Quantification of Extracellular and Intracellular Adenosine Production
Understanding the Transmembranous Concentration Gradient
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Background—Inhibitors of adenosine membrane transport cause vasodilation and enhance the plasma adenosine concentration. However, it is unclear why the plasma adenosine concentration rises rather than falls when membrane transport is inhibited. We tested the hypothesis that the cytosolic adenosine concentration exceeds the interstitial concentration under well-oxygenated conditions.

Methods and Results—In isolated, isovolumically working guinea pig hearts (n=50), the release rate of adenosine and accumulation of S-adenosylhomocysteine (after 20 minutes of 200 μmol/L homocysteine), a measure of the free cytosolic adenosine concentration, were determined in the absence and presence of specific and powerful blockers of adenosine membrane transport (nitrobenzylthioinosine 1 μmol/L), adenosine deaminase (erythro-9-hydroxy-nonyl-adenine 5 μmol/L), and adenosine kinase (iodotubericidine 10 μmol/L). Data analysis with a distributed multicompartment model revealed a total cardiac adenosine production rate of 2294 pmol min⁻¹ g⁻¹, of which 8% was produced in the extracellular region. Because of a high rate of intracellular metabolism, however, 70.3% of extracellularly produced adenosine was taken up into cellular regions, an effect that was effectively eliminated by membrane transport block. The resulting 2.8-fold increase of the interstitial adenosine concentration evoked near-maximal coronary dilation.

Conclusions—We rejected the hypothesis that the cytosolic adenosine concentration exceeds the interstitial. Rather, there is significant extracellular production, and the parenchymal cell represents a sink, not a source, for adenosine under well-oxygenated conditions. (Circulation. 1999;99:2041-2047.)

Key Words: adenosine □ blood flow □ inosine □ S-adenosylhomocysteine

Adenosine serves several important functions in the heart that are mediated via stimulation of cell surface receptors. However, because of rapid metabolism and a high degree of compartmentalization, the precise interstitial adenosine concentration that evokes these effects is unknown. Traditionally, it is assumed that the major site of adenosine production is the cytosol, from which the nucleoside diffuses into the interstitial space to finally reach the vascular region, from which a small fraction is washed out with the coronary flow. This concept, however, cannot explain the effects of membrane transport blockers, which increase the vascular adenosine concentration. An explanation for this discrepancy could be that these blockers inhibit only inward membrane transport in the heart. Alternatively, enhanced vascular concentrations seem possible if net uptake of adenosine supplied by extracellular dephosphorylation of adenine nucleotides is inhibited. A previous limitation in discerning between these alternative explanations has been the inability to measure the adenosine concentration on both sides of the membrane. However, because of the invention of the S-adenosylhomocysteine (SAH) technique and formulation of a related comprehensive mathematical model, it has become possible to quantify cytosolic adenosine concentrations. The present study was designed to determine the adenosine concentrations for the different tissue regions and thereby to assess the net transmembranous concentration gradient. Therefore, in a first experimental stage, we determined the coronary venous adenosine concentration as a measure of the intracellular adenosine concentration. Measurements were made before and after blocking of the facilitative membrane transporter in the absence and presence of blockers of adenosine metabolism. In a second stage, the experimental results were analyzed with a mathematical model to calculate the adenosine production of different tissue regions and predict net transport between regions. The interstitial adenosine concentration was related to the measured changes of coronary flow.
Methods

Isolated Heart Experiments
Hearts from 50 guinea pigs (body mass, 250 to 350 g) were perfused with a modified Krebs buffer (perfusion pressure, 70 cm H2O; arterial Po2, >520 mm Hg; 37°C). Coronary flow (electromagnetic probe), perfusion pressure, isovolumic left ventricular pressure development, dP/dt, and heart rate (strain-gauge pressure transducers) were measured continuously. Hearts were included in the study only if (1) the maximum flow overshoot after a 20-second flow stop was at least twice the flow before occlusion, (2) spontaneous heart rate exceeded 210 bpm, and (3) left ventricular developed pressure exceeded 50 mm Hg. In 9 hearts, oxygen consumption was determined according to the Fick principle. For measurement of cardiac adenosine and inosine, release of the coronary effluent perfusate was collected over 1 to 2 minutes. Individual pathways of adenosine metabolism or membrane transport were inhibited by use of 5 μmol/L erythro-9-hydroxy-nonyl-adenine (EHNA) for adenosine deaminase, 10 μmol/L iodotubericidine (ITU) for adenosine kinase,5,11 and 1 μmol/L nitrobenzylthioinosine (NBTI), dipyridamole (DIP), or drafllarine (DRA) for membrane transport.12 Blockers dissolved in physiological saline (except NBTI, which was dissolved in dimethylsulfoxide) were infused at rates of 0.1% (NBTI) or 1% (all others) of coronary flow. EHNA, ITU, and NBTI were from RBI; DIP was from Thomae Pharmaceuticals; and DRA was from Janssen Pharmaceuticals.

