Comprehensive Model of Transport and Metabolism of Adenosine and S-Adenosylhomocysteine in the Guinea Pig Heart

Keith Kroll, Andreas Deussen, and Ian R. Sweet

Regulation of blood flow and mitochondrial respiration in the heart would be clarified by improved knowledge of interstitial concentrations and cellular production rates of adenosine; however, these variables cannot be measured directly. To interpret indexes that are available, a comprehensive mathematical model was developed, based on a large body of experimental data. The model describes most of the important pathways of capillary–tissue transport and cellular metabolism of adenosine in the guinea pig heart. It includes capillary flow, solute transport between tissue regions, nonlinear enzyme kinetics for adenosine kinase and adenosine deaminase, and reversible biunireactant kinetics for S-adenosylhomocysteine hydrolase in cardiomyocytes and endothelial cells, intracellular production of adenosine via AMP hydrolysis and transmethylation, and extracellular production of adenosine. A single set of parameter values for the model was obtained in the first stage of the analysis by taking certain values directly from published sources, other values were subject to specific constraints, and other values were determined by parameter optimization. The effects of flow and endothelial metabolism on the relation between interstitial and venous adenosine concentrations were determined. The relation between myocardial adenosine production rate and S-adenosylhomocysteine accumulation in the presence of excess homocysteine was estimated. In the second stage of the analysis, the model was used to investigate the mechanism of myocardial adenosine production, without changing the parameter values. Cellular adenosine production rates were estimated by fitting measurements of venous adenosine release obtained during altered energetic conditions in experiments by different investigators. The original results showed a dissociation between measurements of cytosolic AMP concentrations and venous adenosine release. It is concluded that 1) it is essential to account for the effect of flow on interstitial and venous adenosine concentrations, since decreased flow may produce effects outwardly resembling inhibition of the enzyme 5’-nucleotidase, 2) adenosine concentrations in epicardial transudate are not in equilibrium with interstitial fluid, and 3) the rate of cellular adenosine production increases monotonically with free cytosolic concentrations of AMP during a variety of alterations in energy balance of the guinea pig heart. (Circulation Research 1992;71:590–604)

KEY WORDS • myocardium • homocysteine • transmethylation • endothelial cell • purines • energetics • kinetics

It has been hypothesized that under certain conditions coronary blood flow, sinus node depolarization, atrioventricular node transmission, the inotropic response due to sympathetic stimulation, the rate of glycolysis, and the generation of oxygen-derived free radicals by activated neutrophils are regulated via the actions of interstitial adenosine on adenosine receptors located on effector cell membranes (for overviews see References 1–3). Experimental tests of these hypotheses rely heavily on assessments of interstitial adenosine concentrations or myocardial adenosine production rates. However, neither quantity can be directly measured because of problems of compartmentalization and the effects of capillary transport and cellular metabolism. The goal of the present study was to develop a comprehensive mathematical model to estimate interstitial adenosine concentrations and cytosolic adenosine production rates in the guinea pig heart.

Previous mathematical models of adenosine in the heart have focused on specific aspects of transport and metabolism. Kohn and Garfinkel developed a nonlinear model for the ischemic rat heart, which described vectorial production of adenosine in the interstitial space from membrane-associated AMP produced by phosphodiesterase and cellular metabolism by adenosine kinase and adenosine deaminase. The model did not describe the S-adenosylhomocysteine (SAH) pathway, intracellular production of adenosine, or endothelial cells and accounted for flow via first-order exchange between the interstitial and vascular compartments.

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Newby developed a nonlinear model to analyze the effects of blockade of membrane adenosine transport by dipyridamole. The model described two cell types: a production site and a trap. The model included adenosine deaminase and adenosine kinase but not SAH hydrolase and did not describe the endothelial metabolic barrier between the vascular and interstitial spaces.

Wangler et al. developed a multiple-region (capillary, endothelial cell, interstitial fluid, and parenchymal cell) axially distributed adenosine model to analyze results of multiple-indicator dilution experiments. The model included linear transport, metabolism, and regional flow heterogeneity but did not describe intracellular adenosine formation or separate enzymatic pathways for adenosine. The present model is an extension of the Wangler model, since flow and transport are accounted for in much the same way. The major differences are that the present model accounts for individual enzymatic pathways for adenosine using nonlinear reversible kinetics, intracellular adenosine production in both endothelial cells and cardiomyocytes, and an additional tissue region for epicardial transudates.

The two-stage design of the present study was as follows. In the first stage, the structure of the model and a single set of parameter values were determined by analyzing a large body of data describing adenosine transport and metabolism in the heart. Because no single set of observations contains sufficient information to describe all the important pathways, analyzing many different measurements was essential to develop a comprehensive model. The experimental observations included 1) results from multiple-indicator dilution studies,7-9 2) morphometric measurements,10-12 3) in vitro measurements of enzyme kinetics,13-17 4) results from cultured cells,18 5) coronary venous adenosine release rates and myocardial SAH content under normoxic and hypoxic conditions,14 6) myocardial SAH content in the presence of a blocker of the enzyme SAH hydrolase,15 7) capillary endothelial cell trapping of tracer adenosine, supplied via the arterial inflow,19 8) epicardial transudate concentrations of adenosine,20 and 9) the endothelial cell contribution to total venous release of adenosine.21

In the second stage of analysis, the model parameter values were left unchanged, and the model was used to clarify an inconsistency observed by different investigators22-25 on the mechanism of cellular production of adenosine. In these experiments, free cytosolic AMP concentrations were determined under conditions of altered myocardial energetics. The venous release rate of adenosine was measured as an index of myocardial adenosine production. During hypoxic perfusion, studied by He et al.,24 and substrate deprivation, studied by Kang et al.,25 venous release increased continuously with AMP concentrations. The inconsistency was that venous release of adenosine decreased at the highest AMP concentrations during coronary underperfusion, studied by Headrick et al.20 and He et al.23 To explain this finding, He et al suggested that cytosolic 5′-nucleotidase, the enzyme producing adenosine from AMP, was subject to allosteric inhibition during ischemia. However, the use of venous adenosine release as an index of cellular production did not account for the effects of flow or capillary endothelial cell metabolism of adenosine. Therefore, the present model was used to estimate the rate of cellular production of adenosine during altered energetics by analyzing the measurements of venous release. The analysis explained the dissociation between venous release and AMP concentrations and revealed that, in all the data sets, myocardial adenosine production was directly related to cytosolic AMP concentrations over the entire range of myocardial energetics.

Materials and Methods

Structure of the Model

Overview of model. The model equations are based on the principle of the conservation of mass and steady plug flow in the capillary and the absence of radial concentration gradients within the model regions. The model, one capillary pathway of which is shown in Figure 1, describes five axially distributed tissue regions: the capillary, endothelial cell, interstitial, parenchymal cell, and epicardial transudate. Exchange of adenosine and homocysteine between the model regions is described by permeability–surface area products (PS) products, which are first-order terms (units of clearance are milliliters per gram of cardiac tissue per minute). Figure 1 shows the pathways for entry and removal of adenosine, homocysteine, and SAH in the model regions. For example, cytosolic adenosine comes from the hydrolysis of 5'-AMP, the hydrolysis of SAH, and transmembrane influx. Cytosolic adenosine is lost by the formation of AMP, inosine, and SAH and by transmembrane efflux. Epicardial transudate was modeled as an extracellular region within which adenosine is formed, exchanging with the interstitial region by diffusion. The model describes luminal and abluminal membrane transport in endothelial cells, membrane transport in parenchymal cells (cardiomyocytes), and extracellular transport between the capillary and interstitial regions via interendothelial gaps (PS). To represent the effects of spatial
heterogeneity of myocardial flow, the model describes up to 10 parallel capillary pathways, identical except for their flow, with a common inflow and a common outflow.\textsuperscript{26}

**Model equations.** The input variables for the model are the capillary flow rate ($F_{\text{cap}}$), the rates of 5'-AMP hydrolysis ($S^*$, zero order) and cellular transmethylation ($S^\circ$) in the cellular regions, and the capillary inflow concentrations of adenosine and homocysteine. The rates of the adenosine kinase ($R^*$) and adenosine deaminase ($R^\circ$) reactions are described by Michaelis-Menten expressions. The reversible SAH hydrolase reaction is modeled using expressions described below, where $R^{\text{syn}}$ and $R^{\text{hyd}}$ are the unidirectional rates in the synthetic and hydrolytic directions, respectively. The same reactions are described in the endothelial cell and cardiomyocyte regions.

