Release of Adenosine and Cyclic AMP From Coronary Endothelium in Isolated Guinea Pig Hearts: Relation to Coronary Flow

Keith Kroll, Jürgen Schrader, Hans Michael Piper, and Michael Henrich

The coronary efflux of radioactive 3',5'-cyclic adenosine monophosphate (cAMP) and adenosine from isolated guinea pig hearts was measured following selective prelabelling of coronary endothelial adenine nucleotides with 10 nM [2,8,5'-3H] adenosine. Intracoronary infusion of adenosine and its derivatives 5'-N-ethyl-carboxamide-adenosine (NECA), (-)-N6-(R-phenyl-isopropyl)-adenosine (R-PIA), and (+)-N6-(S-phenyl-isopropyl)-adenosine (S-PIA) caused dose-dependent parallel increases in both coronary flow and the coronary efflux of radioactive cAMP with a rank order of potency: NECA > R-PIA > adenosine > S-PIA. In contrast, adenosine receptor stimulation of isolated cardiomyocytes in primary culture decreased the cellular release of cAMP below control levels with a rank order of potency: R-PIA > NECA. Under control conditions, coronary efflux of adenosine and cAMP was 34.3 ± 2.3 and 3.9 ± 0.8 pmol/min (mean ± SEM, n = 6), respectively. NECA (12 μM) caused an increase in cardiac cAMP release of 3.8 times and elevated the specific radioactivity of cAMP 5 times to 63.7 ± 6.0 Ci/mol, a value 11 times greater than the specific radioactivity of tissue ATP. Based on these findings, it was concluded that the coronary endothelium possesses adenosine A2 receptors linked to adenylate cyclase, which are activated in parallel with increases in coronary flow and that cardiomyocyte adenosine receptors are predominantly of the A1 subtype. In addition, the contribution of the coronary endothelium to total cardiac adenosine release was calculated to be 14% using the specific radioactivities of adenosine and cAMP released into the effluent perfusate.

(Circulation Research 1987;60:659-665)

The coronary hypothesis for the control of coronary blood flow proposes that adenosine is an extracellular transmitter released by cardiomyocytes into the interstitial fluid of the heart. Adenosine is then thought to diffuse throughout the interstitium and cause relaxation of vascular smooth muscle, which leads to adaptive changes in coronary blood flow. Most of the biologic effects of adenosine appear to be mediated via membrane adenosine receptors that are coupled to adenylate cyclase via the N regulatory proteins. Two receptor subtypes have been recognized: the A1 (or R1) subtype, which inhibits adenylate cyclase, and the A2 (or R2) subtype, which activates the enzyme. The adenosine derivatives 5'-N-ethylcarboxamide-adenosine (NECA) and (-)-N6-(R-phenyl-isopropyl)-adenosine (R-PIA) are frequently used to characterize adenosine receptor subtypes, since NECA is more potent at A2 receptors, while R-PIA is more potent at A1 receptors. Coronary vasodilation in the dog and rabbit is mediated by adenosine A2 receptors based on the rank order of potency of NECA and R-PIA.

The adenosine hypothesis has been complicated by two recent findings. The first is that cardiac adenosine formation is not limited to cardiomyocytes, the dominant cell species of the heart, but is compartmentalized. A recent study demonstrated that the coronary endothelium contributes to cardiac adenosine release in guinea pig hearts, but the magnitude of this contribution is not known. Should the endothelium produce a major portion of the adenosine released by the heart, it would tend to invalidate measurement of coronary venous adenosine as an index of interstitial adenosine concentration. In addition, this situation would further complicate the relation between coronary venous adenosine release and myocardial adenosine production. Therefore, one purpose of the present study was to determine the contribution of the coronary endothelium to total cardiac adenosine release in the isolated guinea pig heart.

The second complication of the adenosine hypothesis concerns the finding that the coronary endothelium forms a metabolic barrier for adenosine between the vascular and interstitial spaces due to its avid uptake and metabolism of adenosine. Should the metabolic barrier to adenosine be complete, it would imply that the vasodilator action of infused adenosine is entirely mediated by the coronary endothelium. In this event, coronary endothelial adenosine receptors should be of the same subtype as those mediating coronary vasodilation. Furthermore, infused adenosine should cause parallel activation of endothelial adenosine receptors and increases in coronary flow. To test these predictions, we used a technique to selectively prelabel coronary endothelial adenine nucleotides of isolated perfused guinea pig hearts using an intracoronary infusion of radioactive adenosine. Following prelabelling,
stimulation of endothelial adenylate cyclase via adenosine receptors was assessed by measuring the release of radioactive cAMP into the coronary effluent perfusate. The effect of adenosine receptor stimulation on cAMP release by isolated cardiomyocytes was also studied.