Rationale and Experimental Protocols

Global Cardiac Adenosine Production
Adenosine is metabolized via adenosine kinase and adenosine deaminase. During complete inhibition of these enzymes, the venous release rate of adenosine represents a minimum estimate of the global production rate. This approach rests on the assumption that a steady-state cardiac adenosine concentration is reached during enzyme inhibition.

Intracellular Versus Extracellular Adenosine Production
During continuous inhibition of adenosine kinase and adenosine deaminase, membrane transport is blocked. If adenosine is produced mainly in the cytosol, then membrane transport block should decrease net cellular adenosine release and consequently venous release. If, however, adenosine production is mainly extracellular, no decrease but possibly even an increase of venous adenosine release is to be expected.

Intracellular adenosine production rate may also be estimated from tissue SAH content. During inhibition of adenosine metabolism and transport, 1-homocysteine thiolactone was infused (intracoronary concentration, 200 μmol/L; 20 minutes) to drive the SAH-hydrolase reaction toward net production of SAH.9 The SAH content after homocysteine infusion represents the integral of the net reaction from adenosine to SAH, serving as an independent estimate of the minimum intracellular adenosine production rate. In control experiments, the SAH content was measured after 20 minutes of homocysteine alone. The implicit assumption in these experiments is that homocysteine thiolactone enters the cells by a pathway separate from that used by adenosine and that this process is unaffected by any of the blockers of transport or metabolism.

Transmembranous Concentration Gradient
The effects of adenosine transport blockers on adenosine release are quantified under control conditions. If the direction of the adenosine concentration gradient is from intracellular to extracellular, then inhibition of adenosine membrane transport should decrease the rate of adenosine release into the coronary venous perfusate. However, if adenosine is produced in the extracellular region and diffuses into the cytosol to be further metabolized, then adenosine release should rise under this intervention. In addition, SAH content was determined after 20 minutes of infusion of 1-homocysteine thiolactone 200 μmol/L without and with block of adenosine membrane transport.

Adenosine Deaminase Experiments
To test whether coronary flow changes were induced by adenosine, purified adenosine deaminase (calf intestinal mucosa (Boehringer Mannheim) was used. In control experiments, adenosine deaminase was infused during coronary dilation with bradykinin 0.1 μmol/L.

Sample Processing and Analytical Determinations
For measurement of adenosine and inosine, coronary effluent samples were desalted and concentrated with C-18 cartridges.4 For measurement of tissue SAH content, hearts were freeze-clamped after 20 minutes of homocysteine infusion and freeze-dried. Left ventricular samples were homogenized in 0.5 mol/L perchloric acid.9 Compounds were quantified in 50- to 100-fold–concentrated samples of effluent coronary perfusate or tissue homogenate (≈0.1 g tissue dry mass/ml final extract) by high-performance liquid chromatographic analysis.9 Data reported are corrected for analytical losses.

Statistics
Data are reported as mean±SD; significance levels are 2-sided. The significance of differences among different experimental groups was determined by use of 1-way ANOVA and post hoc t tests with Bonferroni correction. Within-subject differences of purine release and flow during additive blocking protocols were assessed with ANOVA for repeated measures. If significant intra-individual effects were discovered, conditions were tested against the next preceding condition by Wilcoxon matched-pairs rank test. Linear correlation coefficients were calculated from the individual data points.

Model Analysis
The axially distributed multicompartment model of adenosine transport and metabolism has been described in detail and validated against experimental data.10 As a starting point for the present analyses, the published parameter set was used.10 The general modeling strategy was to fit measured venous adenosine concentrations by constraining the flow term by the measurements and using adenosine production terms as free parameters. To obtain parameter values that fitted the venous concentrations determined under various experimental conditions equally well, several iterations of parameter adjustment were necessary. Once this parameter set had been obtained, it was used to fit the SAH contents without any further adjustment.