The concentrations of adenosine ($[A]$), homocysteine ([H]), and SAH in the five model regions are calculated as functions of time (t) and distance (x) along the length (L) of the capillary (where x varies between 0 and L), based on the enzyme reaction rates and a system of partial differential equations, examples of which are described below. The subscripts indicate capillary (cap), endothelial cell (ec), interstitial (isf), parenchymal cell (pc), and epicardial transudate (trans) regions.

For adenosine in the capillary ([A]$_{\text{cap}}$)

$$V_{\text{cap}} \cdot \frac{\partial [A]_{\text{cap}}}{\partial t} = -F_{\text{cap}} L \cdot \frac{\partial [A]_{\text{cap}}}{\partial x} - PS^g \cdot ([A]_{\text{cap}}-[A]_{\text{isf}})$$

$$-PS_{\text{ec}} \cdot ([A]_{\text{ec}}-[A]_{\text{cap}}) - PS_{\text{pc}} \cdot ([A]_{\text{pc}}-[A]_{\text{isf}})$$

$$-R^e - R^k + R^{\text{syn}} + R^{\text{hyd}} + S^A$$

(1)

where $V_{\text{cap}}$ is capillary volume, $PS^g_{\text{cap}}$ is capillary gap PS for adenosine, and $PS_{\text{ec}}$ is luminal PS of the endothelial cell for adenosine.

For adenosine in the endothelial cell ([A]$_{\text{ec}}$)

$$V_{\text{ec}} \cdot \frac{\partial [A]_{\text{ec}}}{\partial t} = -PS_{\text{ec}} \cdot ([A]_{\text{ec}}-[A]_{\text{cap}}) - PS_{\text{pc}} \cdot ([A]_{\text{pc}}-[A]_{\text{isf}})$$

$$-R^e - R^k - R^{\text{syn}} + R^{\text{hyd}} + S^A$$

(2)

where $V_{\text{ec}}$ is endothelial cell volume and $PS_{\text{ec}}$ is abluminal PS of the endothelial cell for adenosine.

For adenosine in the interstitial region ([A]$_{\text{isf}}$)

$$V_{\text{isf}} \cdot \frac{\partial [A]_{\text{isf}}}{\partial t} = -PS_{\text{pc}} \cdot ([A]_{\text{pc}}-[A]_{\text{cap}}) - PS_{\text{trans}} \cdot ([A]_{\text{isf}}-[A]_{\text{trans}}) + S^A_{\text{isf}}$$

(3)

where $V_{\text{isf}}$ is interstitial fluid volume and $PS_{\text{pc}}$ and $PS_{\text{trans}}$ are membrane PS of the parenchymal cell and PS of the transudate region, respectively, for adenosine.

For adenosine in the parenchymal cell ([A]$_{\text{pc}}$)

$$V_{\text{pc}} \cdot \frac{\partial [A]_{\text{pc}}}{\partial t} = -PS_{\text{pc}} \cdot ([A]_{\text{pc}}-[A]_{\text{isf}}) - R^e_{\text{pc}} - R^k_{\text{pc}}$$

$$-R^{\text{syn}}_{\text{pc}} + R^{\text{hyd}}_{\text{pc}} + S^A_{\text{pc}}$$

(4)

where $V_{\text{pc}}$ is parenchymal cell volume.

For adenosine in the transudate region ([A]$_{\text{trans}}$)

$$V_{\text{trans}} \cdot \frac{\partial [A]_{\text{trans}}}{\partial t} = -PS_{\text{trans}} \cdot ([A]_{\text{trans}}-[A]_{\text{isf}}) + S^A_{\text{trans}}$$

(5)

where $V_{\text{trans}}$ is volume of the transudate region.

There are five equations for homocysteine ([H]), for the capillary, endothelial cell, interstitial, parenchymal cell, and transudate regions, exemplified by the equation for the parenchymal cell:

$$V_{\text{pc}} \cdot \frac{\partial [H]_{\text{pc}}}{\partial t} = -PS_{\text{pc}} \cdot ([H]_{\text{pc}}-[H]_{\text{isf}}) - R^{\text{syn}}_{\text{pc}} + R^{\text{hyd}}_{\text{pc}}$$

(6)

There are two equations for concentration of SAH ([S]), for the endothelial and parenchymal cell regions only, based on the assumptions that SAH hydrolase activity is intracellular and that membrane permeability for SAH is negligible.\textsuperscript{27} The SAH equations are exemplified by the equation for the parenchymal cell:

$$V_{\text{pc}} \cdot \frac{\partial [S]_{\text{pc}}}{\partial t} = -R^{\text{hyd}}_{\text{pc}} + R^{\text{syn}}_{\text{pc}} + \frac{S^A_{\text{pc}}}{K^S + [S]_{\text{pc}}}$$

(7)

The final term in Equation 7 describes product inhibition of transmethylation by SAH.\textsuperscript{16}

For tracer adenosine, homocysteine, and SAH, the transport and enzyme fluxes are governed by the concentrations of the nontracer mother substance and are equal to the specific activity times the flux of the mother substance.

The unidirectional reactions of the SAH hydrolase enzyme are described using a biunimolecular reaction scheme, assuming random order and equilibrium binding of reactants and products:\textsuperscript{28}

$$R^{\text{syn}} = \frac{[H] \cdot [A]}{K^A \cdot K^H} \left( \frac{[H] \cdot [A] \cdot [S]}{K^A + K^H + K^S} \right)$$

(8)

$$R^{\text{hyd}} = \frac{[S]}{K^S}$$

(9)

where $R^{\text{syn}}$ and $R^{\text{hyd}}$ are the unidirectional rates of the SAH hydrolase reaction in the synthetic and hydrolytic directions, $K^A$, $K^H$, and $K^S$ are the $K_n$ values for adenosine, homocysteine, and SAH, and $V_{\text{trans}}$ and $V_{\text{syn}}$ are the maximum velocity ($V_{\text{max}}$) values for the synthetic and hydrolytic reactions, respectively.

**Calculation of tissue contents.** Tissue contents of adenosine, homocysteine, and SAH were calculated based on the solutions for concentrations at each time point, as exemplified by the expression for adenosine ($q^A$):

$$q^A = V_{\text{cap}} [A]_{\text{cap}} + V_{\text{ec}} [A]_{\text{ec}} + V_{\text{isf}} [A]_{\text{isf}} + V_{\text{pc}} [A]_{\text{pc}} + V_{\text{trans}} [A]_{\text{trans}}$$

(10)

For the calculation of tissue contents, spatial averages were used for the concentrations in Equation 10, since the solutes exhibit concentration gradients along the capillary from inflow to outflow. Because the model does not describe the known binding of adenosine to...
protein,\textsuperscript{29} the calculated tissue content of adenosine reflects the free concentrations only.