Materials and Methods

Isolated Guinea Pig Hearts

Hearts from 18 guinea pigs weighing 250–350 g were perfused according to the Langendorff technique with a modified Krebs-Henseleit solution at 37° C and at a constant pressure of 60 cm water.13 Hearts were electrically paced at a rate of 300 beats/min. Left ventricular isovolumetric pressure development and dP/dt were measured using a balloon placed in the left ventricle. Coronary flow was measured with an electromagnetic flow probe incorporated into the aortic cannula. All compounds were infused into the aortic cannula 2–3 cm above the level of the aortic valve at rates not exceeding 0.1 ml/min.

To preferentially label coronary endothelial adenosine nucleotides, [2,8,5-3H] adenosine was infused for 35 minutes at a constant rate of 2.5 μCi/min, yielding a final concentration of approximately 10 nM. Prelabeling was followed by a 20-minute washout period.

The effects of adenosine, NECA, R-PIA, and (+)-N6-(5-phenyl-isopropyl)-adenosine (S-PIA) on coronary flow and on the release of radioactive cAMP into the effluent perfusate were investigated in 12 experiments. Following a control period, during which 3 serial 1-minute samples of coronary effluent perfusate were collected, adenosine derivatives were infused at increasing concentrations. When coronary flow had stabilized, a 1-minute perfusate sample was collected at each dose. At the end of each experiment, the wet weight of the entire heart was measured.

To calculate coronary endothelial adenosine release, 6 experiments were conducted to measure the specific radioactivity of adenosine and cAMP released into the coronary effluent perfusate. Coronary effluent perfusate was collected on ice for 15 minutes during control conditions and for 6 minutes during each of 2 intracoronary infusions of NECA. After stopping the NECA infusion, hearts were frozen between aluminum blocks, cooled in liquid nitrogen, and lyophilized.

Isolated Cardiomyocytes

Calcium-tolerant ventricular muscle cells were isolated from 200–250 g Wistar rats and plated on 60 mm Falcon dishes in M 199 medium containing 0.2% bovine serum albumin and 4% fetal calf serum as previously described.14 After a 4-hour incubation, dishes were washed and filled with 2 ml of a modified Tyrode solution containing (in mM) NaCl 125.0, KCl 2.6, KH2PO4 1.2, MgSO4 1.2, CaCl2 1.0, and HEPES 10.0 (pH 7.4, 37° C), supplemented with 0.5 mM of the phosphodiesterase inhibitor RO 20-1724. After the addition of 1 μM NECA, R-PIA, or isoproterenol, cells were incubated for 30 minutes at 37° C under air while the dish was slowly rotated (1 cycle/5 min). Aliquots of the extracellular medium from 4 dishes that were treated identically were pooled, frozen rapidly in liquid nitrogen, and stored at -20° C until analysis for cAMP. Protein content of the dishes, determined by the method of Lowry at the end of the experiment, averaged 2.4 ± 0.3 mg protein per dish (mean ± SEM).

Purine Analysis

Adenine nucleotides and nucleosides were separated using a high pressure liquid chromatography (HPLC) system (Waters, Milford, Mass.). Purine compounds were quantified by ultraviolet light absorbance at 254 nm.

Coronary efflux of radioactive cAMP was analyzed by concentrating and desalting samples of effluent perfusate on Waters Sep-Pak C18 cartridges. Samples were eluted using 60% methanol and evaporated. Samples were partitioned on a Waters 5-micron C18 column utilizing a 10–60% linear methanol gradient (Solution A: KH2PO4, 10 mM, pH 5.8; Solution B: 60% methanol in water, flow rate 2 ml/min). Radioactivity eluting with 200 pmol carrier cAMP added previously to each sample was then measured.