Results

Experimental Measurements

Cardiac Dynamics and Oxygen Consumption
Control left ventricular pressure development was 84±15 mm Hg, left ventricular dp/dtmax, 1440±330 mm Hg/s, heart rate 249±24 bpm, and coronary flow 6.7±1.2 mL/min·g−1 (n=44). Arterial and coronary venous Po2 were 577±41 and 202±30 mm Hg, respectively, and myocardial oxygen consumption was 75±15 μL·min−1·g−1 (n=9). The functional parameters, except flow and heart rate, were unaffected by the different enzyme or transport inhibitors. Changes in oxygen consumption after possible bradycardia during inhibition of adenosine kinase or membrane transport were prevented by pacing.

Global Cardiac Adenosine Production
In 14 hearts, control adenosine release was 53±24 pmol·min−1·g−1, inosine release 489±239 pmol·min−1·g−1, and coronary flow 6.7±1.2 mL·min−1·g−1. During EHNA, adenosine release was 91±53 pmol·min−1·g−1 (P=0.003 versus control), inosine release 416±235 pmol·min−1·g−1.
(P=0.046 versus control), and flow 6.7±1.3 mL · min⁻¹ · g⁻¹ (P=0.422 versus control). During EHNA plus ITU, adenosine release was 1511±406 pmol · min⁻¹ · g⁻¹ (P=0.001 versus EHNA), inosine release 475±231 pmol · min⁻¹ · g⁻¹ (P=0.213 versus EHNA), and flow 14.3±3.1 mL · min⁻¹ · g⁻¹ (P=0.001 versus EHNA). Results are shown for the NBTI subgroup in Figure 1.

**Intracellular and Extracellular Adenosine Production**

As shown in Figure 1, additional infusion of NBTI during EHNA plus ITU decreased adenosine release from 1744±518 to 297±106 pmol · min⁻¹ · g⁻¹ (P=0.028). Coronary flow remained unaffected (12.8±3.5 versus 13.1±4.0 mL · min⁻¹ · g⁻¹). Inosine release was 649±295 pmol · min⁻¹ · g⁻¹ before and 584±225 pmol · min⁻¹ · g⁻¹ during additional infusion of NBTI (P=NS). Results similar to those with NBTI (n=6) were obtained with DIP (n=8). During inhibition of adenosine deaminase and adenosine kinase, DIP decreased adenosine release from 1337±183 to 429±135 pmol · min⁻¹ · g⁻¹ (P=0.012).

Control tissue contents of adenosine, inosine, and SAH were 1.6±0.7, 1.8±0.5, and 1.5±0.5 nmol/g, respectively (Figure 2). After 20 minutes of homocysteine (n=7), SAH content was 7.9±1.6 nmol/g (P<0.05 versus control). Tissue contents of adenosine and inosine remained unchanged. During treatment with EHNA plus ITU plus NBTI (n=6), the SAH content after 20 minutes of homocysteine was 23.7±2.8 nmol/g (P<0.05 versus homocysteine). Similarly, the SAH content was 17.5±5.9 nmol/g during EHNA plus ITU plus DIP treatment after 20 minutes of homocysteine (P>0.05 versus homocysteine, P<0.05 versus EHNA plus ITU plus NBTI), during treatment with EHNA plus ITU plus NBTI plus homocysteine, tissue adenosine and inosine contents were 3.9±1.1 and 14.6±5.0 nmol/g, respectively (both P<0.05 versus homocysteine). Tissue inosine content correlated linearly with duration (20 to 65 minutes) of membrane transport inhibition (r=0.886, P=0.0001, n=19). Twenty minutes of NBTI increased the tissue inosine content from 1.4±0.5 to 14.6±5.0 nmol/g and decreased the coronary venous inosine concentration from 97±72 to 50±28 nmol/L (n=6). The tissue inosine content (nmol/g) was converted into a concentration (μmol/L), assuming that tissue inosine was restricted to intracellular regions, which constitute 0.60 mL/g tissue. The respective tissue/venous perfusate concentration ratio increased from 24.5 (control) to 488 after 20 minutes of NBTI (Figure 3).