**Epidermal transudate region.** The epidermal transudate region in the model is an extracellular region within which adenosine is formed, like the interstitial fluid region but comprising a much smaller volume (0.01 versus 0.11 ml/g). A PS product between the transudate and interstitial regions (\(PS_{\text{trans}}\)) was used to represent the effect of long diffusion distances impeding adenosine exchange. While the interstitial region exchanged directly with capillary, endothelial cell, cardiomyocyte, and transudate regions along the length of the capillary, the transudate region exchanged only with the interstitial region. This arrangement was needed to model epidermal transudate adenosine concentrations in the normal range of 70–200 nM.\textsuperscript{20,21,25,30–34} and at the same time to obtain correct estimates of normoxic venous adenosine concentrations and SAH accumulation in the presence of excess homocysteine.

**Multicapillary model.** The multicapillary configuration was similar to that described by Wangler et al.\textsuperscript{7} except that 10 pathways were used to improve on the representation of flow heterogeneity, and nonexchanging large vessels were not included in the present model. The flow distribution used was a slightly right-skewed lagged normal density function, with a relative dispersion (standard deviation/mean) of 0.55, based on a fractal analysis\textsuperscript{35} of the regional microsphere deposition densities measured in guinea pig hearts.\textsuperscript{36}

The lagrangian sliding fluid element approach of Bassingthwaighte et al\textsuperscript{17} was used to solve the convective component of Equation 1, which fixes the primary time step of the model to equal \(V_{\text{cap}} \cdot N_{\text{seg}}/F_{\text{cap}}\), where \(N_{\text{seg}}\) is the number of longitudinal segments into which the capillary is divided. The radial exchanges and nonlinear reactions were solved numerically, using the Runge-Kutta-Fehlberg algorithm. This approximation works well, as long as the secondary time steps used for the radial exchange reactions are small compared with the rate of change of concentrations. This was ensured by setting the size of the secondary time step to equal no more than half of the shortest time constant of all the enzymatic and transport processes.

**Compartmental model.** For comparison with the distributed model described above, a reduced compartmental form of the model was also used consisting of parenchymal cell and interstitial regions and a nonflowing vascular region having constant concentrations. The same transport and enzyme rate expressions were used as in the distributed model; the difference was that the partial differential equations were reduced to the ordinary differential equations of stirred tank modeling.

**Assumptions of the model.** The model is based on a series of assumptions common to previous axially distributed multiple-region models: 1) There is steady plug flow in capillaries. 2) There is instantaneous radial (perpendicular to the length of the capillary) diffusion equilibrium within regions. 3) There is first-order transport between regions. The first two assumptions are based on an analysis by Bassingthwaighte and Goretsky.\textsuperscript{26} The third assumption may introduce a small degree of error in the modeling of the experimental results of intracoronary administration of adenosine (100 \(\mu\)M), since recent multiple-indicator dilution experiments show an apparent \(K_m\) of the endothelial membrane transporter for adenosine in the range 100–200 \(\mu\)M.\textsuperscript{9} In addition, there are assumptions that are specific to the present model: 4) Adenosine is produced in cardiomyocytes and endothelial cells and extracellularly in the interstitial region (considered in “Discussion”). 5) Extracellular transport of adenosine via interendothelial clefts is similar to that of sucrose (considered in “Discussion”). 6) Adenosine is not metabolized extracellularly. (Extracellular adenosine deaminase activity appears to be low.) 7) The simplest kinetically appropriate assumptions were made for the three enzymes: Michaelis-Menten kinetics for adenosine deaminase and adenosine kinase (ATP is saturating) and random-order equilibrium binding and reversible bimolecular kinetics\textsuperscript{28} for SAH hydrolyase. 8) There is no subcellular compartmentalization (protein binding). Since the dissociation half-time of adenosine protein binding in the heart was at least 2.5 hours,\textsuperscript{29} exchange with this site is not expected to play an important role in the processes modeled in the present study.

**First Stage of Analysis: Selection of Parameter Values**

The first stage of analysis consisted of the selection of a single set of model parameter values, following the strategy described below. The parameter values that were selected are listed in Table 1 and were left unchanged during the second stage of analysis.

**Rationale for the strategy.** A number of parameters were assigned values taken from direct measurements in published sources. Parameters in class I included PS products determined by multiple indicator dilution experiments, region volumes determined by morphometric studies, and enzyme \(K_m\) values determined by in vitro kinetic assays. Parameters in class II were assigned values coupled directly to other parameter values, as described below. Parameters in class III were assigned values by parameter optimization, based on the following rationale. If the model structure is correct and if variables can be measured that are sensitive to processes described by the model, then parameter values can be estimated by adjusting their values to provide the best model fit to the data. In the present study, parameters were optimized to fit measurements of adenosine and SAH in guinea pig hearts. Most measurements were fit to within 20% accuracy. Parameter optimization was necessary because several important parameters cannot be measured or have not been measured under the appropriate conditions or because measurements by different investigators do not agree. No parameters were assigned values outside the range of published values, where these were available.

**I: Parameters assigned published values.** Parameters assigned values taken directly from published sources are identified by an asterisk in Table 1.

**II: Coupled parameters.** The following parameters were assigned values coupled directly to other parameter values: 1) It was assumed that endothelial cell \(V_{\text{ms}}\) of adenosine deaminase was 20 times higher than in cardiomyocytes, when activities were expressed per milliliter cell volume, based on isolated cell studies.\textsuperscript{18} 2) It was assumed that the \(K_{S}\) for adenosine deaminase, adenosine kinase, and SAH hydrolyase were identical in endothelial and cardiomyocyte regions. 3) The sum of the endothelial and cardiomyocyte \(V_{\text{ms}}\) of SAH hydrolyase for SAH synthesis in the model (61 nmol \(\cdot g^{-1} \cdot \text{min}^{-1}\)) was taken almost directly from estimates made by Deussen et al\textsuperscript{14} of the global \(V_{\text{ms}}\) in the guinea pig heart (59 nmol \(\cdot g^{-1} \cdot \text{min}^{-1}\)). 4) The Haldane equation,
which describes the relation between the enzyme kinetic parameters of a reversible reaction and the equilibrium constant $K_{eq}$ of the reaction, was used to estimate the $V_{max}$ of SAH hydrolase for SAH hydrolysis. The Haldane equation has the general form

$$K_{eq} = \frac{V_f}{V_r} = \frac{K_{eq}^f}{K_{eq}^r}$$  \hspace{1cm} (11)$$

where the superscripts $f$ and $r$ refer to the forward and reverse directions of the reversible reaction, respectively. If kinetic rate expressions are used to describe a system that reaches equilibrium, correct equilibrium concentrations will only be obtained if the kinetic parameters obey the Haldane equation. In the present model, the Haldane equation was used in the form