For measurement of the specific radioactivity of cAMP released by the heart, samples were desalted and concentrated on Sep-Pak cartridges and then separated by HPLC on a 5-micron C18 column as above, except that Solution A was 2.5 mM KH2PO4. Since cAMP comigrated with other UV-absorbing material, the fractions containing cAMP were passed over a Waters SAX strong anion exchange column (linear buffer gradient beginning with 7.0 mM KH2PO4 and 6.7 mM KCl, pH 4.0 and ending with 0.19 M KH2PO4 and 0.38 M KCl, pH 5.0, flow rate 3 ml/min), followed by rechromatography on a C18 column (10 mM KH2PO4 buffer, pH 5.8, 10–60% linear methanol gradient, flow rate 2 ml/min). The symmetrical cAMP peaks obtained by this procedure were quantified by peak height and were counted for radioactivity. Fractions eluting immediately before and after cAMP contained only background radioactivity; cAMP losses incurred for all analytical steps were 60–70%, and data reported were not corrected for these losses.

Enzymatic peak shifting procedures were carried out in 3 additional experiments by modifying the above analytical techniques to include incubation of coronary effluent samples for 30 minutes at room temperature with 0.04 U phosphodiesterase. In these samples, 98 ± 1% (n = 4) of the radioactive cAMP was converted to AMP.

Adenosine was measured during the first of the above described chromatographic steps for cAMP. Adenosine was quantified by peak height, and fractions containing adenosine were counted for radioactivity.

For measurement of the specific radioactivity of cardiac adenosine nucleotides, lyophilized hearts were homogenized in 0.5 N perchloric acid and neutralized with potassium hydroxide. Samples were separated on a Waters 10-micron SAX column using a nonlinear buffer gradient, starting with 7.0 mM KH2PO4, 6.7
mM KCl, pH 4.0 and ending with 0.25 M KH₂PO₄, 0.50 M KCl, pH 5.0, flow 3 ml/min. ATP, ADP, and AMP were quantified by peak area, and their respective fractions were counted for radioactivity. These results were expressed in terms of tissue wet weight by assuming a wet to dry weight ratio of 5.0.

In experiments with isolated cardiomyocytes, samples of incubation medium were concentrated on Sep-Pak cartridges, and duplicate determinations were made of the cAMP content using a cAMP radioactive ligand binding assay (Amersham, Braunschweig, Federal Republic of Germany).

Statistics

The nonparametric Wilcoxon test for paired data was used to determine the p values of cAMP measurements in the isolated cardiomyocyte experiments. A paired Student's t test was used to obtain all other p values. Results are expressed as mean ± SEM.

Chemicals

Adenosine, R-PIA, S-PIA, and phosphodiesterase were purchased from Boehringer Mannheim GmbH, Federal Republic of Germany. NECA was a gift from Byk Gulden Pharmazeutika. RO 20-1724 was a gift from Hoffman-LaRoche. [2,8,5'-3H] adenosine (specific activity 40-60 Ci/mmol) was purchased from Amersham. All other chemicals were of the highest available purity.

Results

Prelabelling isolated guinea pig hearts by intracoronary infusion of [2,8,5'-3H] adenosine at a concentration of 10 nM for 35 minutes resulted in the incorporation of substantial radioactivity into the adenine nucleotides of cardiac tissue (Table 1). Measurement of the arterial-venous radioactivity difference showed that 68.9 ± 2.0% (mean ± SEM; n = 6) of the total radioactivity infused during the prelabelling period was taken up by the heart. Over 95% of the radioactivity extracted from cardiac tissue with perchloric acid was recovered in the adenine nucleotides. While most (89%) of the radioactivity was incorporated into cellular ATP, the highest specific radioactivity was observed in the AMP fraction (Table 1).

To evaluate the relation between the activation of endothelial adenosine receptors and increases in coronary flow, a series of cumulative dose-response experiments were conducted in prelabelled hearts using NECA, adenosine, R-PIA, and S-PIA. As summarized in Figure 1, all 4 adenosine derivatives caused dose-dependent increases in coronary flow with the following rank order of potency: NECA > R-PIA > adenosine > S-PIA, which is characteristic for adenosine A2 receptors. The same rank order of potency was also found for the release of radioactive cAMP from prelabelled guinea pig hearts. Measurements for 16 complete dose-response curves were made in 12 hearts, electrically paced, and working isovolumetrically. Coronary flow increased from control value 5.90 ± 0.29 to 11.80 ± 0.42 ml/min/g (mean ± SEM; n = 16) during maximal vasodilation.

