridamole, constant flow and low pressure ($V_p = 0.035$ ml/g, $V'_{ec} = 0.0175$ ml/g; $RD = 63\%$)</i>						
16d1	2.100	0.01	0.12	1.56	0.40	11.9
17d1	1.530	0.56	0.11	0.01	0.01	14.2
19d1	1.450	0.16	0.17	0.04	0.01	5.7
20d1	3.030	0.06	0.13	0.02	0.47	5.8
21d1	2.200	0.01	0.10	4.72	0.01	6.8
34d2	2.000	0.20	0.21	0.01	0.05	10.3
35d2	1.920	0.46	0.21	0.01	0.16	3.3
Means $\pm$ SD	2.033 $\pm$ 0.522	0.21 $\pm$ 0.22	0.15 $\pm$ 0.05	0.91 $\pm$ 1.78	0.16 $\pm$ 0.20	8.3 $\pm$ 3.9
<i>Dipyridamole, constant pressure and high flow ($V_p = 0.035$ ml/g, $V'_{ec} = 0.0175$ ml/g; $RD = 30\%$)</i>						
16d2	1.530	0.73	0.210	1.63	0.01	5.5
17d2	3.170	0.01	0.200	0.76	1.59	13.3
19d2	2.870	1.27	0.210	0.48	8.38	5.7
20d2	1.970	0.01	0.130	0.01	3.25	5.3
21d2	2.560	0.03	0.210	2.90	2.28	7.7
34d1	2.350	0.28	0.210	0.01	0.01	11.8
35d1	2.490	1.67	0.210	0.01	0.27	5.8
Means $\pm$ SD	2.420 $\pm$ 0.546	0.571 $\pm$ 0.674	0.197 $\pm$ 0.030	0.83 $\pm$ 1.09	2.26 $\pm$ 2.97	7.9 $\pm$ 3.3

Definitions are as in Fig. 1. All parameters are free and unconstrained, but $\bar{G}_{ec}/G_{pc} = 5$.

TABLE 4

Estimated parameters for adenosine in guinea pig heart with four-region model (intracellular consumption fixed)

Expt No.	PS_g , $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	V'_{ist} , ml/g	PS_{ec} , $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	V_{pc} , ml/g	CV, %
<i>Control ($V_p = 0.04 \text{ ml/g}$, $V'_{\text{ec}} = 0.02 \text{ ml/g}$; $RD = 50\%$)</i>					
16c	1.315	0.095	5.13	0.700	9.2
17c	2.181	0.098	4.56	0.660	9.9
19c	1.734	0.110	2.58	0.700	7.8
20c	1.154	0.097	3.80	0.680	8.5
21c	2.109	0.168	4.78	0.700	10.6
34c	2.211	0.210	5.22	0.580	8.9
35c	2.452	0.169	3.94	0.620	9.2
Means $\pm$ SD	1.879 $\pm$ 0.491	0.135 $\pm$ 0.046	4.29 $\pm$ 0.93	0.663 $\pm$ 0.047	9.2 $\pm$ 0.9
<i>Dipyridamole, constant flow and low pressure ($V_p = 0.03 \text{ ml/g}$, $V'_{\text{ec}} = 0.015 \text{ ml/g}$; $RD = 63\%$)</i>					
16d1	1.475	0.138	0.021	0.615	6.5
17d1	2.075	0.105	0.000	0.615	12.4
19d1	1.716	0.210	0.000	0.614	6.5
20d1	2.849	0.137	0.004	0.613	4.5
21d1	1.730	0.095	0.028	0.613	5.7
34d2	2.127	0.210	0.000	0.623	3.9
35d2	2.050	0.210	0.000	0.623	3.8
Means $\pm$ SD	2.003 $\pm$ 0.442	0.158 $\pm$ 0.051	0.008 $\pm$ 0.012	0.617 $\pm$ 0.004	6.2 $\pm$ 3.0
<i>Dipyridamole, constant pressure and high flow ($V_p = 0.05 \text{ ml/g}$, $V'_{\text{ec}} = 0.025 \text{ ml/g}$; $RD = 30\%$)</i>					
16d2	1.886	0.210	0.003	0.623	9.4
17d2	2.486	0.210	0.021	0.625	14.6
19d2	3.223	0.210	0.143	0.623	12.9
20d2	1.850	0.191	0.000	0.623	6.4
21d2	1.915	0.210	0.050	0.623	9.6
34d1	2.824	0.210	0.000	0.609	12.4
35d1	2.980	0.210	0.003	0.610	8.4
Means $\pm$ SD	2.452 $\pm$ 0.575	0.207 $\pm$ 0.007	0.031 $\pm$ 0.052	0.619 $\pm$ 0.007	10.5 $\pm$ 2.9

Values of PS_{eca} and PS_{pc} fixed with $PS_{\text{eca}}/PS_{\text{pc}} = 10$; $PS_{\text{pc}} = PS_{\text{ec}}$; $G_{\text{ec}} = 40 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$; and $G_{\text{pc}} = 4 \text{ ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. Definitions are as in Fig. 1.

TABLE 5

Adenosine curves in absence of dipyridamole

Expt No.	$PS_{\text{ecf}} = PS_{\text{eca}}, \text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	$G_{\text{ec}}, \text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	$V'_{\text{pc}}, \text{ml/g}$	CV, %
16c	5.3	19.8	0.66	8.0
17c	7.1	3.2	0.70	11.0
19c	2.7	13.0	0.65	7.3
20c	5.5	4.33	0.61	7.7
21c	5.3	12.8	0.60	9.4
34c	9.6	1.6	0.70	10.5
35c	7.8	1.0	0.70	8.8
Means $\pm$ SD	6.2 $\pm$ 2.2	8.0 $\pm$ 7.2	0.66 $\pm$ 0.04	9.0 $\pm$ 1.4

Table 5 shows test of goodness of fit under assumptions that 1) densities of transporters are same on all surfaces and 2) clearance per unit volume is same in endothelial and parenchymal cells. Definitions are as in Fig. 1.