$$\frac{V_{hyd}}{V_{syn}} = \frac{K_{eq}^f}{K_{eq}^r}$$

Using the $K_{eq}$ values listed in Table 1 and $K_{eq}=0.8 \mu M$, the ratio of the $V_{max}$ was equal to 0.01; the absolute value of the $V_{max}$ for synthesis was determined as described in point 3 above. 5) The $V_{max}$ for endothelial adenosine deaminase and adenosine kinase in the model were constrained by multiple indicator dilution measurements by Wangler et al. who estimated the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Values and units</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{max}^{syn}$</td>
<td>$V_{max}$ for SAH synthesis in ec</td>
<td>$6.0 \text{ nmol} \cdot 1^{-1} \cdot \text{min}^{-1}$</td>
<td>Constrained by $V_{max}^{syn}$</td>
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<td>$V_{max}$ for SAH hydrolysis in ec</td>
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<td>Haldane equation</td>
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<td>$K_{m}^{a}$</td>
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<td>$K_m$ of SAH hydrolase for Hcy</td>
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<td>$K_{m}^{d}$</td>
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<td>$K_{m}^{k}$</td>
<td>$K_m$ for Ado kinase</td>
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<tr>
<td>$P_{SA}^{l}$</td>
<td>Capillary gap PS for Ado</td>
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<td>$P_{SA}^{a}$</td>
<td>Abluminal PS of ec for Ado</td>
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<tr>
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<td>Membrane PS of pc for Ado</td>
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<td>$V_{capp}^{l}$</td>
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<tr>
<td>$V_{capp}^{a}$</td>
<td>Volume of ec region</td>
<td>$0.030 \text{ ml/g}$</td>
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<tr>
<td>$V_{isf}^{a}$</td>
<td>Volume of isf region</td>
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<td>$V_{isf}+V_{isf}=0.12 \text{ ml/g}$, 12</td>
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<td>$V_{trans}^{a}$</td>
<td>Volume of trans region</td>
<td>$0.01 \text{ ml/g}$</td>
<td>Constrained by $V_{trans}^{a}$</td>
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<td>$V_{pc}^{l}$</td>
<td>Volume of pc region</td>
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<td>$S_{pc}^{a}$</td>
<td>Rate of cardiomyocyte Ado formation from AMP</td>
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<tr>
<td>$S_{pc}^{a}$</td>
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<td>Constrained by $S_{pc}^{a}$</td>
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<td>Rate of Ado formation in isf region</td>
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<td>Rate of Ado formation in trans region</td>
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<td>Constrained by $S_{transf}^{a}$</td>
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<tr>
<td>$S_{transf}^{a}$</td>
<td>Rate of Ado formation in trans region</td>
<td>$190 \text{ pmol} \cdot 1^{-1} \cdot \text{min}^{-1}$</td>
<td>15</td>
</tr>
<tr>
<td>$S_{ec}^{a}$</td>
<td>Rate of endothelial transmethylation</td>
<td>$29 \text{ pmol} \cdot 1^{-1} \cdot \text{min}^{-1}$</td>
<td>Constrained by $S_{ec}^{a}$</td>
</tr>
<tr>
<td>$F_{uc}^{l}$</td>
<td>Perfusion flow rate</td>
<td>Input variable, $\text{ml} \cdot 1^{-1} \cdot \text{min}^{-1}$</td>
<td>. . .</td>
</tr>
<tr>
<td>[Ado]_{in}</td>
<td>Inflow conc of Ado</td>
<td>Input variable, mol/ml</td>
<td>. . .</td>
</tr>
<tr>
<td>[Hcy]_{in}</td>
<td>Inflow conc of Hcy</td>
<td>Input variable, mol/ml</td>
<td>. . .</td>
</tr>
</tbody>
</table>

$V_{max}$, maximum velocity; SAH, S-adenosylhomocysteine; ec, endothelial cell; pc, parenchymal cell; Ado, adenosine; Hcy, homocysteine; PS, permeability--surface area product; trans, transudate; isf, interstitial fluid; conc, concentration.

*Values taken directly from published sources.
combined activity of the enzymes using the consumption parameter $G_e$. First-order rate constants such as $G_e$ (with units given as ml $\cdot$ g$^{-1} \cdot$ min$^{-1}$) are equivalent to $V_{ma}/K_m$ (with identical units) for tracer kinetics (concentration far below $K_m$). Since $G_e$ was the sum of the activities of adenosine kinase and adenosine deaminase, it was assumed that $V_e^{k}/K_e^{k}+V_{ed}^{d}/K_{ed}^{d}=25$ ml $\cdot$ g$^{-1} \cdot$ min$^{-1}$, based on the Wangler measurements, where the superscripts k and d indicate adenosine kinase and adenosine deaminase, respectively. Using $K_m$ values from the literature, the $V_{max}$ were constrained to agree with the expression. 6) It was assumed that the transudate region was part of the interstitial space and, therefore, that $V_{ist}+V_{trans}=0.12$ ml/g. 7) The production of adenosine from AMP in both the endothelial and parenchymal cell regions was constrained to be equal under all conditions, when production was expressed per milliliter of cell volume. 8) It was assumed that abluminal endothelial PS values were equal to the luminal values.

### III: Procedures for parameter optimization

The following measurements of adenosine and SAH in isolated guinea pig hearts were used for parameter optimization. Experimental conditions were modeled explicitly by setting the model inflow concentrations of adenosine and homocysteine, the flow rate, and the duration of the simulation equal to the values used in the experiments. Two studies were particularly useful, since they reported simultaneous measurements of venous adenosine release and tissue SAH content under six experimental conditions in the same preparation in the same laboratory (Deussen and colleagues14,15). Table 2 summarizes the measurements of Deussen and colleagues that were fit by the model and lists predicted adenosine concentrations and flux rates for each fit. Measurements in Table 2 fit within 20% are identified by an asterisk.

1: Adenosine release and SAH content under control conditions. Experimental measurements were obtained by Deussen and colleagues14,15 under steady-state control conditions using normoxic perfusate containing no exogenous homocysteine or adenosine at a flow rate of 5.0 ml $\cdot$ g$^{-1} \cdot$ min$^{-1}$. 14 Measurements of control steady-state SAH content (Table 2, column 1) provided constraints on the rate of myocardial transmethylation and the $V_{max}$ of SAH hydrolysis in the hydrolytic direction. If transmethylation was increased, then SAH content was overestimated. If the $V_{max}$ was increased, then SAH content was decreased. Measurements of venous adenosine release provided constraints on adenosine production rates and the parameters describing adenosine kinase in parenchymal and endothelial cells.

2: Adenosine release and SAH accumulation during infusion of homocysteine. The accumulation of SAH in isolated hearts was determined during perfusion with normoxic medium containing 400 $\mu$M l-homocysteine thiolactone for periods of 5, 10, 20, and 30 minutes at a flow rate of 5.5 ml $\cdot$ g$^{-1} \cdot$ min$^{-1}$. The results shown in Table 2, column 2, are the final values.
after 30 minutes. There were two kinetic requirements for fitting the measurements of SAH accumulation in the presence of excess homocysteine. The first was that SAH hydrolase is partially saturated by control SAH concentrations. The second requirement was that the ratio of the $V_{\text{max}}$ for the synthetic and hydrolytic directions of the SAH hydrolase reaction was approximately 100:1. Walker and Duerre reported that $V_{\text{max}}$ for SAH synthesis were 50–120 times greater than for SAH hydrolysis in studies of SAH hydrolase purified from a variety of organs (including heart) in rats, dogs, and rabbits. However, other studies including our own have shown similar rates. The cause for the discrepancy is not clear.

3: SAH ACCUMULATION DURING INFUSION OF ADENOSINE, HOMOCYSTEINE, AND ERYTHRO-9-(2-HYDROXY-3-NONYL) ADENINE (EHNA). Experimental measurements were obtained during increased rates of SAH formation in hearts perfused for 10 minutes with normoxic medium containing 1 mM l-homocysteine thiolactone and 10 $\mu$M (Table 2, column 3) or 100 $\mu$M (Table 2, column 4) adenosine, at a flow rate of 10 ml · g$^{-1}$ · min$^{-1}$ in the presence of 5 $\mu$M EHNA, a selective inhibitor of the enzyme adenosine deaminase. Because cytosolic concentrations of adenosine were estimated to be approximately 10 times higher than the $K_m$ for adenosine kinase and SAH hydrolase during 100 $\mu$M adenosine infusion, these measurements constrained estimates of the in vivo $V_{\text{max}}$ of the enzyme. Measurements during 10 $\mu$M adenosine infusion constrained parameters describing the endothelial barrier for adenosine.