Table 1. Incorporation of Radioactivity Into Adenine Nucleotides (AN) of Isolated Guinea Pig Hearts After Prelabelling With 10 nM Radioactive Adenosine

<table>
<thead>
<tr>
<th>Content (nmol/g wet)</th>
<th>Radioactivity (μCi/g wet)</th>
<th>Specific radioactivity (Ci/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP 3,960 ± 300</td>
<td>22.4 ± 2.4</td>
<td>5.62 ± 0.51</td>
</tr>
<tr>
<td>ADP 610 ± 32.0</td>
<td>2.44 ± 0.24</td>
<td>4.00 ± 0.38</td>
</tr>
<tr>
<td>AMP 44.6 ± 4.60</td>
<td>0.338 ± 0.032</td>
<td>8.16 ± 1.39</td>
</tr>
<tr>
<td>ΣAN 4,620 ± 320</td>
<td>25.2 ± 2.6</td>
<td>5.42 ± 0.49</td>
</tr>
</tbody>
</table>

Following NECA stimulation, hearts were quickly frozen and processed further as described in "Materials and Methods." Mean ± SEM; n = 6.
regression analysis yielded correlation coefficients of \( r = 0.718 \) and \( r = 0.884 \) for NECA and adenosine, respectively. Calculation of 95% confidence intervals indicated that the adenosine curve was significantly different from the NECA curve, while the effects of R- and S-PIA appeared similar to NECA.

To determine whether adenosine agonists also stimulate cAMP release from cardiomyocytes, experiments were conducted on isolated ventricular muscle cells in primary culture. Stimulation of these cells with R-PIA significantly \((p < 0.05)\) decreased the release of cAMP into the culture medium below control levels, while NECA at the same concentration caused no significant change (Table 2). In contrast to the effects of the adenosine analogs, stimulation with isoproterenol led to a twenty-fourfold increase in cAMP release.

In isolated hearts perfused with normoxic medium, coronary flow averaged 9.5 ± 1.1 ml/min (mean ± SEM, \( n = 6 \)), and cAMP and adenosine were released into the effluent perfusate at constant rates of 3.9 ± 0.8 and 34.3 ± 2.3 pmol/min, respectively. The mean specific radioactivity of released cAMP was 12.6 ± 3.1 Ci/mol (\( n = 6 \)).

The rank order of potency for coronary vasodilation was NECA > R-PIA > adenosine > S-PIA (Figure 1), which is characteristic for the adenosine A2 receptor subtype, thus confirming similar findings in the dog and rabbit. Because of the selectivity of the radioactive prelabelling technique used in these experiments, radioactive cAMP was most likely derived from the coronary endothelium. Since the rank order of potency for radioactive cAMP release was also NECA > R-PIA > adenosine > S-PIA, this provides evidence that coronary endothelial cells also possess adenosine A2 receptors.

In contrast to endothelium, stimulation of isolated cardiomyocytes by R-PIA decreased cellular cAMP release below control values (Table 2). Both the direction of the effect (inhibition of adenylate cyclase) and the apparent rank order of potency (R-PIA > NECA) clearly indicate that on myocardial cells, adenosine receptors of the A1 subtype predominate. Thus, there appears to be a distinct cellular distribution of adenosine receptors in the heart. Vascular cells, such as smooth muscle and coronary endothelium, are characterized by adenosine A2 receptors, while cardiomyocyte adenosine receptors are predominantly of the A1 subtype. An important implication of this finding is that, due to the opposite effects of A1 and A2 receptor activation on cAMP formation, measurement of adenylate cyclase activity in whole-tissue preparations may lead to erroneous conclusions. In view of the divergent effects of adenosine on cardiac adenylate cyclase reported in the literature, it is likely that cellular heterogeneity of the heart has been an uncontrolled variable influencing the results.

Measurement of radioactive cAMP release as an
index of endothelial adenylate cyclase activation in the intact heart is supported by three lines of evidence: 1) the specificity of the endothelial prelabelling technique, 2) the selective release of cAMP by endothelial cells during adenosine stimulation, and 3) the exclusion of other cell types of the heart as major contributors. Evidence in favor of the specificity of the radioactive adenosine prelabelling technique is available from studies using autoradiography, cell fractionation techniques, and multiple indicator dilution measurements. These studies indicate that at the concentration used for prelabelling in the present study, infused adenosine is selectively incorporated into the coronary endothelium. The selectivity of the prelabelling technique is also supported by data obtained in this study. The specific radioactivity of cAMP (63.7 Ci/mol, Table 3) released in the presence of NECA was 11 times greater than that of its immediate precursor, ATP (5.62 Ci/mol, Table 1), in cardiac tissue. This indicates that cAMP must have been formed from a highly labelled adenine nucleotide compartment constituting only a small fraction of the total adenine nucleotides of the heart, most likely the coronary endothelium. The specificity of the prelabelling technique employed is further supported by the finding that the calculated sizes of the prelabelled adenine nucleotide compartment and the endothelial adenine nucleotide compartment were the same. To make this calculation, it was assumed that all the radioactivity incorporated into cardiac tissue adenine nucleotides (25.2 μCi/g, Table 1) was confined to the precursor compartment of the cAMP (specific radioactivity 63.7 Ci/mol) released during NECA stimulation. The size of this prelabelled compartment was calculated to be