**Transmembranous Concentration Gradient**

In 16 hearts, control cardiac adenosine release was 46±22 pmol · min⁻¹ · g⁻¹ and coronary flow 6.6±1.3 mL · min⁻¹ · g⁻¹ (Figure 4). NBTI enhanced adenosine release to 179±72 pmol · min⁻¹ · g⁻¹ and flow to 12.4±3.1 mL · min⁻¹ · g⁻¹ (both P=0.008). Similar effects on adenosine release and flow were obtained with DIP and DRA. Infusion of homocysteine resulted in SAH contents of 8.5±1.1 nmol/g during NBTI plus EHNA (n=5), 7.7±2.0 nmol/g during DIP plus EHNA (n=4), and 9.3±2.1 nmol/g during EHNA alone (n=3). These concentrations were not significantly different from each other.

**Adenosine Deaminase Experiments**

NBTI (n=2) or DIP (n=2) increased coronary flow 113% to 149% above control levels. Additional infusion of adenosine deaminase (10 U/mL) blunted this response (flow, 0% to 23%
above control), and adenosine was undetectable in the coronary venous perfusate. During bradykinin infusion (0.1 μmol/L), flow rose by 90% to 136% (n=2) and remained elevated (86% to 124% above control) during additional infusion of adenosine deaminase.

Model Calculations
Starting with a previously published parameter set, the effects of transport inhibition were simulated by setting the parameter values of the membrane permeability–surface area products to 2% of the starting values (98% inhibition of transport at basal adenosine concentrations) and flow rate to the measured value. An interstitial adenosine production term of 185 pmol min⁻¹ g⁻¹ fitted the mean measured release rate within 3%. This value was kept constant for all further simulations. Next, the intracellular adenosine production rates were estimated. Production rates of 1800 and 90 pmol min⁻¹ g⁻¹ for the parenchymal and the endothelial-cell regions, respectively, fitted the measurements of adenosine release made during EHNA and ITU treatment, simulated by increasing the respective enzyme Kₘ values 100-fold, within 1%. With transmethylation rates of 190 and 30 pmol min⁻¹ g⁻¹ in parenchymal and endothelial cell regions, respectively, total cardiac production rate was 2295 pmol min⁻¹ g⁻¹ (Table 1).

With these production rates, venous release rates calculated for control conditions were slightly higher than those measured (90 versus 53±24 pmol min⁻¹ g⁻¹). This misfit was brought about by too-high intracellular and/or interstitial concentration estimates, which resulted in a too-high venous release rate. Likely explanations for this moderate discrepancy were higher intracellular rates of adenosine degradation and/or a lower interendothelial gap permeability–surface area product for adenosine than previously thought. Assuming a combination of both effects, the parameter values for the Vₘₐₓ of adenosine kinase were enhanced by 50% (set to 150 and 56 nmol min⁻¹ g⁻¹ for the parenchymal and endothelial-cell regions, respectively), and the endothelial gap permeability–surface area product was lowered by 20% (set to 2.0 mL min⁻¹ g⁻¹). With these moderate adjustments, the model fitted the adenosine release rates determined under the different conditions almost equally well (Table 2). Furthermore, SAH contents after 20-minute step inputs of 200 μmol/L homocysteine were predicted reasonably well for all conditions without any further adjustment of model parameters. This parameter set also fitted the adenosine release rate and the tissue SAH content of the previous study within 10%.

Figure 5 shows measured coronary flows plotted versus calculated adenosine concentrations of capillary and interstitial regions, respectively. For a control coronary venous adenosine concentration of 10.1 nmol/L, calculated capillary and interstitial concentrations were 7.3 and 34 nmol/L, respectively. For membrane transport block, capillary and interstitial concentrations were 9.3 and 94 nmol/L, respectively. For simultaneous inhibition of adenosine kinase and adenosine deaminase, concentrations of 74 and 420 nmol/L, respectively, were calculated. Simulation of concerted block-
ing of transport and metabolism predicted that the capillary and interstitial adenosine concentrations should decrease but still remain above those calculated for inhibition of adenosine membrane transport alone (11.3 and 121 nmol/L, respectively). This explains the observation that NBTI in the presence of EHNA plus ITU reduced venous adenosine release but left coronary flow unchanged (Figure 1).