4: ADENOSINE RELEASE AND SAH ACCUMULATION DURING INHIBITION OF SAH HYDROLASE AND INFUSION OF EHNA. Measurements during selective inhibition of SAH hydrolase were obtained by perfusion with normoxic medium containing the blocker adenosine dialdehyde (10 $\mu$M) in the presence of EHNA for 30 minutes at a flow rate of 10.5 ml · g$^{-1}$ · min$^{-1}$ (Table 2, column 5). Tissue SAH content was increased by adenosine dialdehyde sixfold over control levels because of ongoing transmethylation. The effects of adenosine dialdehyde were modeled on in vitro measurements showing that it increased the enzyme $K_m$ 31-fold. Although the model correctly described SAH accumulation under these conditions, the estimate of venous adenosine release was 2.1 times greater than the measured value. The error in this estimate was mostly due to a lower control adenosine release rate in this experimental group (38 pmol · g$^{-1}$ · min$^{-1}$) and to an underestimation in the decrease in adenosine release caused by adenosine dialdehyde. The fit could be improved if the model adenosine production rates were decreased to fit the control conditions of this experimental group. However, the standard production rates shown in Table 1 were not changed.

5: ADENOSINE RELEASE AND SAH ACCUMULATION DURING INFUSION OF HOMOCYSTEINE DURING HYPOXIA. SAH accumulation during hypoxia was determined by perfusion of hearts with medium equilibrated with 30% oxygen containing 1 mM l-homocysteine thiolactone for 5, 10, 20, and 30 minutes at a flow rate of 10.5 ml · g$^{-1}$ · min$^{-1}$. After 30 minutes, SAH content was elevated 10-fold compared with normoxic values, and adenosine release was elevated ninefold (compare columns 2 and 6, Table 2). Modeling these results provided constraints for estimating the rate of myocardial adenosine production, kinetic parameters for SAH hydrolase, and the parameters describing the endothelial cell barrier for adenosine.

6: ENDOTHELIAL INCORPORATION OF INFUSED TRACER ADENOSINE. Nees et al. reported that 80–90% of the sequestration of infused tracer adenosine was accounted for by capillary endothelial cells; this value was based on cell fractionation studies and autoradiography. The model solution describing the experimental conditions indicated that 80% of the total incorporation of infused tracer adenosine was accounted for by endothelial cell adenosine kinase. The selectivity of endothelial uptake was partly due to higher tracer concentrations in endothelial cells compared with cardiomyocytes. It was also due to a sevenfold higher activity of adenosine kinase in endothelial cells compared with cardiomyocytes; activity was expressed per milliliter cell volume.

7: EPICARDIAL TRANSUDATE AND VENOUS ADENOSINE CONCENTRATIONS. Simultaneous measurements of epicardial transudate and venous adenosine concentrations were obtained by Headrick et al. during normoxic control conditions at a flow rate of 9.6 ml · g$^{-1}$ · min$^{-1}$. Venous concentrations were 17 nM, and transudate concentrations were 150 nM. It was possible to model interstitial concentrations of 150 nM without a transudate region. However, to do so, it was necessary to increase cellular adenosine production to such a high value (18 nmol · g$^{-1}$ · min$^{-1}$) that the model greatly overestimated measurements of normal venous adenosine concentrations and SAH accumulation during excess homocysteine. Model solutions within 20% of normal values for all three variables were possible by modeling extracellular adenosine production in the interstitial and transudate regions at rates of 100 and 10 pmol · g$^{-1}$ · min$^{-1}$ (Table 1).

8: VENOUS, TRANSUDATE, AND INTERSTITIAL CONCENTRATIONS DURING INFUSION OF ADENOSINE. Mohrman and Heller reported simultaneous measurements of venous and transudate adenosine concentrations during intracoronary infusion of adenosine at a flow rate of 15 ml · g$^{-1}$ · min$^{-1}$. Their published findings and the model fits based on their experimental conditions are shown in Table 3. Table 3 includes estimates of interstitial concentrations. Parameter values were identical to those in Table 1. Inflow adenosine concentrations were set to equal those used in the experiments. The authors noted that only at an inflow concentration of 300 nM was the venous concentration lower than either the inflow or the transudate concentration. This qualitative behavior was also described by the model. These measurements provided constraints on the parameters describing the endothelial barrier for adenosine and parenchymal cell PS for adenosine (PS$_m$).

9: CONTRIBUTION OF ENDOTHELIAL CELLS TO VENOUS RELEASE OF ADENOSINE. Kroll et al. estimated that capillary endothelial cells contribute 14% of the total venous release of adenosine under control conditions. Modeling steady-state control conditions using the parameters listed in Table 1 indicated that the endothelial cell contribution was 15%. This measurement provided a constraint on the endothelial cell production rate of adenosine.
Sensitivity analysis. A sensitivity analysis was carried out to determine which parameters had the greatest influence on model solutions. Using the parameter values obtained in the first stage, test solutions were obtained by incrementing each parameter in turn by 10%. The differences between original and test solutions for adenosine concentrations and SAH contents were noted. One test solution was obtained for each model parameter. Links between parameters were removed, so that only one parameter was changed for each solution.

Second Stage of Analysis: Mechanism of Myocardial Adenosine Production

In the second stage of analysis, the parameter values listed in Table 1 were used to model measurements of venous adenosine release reported in completely independent studies of altered myocardial energetics in isolated perfused guinea pig hearts.20,23–25 To model these data, \( F_{cap} \) was set equal to the values reported for each experiment. The only parameter used to fit the measured venous release rates was the cardiomyocyte production rate of adenosine. Production rate was adjusted until the model solution for venous release exactly matched the measured value. The production rate obtained by this method was plotted against the reported free cytosolic AMP concentration in each experiment. The measurements of AMP played no role in the modeling. Production rates were estimated only by fitting the venous release measurements. All other parameter values were left unchanged, except for the endothelial cell production of adenosine, which was kept equal to the cardiomyocyte rate when expressed per milliliter of cell volume.

Results

Model fits of measurements of SAH accumulation. Most of the experimental results used for parameter optimization were fit by the model to within 20% accuracy. An example would be the fits to the time course of SAH accumulation in isolated hearts measured by Deussen et al14 shown in Figure 2. Symbols show measurements of SAH during perfusion with medium containing excess L-homocysteine thiolactone beginning at time = 0, under normoxic (solid symbols) and hypoxic (open symbols) conditions. Model solutions are shown by the continuous curves. The only differences between the two model solutions were the cellular adenosine production rates (20-fold higher for hypoxia solution), the flow rates, and the inflow homocysteine concentrations, which were set to measured values. The identical model solutions also fit the measured venous release rates of adenosine shown in Table 2.

Relation between venous and interstitial adenosine concentrations. Effect of cellular adenosine production. The model was used to estimate steady-state venous and interstitial adenosine concentrations when cellular adenosine production and flow rates were altered and the arterial concentration was zero. In Figure 3A, isoflow lines (along which production increases) slope upward linearly from the origin. Isoproduction lines (along which flow decreases) curve more steeply upward from the x axis. Increasing flow at a constant production rate decreases venous concentrations more than interstitial concentrations; i.e., washout of interstitial adenosine is minor. The figure may be used as a nomogram to estimate interstitial adenosine concentrations by drawing a vertical line from the intersection of a measured venous concentration (y axis) and the corresponding isoflow line. The model estimate of the corresponding myocardial adenosine production rate may be interpolated from the neighboring isoproduction lines. (Figure 3 enlargement is available by request.)

Effect of intracoronary adenosine infusion. The model was also used to estimate venous and interstitial adenosine concentrations when arterial adenosine concentrations and flow rates were altered and myocardial production was held constant (Figure 3B). Isoflow lines again radiate linearly from the vicinity of the origin, and isoconcentration lines (along which flow increases) sweep upward from the vicinity of the origin. Below flow

![Figure 2](image-url)
rates of approximately 1 ml·g⁻¹·min⁻¹, venous concentrations are largely independent of the arterial concentrations. Above 1 ml·g⁻¹·min⁻¹, increasing flow at a constant arterial concentration causes increases in both venous and interstitial adenosine concentrations. The figure may be used to estimate interstitial adenosine concentrations by drawing a vertical line from the intersection of corresponding isoflow and isoconcentration lines.