\[
\frac{25.2 \mu\text{Ci/g}}{63.7 \text{ Ci/mol}} = 396 \text{ nmol/g heart}
\]

An almost identical value can be estimated for the size of the endothelial adenine nucleotide compartment based on the following known morphometric and biochemical data. The coronary endothelium comprises 2.8% of heart volume, and the adenine nucleotide content of coronary endothelial cells is 14.2 μmol/g. On this basis, the size of the endothelial adenine nucleotide compartment was calculated to be 398 nmol/g heart. The similarity of these calculated values not only supports the selectivity of the prelabelling technique but also provides indirect evidence against important subcellular compartmentalization of coronary endothelial adenine nucleotides.

The use of radioactive cAMP release to determine activation of endothelial adenylate cyclase is further supported by the finding that cAMP formation by endothelial cells in culture was greatly enhanced by adenosine. Virtually all the cAMP formed was released into the extracellular medium, indicating that endothelial cells are highly permeable to cAMP. Egress of cAMP from a variety of cell types during adenylate cyclase stimulation has been characterized as a specific, unidirectional transport mediated process.

Cell types of the heart other than the coronary endothelium were unlikely to have interfered with the use of the coronary efflux of radioactive cAMP to assess the activation of endothelial adenylate cyclase. Results of the present study clearly demonstrate that NECA and R-PIA did not increase cAMP release by isolated cardiomyocytes (Table 2). In contrast, these results suggest that cardiomyocytes possess adenosine A1 receptors, which inhibit adenylate cyclase. Furthermore, intracoronary infusion of NECA did not increase ventricular contractility (Table 3). Since cAMP is causally implicated in the hormonal stimulation of cardiac contractile force, this finding argues against the activation of cardiomyocyte adenylate cyclase by NECA. Another source of cAMP could be vascular smooth muscle. However, it is unlikely that these cells interfered in the present experiments since they lack a high affinity adenosine uptake mechanism. Therefore, these vascular smooth muscle adenine nucleotides were either not at all or only weakly prelabelled by radioactive adenosine.

The parallel nature of the dose-response curves for coronary flow and radioactive cAMP release reported here (Figure 1) demonstrates that the coronary adenosine receptors eliciting vasodilation are activated simultaneously with those that stimulate endothelial adenylate cyclase. If the vasodilator action of adenosine were entirely mediated by the activation of coronary endothelial adenylate cyclase, then the curves in Figure 2 relating coronary flow and endothelial activation (radioactive cAMP release) should be the same for adenosine and NECA. Instead, the position of the adenosine curve is significantly different from that of NECA. This difference is most likely a consequence of the more rapid endothelial uptake and metabolism of adenosine compared with NECA. NECA most likely approached equilibrium between the vascular and in-

<table>
<thead>
<tr>
<th>cAMP release (pmol/min)</th>
<th>cAMP release (μCi/min)</th>
<th>cAMP specific radioactivity (Ci/mol)</th>
<th>LVP (mm Hg)</th>
<th>LV dP/dt max (mm Hg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9 ± 0.8</td>
<td>41 ± 12</td>
<td>12.6 ± 3.1</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>NECA 2.3 μM</td>
<td>12.3 ± 2.1†</td>
<td>748 ± 137†</td>
<td>64.0 ± 9.1†</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>NECA 11.6 μM</td>
<td>14.8 ± 2.7†</td>
<td>903 ± 146†</td>
<td>63.7 ± 6.0†</td>
<td>91 ± 4*</td>
</tr>
</tbody>
</table>