**Discussion**

We tested the hypothesis that the cytosolic adenosine concentration exceeds the interstitial in the well-oxygenated heart. We found that nucleoside transport blockers effectively inhibit cellular efflux of adenosine when the cytosolic concentration is raised. However, during physiological conditions, nucleoside transport blockers raise the interstitial adenosine concentration by inhibiting net cellular uptake of extracellularly produced adenosine. The findings are consistent with the assumption of a bidirectional block of membrane transport and indicate that the parenchymal cell represents a sink rather than a source of adenosine under well-oxygenated conditions. The transmembranous concentration gradient from extracellular to intracellular is in contrast to our intuition that there is a concentration gradient from the parenchymal cell to the capillary region, because the largest production site is in the parenchymal cell.

**Global Adenosine Production Rate**

During EHNA plus ITU, adenosine release averaged 1511 pmol/min·g⁻¹·g⁻¹ (Figure 1). This release rate is lower than those of 2 previous studies⁵,⁶ that reported 3.3 and 3.48 nmol/min·g⁻¹·g⁻¹, respectively, during the same inhibitor protocol. Reasons for these discrepancies are not entirely clear but are probably related to the quality of the isolated heart preparation (see Methods, Isolated Heart Experiments). Modeling adenosine production rates by fitting venous release rates revealed an estimate of 2294 pmol/min·g⁻¹·g⁻¹ (Table 1). This analysis accounted for incomplete enzyme inhibition because of increased substrate concentrations. Global production rate would have been underestimated by 31% if inferred from direct measurement of the release rate (1511 pmol/min·g⁻¹·g⁻¹). The present estimate of the global production rate is 67% of those reported before,⁵,⁶ which did not take the effects of increased substrate concentrations into account.

**Intracellular Adenosine Production**

During EHNA plus ITU, NBTI reduced coronary venous adenosine release from 1744 to 297 pmol/min·g⁻¹·g⁻¹ (Figure 1). Thus, NBTI blocked adenosine cell outward transport effectively, and ≥83% of the global adenosine production originated from an intracellular site. This conclusion is supported by measurements of SAH content after 20 minutes of homocysteine. SAH content differed by 15.8 nmol/g in the presence and absence, respectively, of concerted block of adenosine metabolism and transport (Figure 2). Because the rate of SAH production is rather linear during 20 minutes of homocysteine treatment under steady-state conditions,⁹ a mean production rate of 790 pmol/min·g⁻¹·g⁻¹ may be assumed. This value is 55% of the decrease of venous adenosine release after NBTI (1447 pmol/min·g⁻¹·g⁻¹). The difference is probably largely accounted for by a decreasing effectiveness of enzyme inhibition at an increased cytosolic adenosine concentration (Figure 2). If the measured SAH content was fitted by the model, thereby accounting for incompleteness of enzyme inhibition, a global production rate of 1623 pmol/min·g⁻¹·g⁻¹ was obtained. This figure is 70.7%
of that estimated from fitting measurements of the venous adenosine release rate (Table 1).

Effective block of outward membrane inosine transport by NBThI is suggested by the reciprocal changes of tissue and effluent perfusate concentrations (Figure 3). During well-oxygenated conditions, inosine is derived largely from inosine monophosphate, not adenosine. This is indicated by the following lines of evidence. (1) At a concentration of 5 \( \mu \text{mol/L} \) EHNA, no residual activity of adenosine deaminase was found in the guinea pig heart.\(^6\) (2) Although EHNA has only small effects on coronary venous adenosine release under well-oxygenated conditions, it doubles adenosine release during tissue hypoxia\(^14\) when inosine production from adenosine is increased. Thus, it is not a valid approach to determine adenosine production under well-oxygenated conditions by summing values of adenosine, inosine, (hypoxanthine, and uric acid.\(^15\)

### Extracellular Adenosine Production

Under control conditions, NBThI enhanced adenosine release from 46 to 179 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) (Figure 4), whereas SAH content remained unchanged (8.5 ± 1.1 versus 9.3 ± 2.1 nmol/g). This suggested an adenosine concentration gradient directed from extracellular to intracellular. Because 1 \( \mu \text{mol/L} \) NBThI blocked adenosine membrane transport sites nearly completely,\(^12\) the adenosine release rate of 179 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) is probably a rather accurate figure of the extracellular production rate in the isolated guinea pig heart (model estimate, 185 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \)). Compared with the above estimates of the global adenosine production rate (1623 and 2294 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \), respectively), extracellular production contributed 8% to 11% under well-oxygenated conditions.