**Effect of flow rate on venous transport and metabolism.** Below flow rates of 2 ml·g⁻¹·min⁻¹, the effect of flow on the relation between interstitial and venous concentrations shown in Figure 3A became much less pronounced. Consequently, the following unexpected prediction emerges. When flow is decreased below 2 ml·g⁻¹·min⁻¹, the calculated venous release rate (venous concentration×flow rate) decreases if cellular production rate remains constant. If the metabolism of adenosine were abolished, then the venous release rate would remain constant. To clarify the effect of flow rate on the venous release of adenosine, model solutions were obtained for flow rates between 0.125 and 8 ml·g⁻¹·min⁻¹. When cellular production rate was held constant (Figure 4, solid curve), the model solution showed a striking decrease in release rate as flow was decreased.

\[
S_0^A = S_0^A \times e^{\frac{1}{\alpha}}
\]

where \(S_0^A\) and \(F_0\) denote adenosine production (1.6 nmol·g⁻¹·min⁻¹) and coronary flow (8 ml·g⁻¹·min⁻¹) at the highest flow, \(S_0^A\) and \(F_0\) are production and flow as flow was reduced, and \(\alpha\) is a coefficient determining the steepness of the function. For the steepest function (\(\alpha = 2.5\)), adenosine production increased 244-fold over control levels at the lowest flow. Parenchymal and endothelial cell adenosine production rates were maintained in the ratio 20.5:1 to model equal rates, expressed per milliliter volume. Model parameter values are listed in Table 1. Arterial concentrations were equal to zero. The results predict that venous release of adenosine may decrease as flow is reduced, even though cellular production increases.

**FIGURE 4.** Graph showing the model estimate of the effect of flow rate on venous release rate (venous concentration×flow) of adenosine. Main panel shows one solution for constant cellular adenosine production (solid curve) and three solutions in which cytosolic adenosine production was increased as flow was decreased. Inset shows the functions used to define the increases in adenosine production as flow was decreased, calculated from the expression:

\[
F_n = \frac{\log(F_0)}{\alpha}\\
S_n^A = S_0^A \times e^{\frac{1}{\alpha}}
\]

The model solutions are used to show that the release rate decreases as flow is reduced.
To describe the relation between flow and adenosine production in a heart more realistically, a series of curves was used to describe increasing cellular adenosine production rates as flow was lowered (Figure 4, inset). These curves were used to produce the other three solutions in Figure 4 to show the qualitative effect of increasing adenosine production as flow was lowered. Only for the solution in which adenosine production was increased most steeply, 244-fold at the lowest flow rate (dotted curve), did venous release rate increase continuously. Results in Figure 4 show that venous release of adenosine may decrease, even though cellular production increases.

The reason for the marked effect of flow rate on adenosine release is clarified by observing the concentration gradients along the length of the capillary (Figure 5). At a flow rate of 16 ml·g\(^{-1}\)·min\(^{-1}\) (top panel), the endothelial cell concentration was higher than that in the capillary along the entire length of the capillary because of endothelial cell production of adenosine. The arrows indicate the direction of the concentration gradient for adenosine diffusion. The endothelial cell was a source for adenosine, and the capillary concentration increased continuously from inflow (at the left) to outflow, without reaching a stable level. At a lower flow rate of 4 ml·g\(^{-1}\)·min\(^{-1}\) (middle panel), endothelial cells were a source at the inflow end of the capillary and a sink at the outflow. The capillary concentration still did not reach a stable level. At the lowest flow rate of 0.1 ml·g\(^{-1}\)·min\(^{-1}\) (bottom panel), the endothelial cell was a sink along most of the length of the capillary. The capillary concentration rose to a high stable level near the inflow. The source for adenosine was cardiomyocyte and extracellular production. At a flow rate of 2 ml·g\(^{-1}\)·min\(^{-1}\) (not shown), the outflow concentration was nearly the same, but it was only reached at the end of the capillary. Calculated venous release of adenosine was 20-fold higher at a flow rate of 2 ml·g\(^{-1}\)·min\(^{-1}\) compared with 0.1 ml·g\(^{-1}\)·min\(^{-1}\), although adenosine production rate was identical. It should be noted that adenosine production rates were unchanged in the three panels of Figure 5.

**Flow heterogeneity.** To account for the normal fivefold range in regional flow, the model included 10 parallel capillary tissue exchange units, identical except for their flow, an approach used by Wangler et al. Adenosine production was assumed to be equal in all flow pathways. The effect of flow heterogeneity was to decrease total venous release by approximately 5% compared with conditions of uniform flow distribution. The effect of heterogeneity was due to reduced venous release of adenosine in low-flow pathways. Flow heterogeneity had a negligible effect on SAH accumulation.

**Compartmental model.** Solutions of the compartmental and distributed models were compared for cytosolic adenosine concentration and SAH accumulation in the presence of exogenous homocysteine, using identical parameter values for the cardiomyocyte region and modeling the vascular concentration of homocysteine as a step function. Solutions of the compartmental model, which does not describe flow, were within approximately 1% of solutions of the distributed model for flow rates above 1 ml·g\(^{-1}\)·min\(^{-1}\). However, at a flow rate of 0.1 ml·g\(^{-1}\)·min\(^{-1}\), SAH accumulation was decreased by 10% in the distributed model and by 60% at a flow rate of 0.01 ml·g\(^{-1}\)·min\(^{-1}\). The decreases, not accounted for by the compartmental model, were due to effects of flow on homocysteine availability; adenosine production was not changed. Because of limitations in the compartmental model, it is inappropriate for modeling conditions where arteriovenous concentration gradients or endothelial cell metabolism are important.

**Parameter sensitivity analysis.** The relative importance of model parameters was determined by incrementing each parameter in turn by 10%. All parameters causing more than a 5% change in the solutions are listed in Table 4, together with the output variable exhibiting the change and the magnitude of the change (in percent of control value). Fifteen other parameters caused less than a 1% change in any output variable. A comparison was made of the relative responses of three experimental indexes to a 10% increase in cardiomyocyte adeno-

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**Figure 5.** Model predictions of adenosine concentration gradients along the length of the capillary at three flow rates (16 [top panel], 4 [middle panel], and 0.1 [bottom panel] ml·g\(^{-1}\)·min\(^{-1}\)) with constant adenosine production. Concentration gradients are shown for capillary (cap, dashed lines) and endothelial cells (endo, solid lines). Model parameter values are listed in Table 1. Arrows indicate the direction of the diffusion gradient between capillary and endothelial cells. At high flow rate (top panel), endothelial cells function as a source for adenosine, whereas at a low flow rate (bottom panel), endothelial cells function as a sink for adenosine. Between flow rates of 2 and 0.1 ml·g\(^{-1}\)·min\(^{-1}\), nearly identical capillary outflow concentrations are reached but at different points along the capillary. As flow is increased in this range, the steady-state concentration is reached nearer to the outflow end of the capillary.
TABLE 4. Sensitivity Analysis: Model Parameters With the Greatest Influence on Solutions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect of 10% increment in parameter value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{pc}$</td>
<td>$q_{st}^a + 9.5%$</td>
</tr>
<tr>
<td>$V_{cap}$</td>
<td>$[A]_{venous} - 9.1%$</td>
</tr>
<tr>
<td>$S_{trans}$</td>
<td>$[A]_{trans} + 8.6%$</td>
</tr>
<tr>
<td>$S_{pc}$</td>
<td>$q_{st}^a + 8.5%$</td>
</tr>
<tr>
<td>$K^a$</td>
<td>$[A]_{i} + 8.4%$</td>
</tr>
<tr>
<td>$S_{pc}$</td>
<td>$q_{st}^a + 8.3%$</td>
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<td>$V_{hyd}$</td>
<td>$q_{st}^a - 8.1%$</td>
</tr>
<tr>
<td>$V_{pc}$</td>
<td>$[A]_{i} - 7.9%$</td>
</tr>
<tr>
<td>$PS_{trans}$</td>
<td>$[A]_{trans} - 7.7%$</td>
</tr>
<tr>
<td>$V_{vc}$</td>
<td>$[A]_{i} - 5.5%$</td>
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<td>$K_{ec}$</td>
<td>$q_{st}^a + 5.5%$</td>
</tr>
<tr>
<td>$S_{st}$</td>
<td>$[A]_{i} + 5.4$</td>
</tr>
</tbody>
</table>