LVP, left ventricular isovolumetric developed pressure; H-cAMP, radioactive cAMP. Mean ± SEM, n = 6; *p<0.025 vs. control; †p<0.005 vs. control.
terstitial spaces during intracoronary infusion and caused equivalent stimulation of endothelial and smooth muscle adenosine receptors. In contrast, it is highly probable that infused adenosine was hindered from reaching the interstitial space due to endothelial trapping. This would lead to a greater stimulation of endothelial receptors by adenosine than those of smooth muscle cells. Consequently, at equivalent levels of endothelial adenosine receptor activation (equal radioactive cAMP), NECA most likely caused greater smooth muscle stimulation than adenosine and, therefore, greater coronary flow (Figure 2). Our results suggest that a component of the coronary flow increase caused by adenosine may be mediated by the endothelium but that at least part of the vasodilatory response must be endothelium-independent. This interpretation is consistent with the finding that the relaxation of a number of isolated arteries caused by adenosine is reduced by removal of the endothelium but not abolished. Possible mechanisms for endothelial mediation might involve endothelium-derived relaxing factor or direct interaction between endothelial and smooth muscle cells via gap junctions. Extracellular cAMP itself is probably not involved since it is only a weak coronary vasodilator when infused intracoronarily. The parallelism between coronary flow and radioactive cAMP release was not caused by changes in coronary flow itself since a recent study using the identical preparation reported similar results in hearts perfused with constant coronary flow (12 ml/min).

The present study shows that NECA increased cardiac cAMP efflux 3.8 times (Table 3). Intai and coworkers reported that intracoronary norepinephrine increased cAMP release from isolated guinea pig hearts 2.3 times. While cardiac cAMP release paralleled inotropic effects during norepinephrine stimulation, cAMP and inotropism were fully dissociated during NECA stimulation (Table 3). These observations, together with the finding that coronary endothelial cells possess $\beta_1$-adrenergic receptors (authors' unpublished observations), suggest that the coronary endothelium plays an important role in cardiac cyclic nucleotide metabolism not previously appreciated.

Data reported in this experiment permit the calculation of the coronary endothelial contribution to total cardiac adenosine release. The rationale underlying this calculation is that the large selective increase in the release of endothelial cAMP due to NECA stimulation made endothelial ATP the predominant precursor for cAMP in the effluent perfusate. Therefore, the specific radioactivity of cAMP released during NECAstimulation was assumed to equal that of the endothelial precursor adenine nucleotides from which adenosine is also formed. Any difference between the specific radioactivity of released adenosine and that of endothelial precursor adenine nucleotides must be due to dilution of the endothelium-derived adenosine (labelled) with adenosine formed by unlabelled cardiomyocytes. Using the specific radioactivities of coronary effluent adenosine (9.04 Ci/mol, see “Results”) measured under control conditions and of cAMP (63.7 Ci/mol, Table 3) released during NECA infusion, the fraction of total cardiac adenosine release that was derived from the coronary endothelium can be calculated as follows:

$$\frac{9.04 \text{ Ci/mol}}{63.7 \text{ Ci/mol}} = 0.14$$

This ratio indicates that in the well-oxygenated heart, 14% of total cardiac adenosine released into the coronary effluent perfusate is derived from the coronary endothelium. This calculation assumes that endothelium-derived adenosine and cAMP were formed from the same precursor adenine nucleotides. Indirect evidence for the validity of this assumption is the similarity between the calculated sizes of the endothelial and the prelabelled adenine nucleotide pools. If endothelium-derived adenosine were produced by the hydrolysis of $S$-adenosylhomocysteine, the above calculation would still be valid since adenosine formed from the transmethylation pathway is also derived from cellular ATP.

The physiologic role of endothelium-derived adenosine is difficult to assess. According to the present results, most (86%) of the adenosine released from the normoxic heart into the coronary vascular space is derived from cardiomyocytes. In view of the active adenosine uptake system, it appears that the coronary endothelium serves predominantly as a sink for adenosine released from cardiomyocytes rather than as a source itself. On the other hand, because of the close contacts that exist between endothelial and vascular smooth muscle cells in vivo, it is conceivable that adenosine released from endothelial cells may influence vascular tone.

References

4. Van Calker D, Muller M, Hamprecht B: Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J Neurochem* 1979;33:999–1005


**KEY WORDS** • endothelial cells • heart • adenylate cyclase • adenosine receptors • coronary flow
Release of adenosine and cyclic AMP from coronary endothelium in isolated guinea pig hearts: relation to coronary flow.

K Kroll, J Schrader, H M Piper and M Henrich

Circ Res. 1987;60:659-665
doi: 10.1161/01.RES.60.5.659

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/60/5/659