Adenosine produced in the interstitial region is most likely derived from 5'-AMP. Membrane-bound 5'-nucleotidase has been documented histochemically on the cell surface of cardiomyocytes and vascular cells of guinea pig heart.\(^16\) In this species, AMP is recovered from an extracellular fluid region,\(^7\) and coronary venous adenine nucleotide release is 40 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) under control conditions and 430 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) during ecto-5'-nucleotidase inhibition by \( \alpha,\beta\)-methyleneadenosine diphosphate.\(^8\) This release is quantitatively sufficient to account for the extracellular adenosine production rate estimated in the present experiments. Concerning possible sources of extracellular nucleotides, our own experiments indicate that endothelial cells\(^11\) and smooth muscle cells\(^17\) need to be considered. Whether the multidrug resistance (mdr1) gene product mediates cell nucleotide release\(^18\) in cardiac tissues remains to be shown.

### Transmembranous Adenosine Concentration Gradient

Figure 6 summarizes interregional adenosine flux rates calculated by model analysis from experimental measurements (Figures 1 through 4). These net exchange rates are averages over the entire capillary length. With an inflow adenosine concentration of zero, regional concentrations increase monotonically along the capillary length.\(^10\) Thus, concentrations lower than those shown in Figure 6 would be expected near the arterial inflow and higher concentrations near the venous outflow. In extracapillary regions, this axial concentration gradient is <20%.\(^10\) The present model calculations for well-oxygenated conditions show the highest concentration (34 nmol/L) in the interstitial region. The free adenosine concentration of the parenchymal cell was slightly lower and that of the endothelial cell notably lower because of intracellular metabolism. The steeper concentration gradient across the abluminal endothelial cell membrane reflects the assumption of a 20-fold higher endothelial adenosine deaminase activity per milliliter of cell volume compared with the cardiomyocyte region.\(^19\) Our analyses predict that of extracellularly produced adenosine, 4.9% is taken up into parenchymal cells, 65.4% is taken up into endothelial cells, and 29.7% escapes from the interstitial region through interendothelial clefts into the capillary region.

Similar model analyses conducted in a previous study on the same experimental model\(^20\) revealed interstitial adenosine concentrations of 6.8 nmol/L under control conditions (our estimate, 34 nmol/L) and 191 nmol/L during membrane transport block by dipyridamole (our estimate, 94 nmol/L). Specific differences from the present study were as follows. (1) Dipyridamole was used at a concentration of 10 \( \mu \text{mol/L} \), which has significant side effects on adenosine metabolism\(^21,22\) and therefore probably overestimated extracellular adenosine production (496 versus 185 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \)). (2) The previous study attributed adenosine production exclusively to the interstitial region. (3) The global adenosine production rate (496 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \)) was smaller than that assessed in the present study (2295 pmol \( \cdot \text{min}^{-1} \cdot \text{g}^{-1} \), Table 1). For these reasons, that previous study most likely underestimated the interstitial adenosine concentration under control conditions and overestimated it during membrane transport block.
Study Limitations
The conclusions that may be drawn from inhibitor experiments depend on the potency and specificity of the inhibitors. EHNA 5 μmol/L,6 ITU 1 to 10 μmol/L,6,11 and NBTH 0.1 to 1.0 μmol/L,12 inhibit the respective enzymes or transporter in guinea pig heart specifically and nearly completely. An effectiveness of >90% was reported for DIP in the guinea pig heart at concentrations of 1.0 to 5.0 μmol/L.12 Under our experimental conditions, steady-state extraction of [14C]adenosine before and during DIP 1.0 to 5.0 μmol/L was 56% and 3%, respectively (n=2). However, higher concentrations may inhibit adenosine deaminase21 or phosphodiesterase.22 NBTH 10 μmol/L or DIP 10 μmol/L has no effect on in vitro SAH hydrolase activity extracted from guinea pig liver (A.D., unpublished data). Although the inhibitors were potent and specific at the concentrations used, in intact tissue, block of 1 or several metabolic pathways may enhance the concentration of unmetabolized substrate. Therefore, estimates of adenosine production rates made from outflow measurements must be taken as minimum figures or, even better, should be subjected to a mathematical model analysis that accounts for the effects of substrate accumulation.

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