$V_{pc}$, % volume of parenchymal cell (pc); $q_{st}^a$, steady-state $S$-adenosylhomocysteine (SAH) content in the absence of homocysteine in the inflow; $V_{cap}$, volume of capillary; $[A]_{venous}$, adenosine concentration ([A]) in the model outflow; $S_{trans}$, rate of adenosine formation in the transudate region (trans); $S_{pc}$, rate of cardiomyocyte transmethylation; $K^a$, $K_n$ for adenosine kinase; $S_{pc}^a$, rate of cardiomyocyte adenosine formation from AMP; $q_{st}^a$, SAH accumulation after 30 minutes excess homocysteine in the inflow; $V_{hyd}$, $V_{max}$ for SAH hydrolysis in pc; $V_{pc}^{}\text{c}$, $V_{max}$ for adenosine kinase in pc; $PS_{trans}^{}\text{c}$, permeability/surface area product of trans for adenosine; $V_{vc}^{}\text{c}$, $V_{max}$ for adenosine kinase in endothelial cell (ec); $K_{ec}^{a}$, $K_n$ of SAH hydrolyase for SAH in pc; $S_{st}^{a}$, rate of adenosine formation in interstitial fluid region (stf).

sine production. The predicted response was greatest for SAH accumulation (+4.7%), followed by venous concentration (+2%), while the transudate concentration showed the smallest change (+0.6%).

Second Stage of Analysis: Estimation of Cellular Adenosine Production From Venous Release Measurements

Data from four studies that reported measurements of free cytosolic AMP concentrations and venous release rates of adenosine during altered myocardial energetics are shown as published in Figure 6A. In the studies using underperfusion to alter myocardial energetics, adenosine release was decreased at the highest AMP concentrations (corresponding to the lowest flow rates). However, when hypoxia and substrate reductions were used to alter energetics, adenosine release increased continuously with AMP concentration (Figure 6A).

Model estimates of cellular adenosine production rates, obtained by fitting the venous release data in Figure 6A, increased monotonically with AMP concentrations under all conditions (Figure 6B). During ischemia, myocardial adenosine production increased at the same time venous release decreased. The AMP concentrations in Figure 6 were taken directly from the original sources and are identical in both panels. The AMP concentrations were not used in the analysis. Model parameter values were identical to those obtained in the first stage of analysis.

Discussion

Adenosine Concentrations in Epicardial Transudate Are Not in Equilibrium With Interstitial Fluid

Adenosine concentrations in fluid collected from the epicardial surface of the normoxic heart lie in the range of 70–500 nM and have been hypothesized to represent concentrations in interstitial fluid. Results of the present study do not agree with this hypothesis. Intertitial adenosine concentrations in the range of 150–200 nM were associated with hypoxia in the model analysis. If interstitial concentrations were raised to these levels by increasing adenosine production in the model, SAH accumulation in the presence of excess homocysteine and venous adenosine concentrations in the normoxic heart were overestimated. A possible explanation is that adenosine in epicardial transudate fluid is produced in an extracellular tissue region that is separated from bulk interstitial fluid by an extended diffusion distance.

The following experimental evidence supports this possibility. 1) Headrick et al. presented data showing a linear relation between venous and transudate concen-
trations of adenosine with a nonzero intercept at a transudate concentration of 200 nM (Figure 3 of Reference 33). The model also predicted a linear relation between venous and transudate concentrations (Figure 7). However, nonzero intercepts could only be modeled by including adenosine production in the transudate region (\( S^{\text{trans}} \)). When \( S^{\text{trans}} = 0 \), interstitial and transudate concentrations were equal, and the extrapolated intercept was at the origin. 2) Imai et al.\(^{44} \) showed that chemical sympathectomy using 6-hydroxydopamine in guinea pigs caused reductions in extracellular transudate adenosine concentrations from 68 to 18 nM. In the present study, normal interstitial adenosine concentrations were estimated to be 23 nM. These findings suggest that adrenergic nerves may be responsible for adenosine production in the transudate region. In the Imai study, blocking extracellular AMP hydrolysis decreased transudate adenosine concentrations to 11 nM and increased AMP concentrations over 10-fold. In the present study, interstitial adenosine concentrations modeled in the absence of extracellular adenosine production were 9 nM. These findings suggest that adrenergic nerves produce adenosine by an extracellular mechanism. 3) Anatomic\(^{49} \) and functional\(^{140,41} \) studies showed an abundance of adrenergic nerves in superficial subepicardial and subendocardial layers. A subendocardial plexus is particularly well developed.\(^{39} \) 4) Bünger et al.\(^{42} \) reported that the extracellular production of adenosine is independent of the myocardial energetic state. Based on these findings, it is hypothesized that much of the adenosine normally found in epicardial transudate comes from the extracellular hydrolysis of adenine nucleotides released from adrenergic nerves in a superficial layer beneath the epicardial surface. The hypothesis predicts that adenosine concentrations in endocardial transudate may be even higher than in epicardial transudate. The hypothesis is consistent with a similar hypothesis presented by Nakazawa et al.\(^{44} \) and underscores reservations voiced by Kusachi and Olsson\(^{44} \) regarding the assumption of equilibrium between interstitial and epicardial superfusate adenosine concentrations.

The above hypothesis is based on the assumption that the extracellular transport of adenosine between capillary and interstitial regions is similar to that of extra-

\[
\text{Adenosine Production Between Venous and Epicardial Transudate Concentrations.}
\]

When adenosine production in the extracellular transudate region was zero (\( S^{\text{trans}} = 0 \)), concentrations in interstitial and transudate regions were equal. However, the intercept of transudate and venous concentrations was at the origin, not at 150–200 nM as reported by Headrick et al.\(^{33} \) When adenosine production in the transudate region was made equal to 1% of total myocardial production (\( S^{\text{trans}} = 10 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \)), solutions were displaced to the right, introducing a nonzero intercept at approximately 140 nM and a gradient between interstitial and transudate concentrations.

![Graph showing model estimate of the relations between venous and epicardial transudate adenosine concentrations.](http://circres.ahajournals.org/)

**FIGURE 7.** Graph showing model estimate of the relations between venous and epicardial transudate adenosine concentrations. When adenosine production in the extracellular transudate region was zero (\( S^{\text{trans}} = 0 \)), concentrations in interstitial and transudate regions were equal. However, the intercept of transudate and venous concentrations was at the origin, not at 150–200 nM as reported by Headrick et al.\(^{33} \) When adenosine production in the transudate region was equal to 1% of total myocardial production (\( S^{\text{trans}} = 10 \text{ pmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \)), solutions were displaced to the right, introducing a nonzero intercept at approximately 140 nM and a gradient between interstitial and transudate concentrations.

### Relation Between Cytosolic AMP Concentration and Myocardial Adenosine Production

Four studies investigated the mechanism of myocardial adenosine production by measuring free cytosolic AMP concentrations and the rate of venous adenosine release, as an index of cellular production, during altered myocardial energetics.\(^{20,23–25} \) In the two studies using coronary underperfusion to alter energetics by Headrick et al.\(^{20} \) and He et al.\(^{23} \) venous release of adenosine decreased at the highest AMP concentrations. However, this effect was not observed in experiments using hypoxic perfusion by He et al.\(^{24} \) and substrate deprivation by Kang et al.\(^{25} \) even though cytosolic energetics were decreased similarly. A limitation in the studies is that measuring venous adenosine release rate as an index of myocardial production rate did not account for the effects of flow or the endothelial metabolic barrier for adenosine.\(^{19} \)

To overcome this limitation, the measurements of venous adenosine release were analyzed with the model to estimate rates of cellular adenosine production. The model parameter values were kept at values determined independently in the first stage of analysis, and only flow and cellular production rate were altered. The predicted relation between myocardial adenosine production and cytosolic AMP concentration indicates that the rate of AMP hydrolysis (adenosine production) increases monotonically with AMP concentration. The finding of decreased venous release at the highest AMP concentrations during underperfusion can be explained by the combined effects of reduced flow rate and endothelial metabolism of adenosine. It is not necessary to invoke allosteric inhibition of \( S' \)-nucleotidase, as suggested by He et al.\(^{20} \)
The above findings support the concept that cellular adenosine production is closely linked to myocardial energetics. This is because concentrations of AMP are closely coupled to those of ADP, ATP, and creatine phosphate by the creatine kinase and myokinase reactions. Therefore, global myocardial energetics may be assessed by a complete analysis of coronary venous adenosine and the flow rate. However, under conditions of coronary underperfusion, myocardial energetics are not uniform. The subendocardial layer often exhibits the most severe ischemia, giving rise to a transmural gradient. Regional myocardial energetics can be assessed using the SAH technique to obtain spatial resolution. The parameter sensitivity analysis indicated that a given change in cellular production produces a larger change in SAH accumulation than in venous adenosine. The high spatial resolution of the SAH technique may complement 31P nuclear magnetic resonance spectroscopy, which provides information on energetics with high temporal resolution, but more limited spatial resolution.

Relative Rates of Cytosolic AMP Hydrolysis and Transmethylation

Lloyd et al. presented evidence that during normoxia most myocardial adenosine is formed via the transmethylation pathway rather than via AMP hydrolysis. The evidence was based on the dilution of specific activity of the SAH pool in the guinea pig heart, prelabeled with radioactive adenosine. It was not possible to reconcile this finding with the analysis of findings of Deussen et al. on the rate of SAH accumulation in the presence of a blocker of SAH hydrolase (Table 2, column 5). In the present study, transmethylation was estimated to be one fifth the rate of AMP hydrolysis. The analysis accounted for product inhibition of SAH on transmethylation but not for the increase in S-adenosylhomocysteine caused by adenosine dialdehyde. Consequently, the normal rate of transmethylation may be underestimated in the present study.

Parameter Sensitivities

The parameter sensitivity analysis identified the most important parameters in the model. These included region volumes, kinetic parameters for adenosine kinase and SAH hydrolase, and adenosine production rates. Volumes and adenosine kinase parameters were rather well constrained by morphometric studies and in vitro enzyme assays. The parameters to which the most interest is attached, adenosine production rates, are among the most influential in the model. High sensitivity is essential if they are to be identified by fitting experimental measurements. Because adenosine production in both parenchymal cells and the interstitial region influenced venous concentrations, it may not be possible to distinguish between them on the basis of venous measurements alone. They may be distinguished by additional measurements of SAH accumulation in the presence of excess homocysteine, which is more sensitive to cytosolic adenosine than interstitial adenosine. A test was made of model sensitivity to the possibility that adenosine deaminase is absent in cardiomyocytes by setting Vm,a=0. The largest effect was a 17% increase in cytosolic adenosine concentration in the modeling of hypoxia.

Strengths and Weaknesses of the Model

To assess the role of adenosine as a physiological regulator and energetic marker in the heart, it is of paramount importance to estimate interstitial concentrations of adenosine and myocardial production rates. Because these variables cannot be directly measured, venous concentrations have usually been used as an index. The present model makes it possible to overcome serious weaknesses in the index by accounting for the effects of capillary transport and cellular metabolism of adenosine in a comprehensive and physically realistic manner. A major advantage of the present model is that it is based on a large number of the key experimental observations on adenosine in the heart. The ability of the model to resolve the inconsistency regarding the mechanism of myocardial adenosine production shown in Figure 6 demonstrates the utility of model analysis of adenosine measurements. An additional strength of the model is that it makes it possible to assess in vivo enzyme kinetic behavior. The true power of this approach requires that the design of experiments be developed with the aid of the model, to optimize the sensitivity of the measurements.

The model involves a number of limitations. 1) The model does not describe extracellular adenosine deaminase, the regulation of extracellular production of adenosine, or adenosine uptake and metabolism in the transmural region. Although these processes must occur, their role under normal conditions may be minor. To extend the model to include these processes, detailed measurements of extracellular production and metabolism of adenosine will be necessary. 2) The parenchymal cell Vm,a for adenosine kinase is not well-defined experimentally. However, the Vm,a and cellular adenosine production rate are the key determinants of steady-state cytosolic concentration of adenosine. Therefore, the production/Vm,a ratio is constrained by the measurements of SAH accumulation in the presence of excess homocysteine. There is less uncertainty in the above ratio than in the absolute value of myocardial adenosine production. 3) Another limitation is the problem of assigning specific values to the kinetic parameters for SAH hydrolase. The best fits to all the experimental observations in the present study were obtained with K values for adenosine, 150 μM for homocysteine, and 3.75 μM for SAH, whereas reported values for SAH hydrolase from guinea pig heart were 1.3 μM for adenosine, 260 μM for homocysteine, and 2.9 μM for SAH. The model solutions for SAH content in the absence and presence of excess homocysteine were decreased by 21% and 10%, respectively, if the reported parameter values were used directly. If, in addition, the Vm,a for the enzyme in the hydrolytic direction was adjusted to conform to the constraint imposed by the Haldane equation, then the solutions were increased by 35% and 1%, respectively. 4) Region volumes were taken from studies of normal hearts, which may not be appropriate for buffer-perfused hearts, since such hearts may exhibit considerable edema. The sensitivity analysis indicated that increasing the interstitial volume by 10% did not change any model solutions by more than 0.1%. However, if the parenchymal cell volume was reduced by 0.2 ml/g, to make up for a possible 0.2 ml/g increase in the interstitial volume,
then the estimate of steady-state tissue SAH content in the absence of homocysteine in the inflow would be decreased by approximately 30%. The estimate of SAH accumulation in the presence of excess homocysteine would be decreased by 17%, and the estimates of adenosine concentrations would be changed by less than 0.01%. 5) Another limitation is that the model does not describe the effects of high-energy phosphate compounds on cytosolic 5'-nucleotidase, substrate inhibition of adenosine kinase by adenosine, or effects of adenine and adenosine nucleotides on SAH hydrolysis. The concentration ranges over which these effects are expected generally lie outside the physiological range. 6) The model does not explicitly describe inosine or AMP. 7) Finally, because the model was based on measurements obtained in guinea pig hearts, if it is applied to hearts of other species, inaccuracies are possible.

In summary, the present study provides comprehensive descriptions of the expected effects of the major pathways of transport and metabolism of adenosine in the heart. Improved estimates of interstitial adenosine concentrations and myocardial adenosine production rates were determined by accounting for the effects of flow and endothelial metabolism. The results are presented in the form of a nomogram to estimate these variables graphically. It is concluded that, although epicardial transudate and interstitial adenosine concentrations change in parallel, they are not in equilibrium, and localized adenosine production makes transudate measurements insensitive for physiological changes in interstitial adenosine. It is also concluded that cellular adenosine production is directly related to free cytosolic concentrations of AMP. Therefore, the SAH technique may provide spatially resolved information on myocardial energetics.

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