Adenosine Metabolism in Canine Myocardial Reactive Hyperemia

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SUMMARY In pentobarbital-anesthetized open-chest dogs, myocardial adenosine content is elevated by 5 or 15 seconds of left coronary artery occlusion and falls exponentially to control levels during reactive hyperemia. The rate constants for adenosine dissipation are (mean ± SEM): −0.08 ± 0.01 and −0.034 ± 0.007 sec⁻¹ after 5- and 15-second occlusion, respectively. Kinetic analysis of the reactive hyperemia flow curves (Circ Res 14/15 (suppl 1): 81-85, 1963) predicts rates of −0.069 ± 0.009 sec⁻¹ and −0.04 ± 0.009 sec⁻¹, indicating that changes in adenosine levels can account for the way coronary flow changes during this response. The log (dose-) response curve relating reactive hyperemia flow to tissue adenosine concentration has a steeper slope and is half-maximal at a lower adenosine concentration than the dose-response curve obtained by intracoronary infusions of adenosine in oxygenated hearts, indicating that the coronary vasoactivity of adenosine is enhanced during reactive hyperemia. This could explain why theophylline antagonizes the coronary vasodilatory effect of adenosine in oxygenated hearts but has relatively little effect on reactive hyperemia.

ADENOSINE has been detected in coronary venous blood during myocardial reactive hyperemia, a finding which has been interpreted as evidence that this nucleoside may regulate coronary vascular tone during the hyperemia response. In support of this, adenosine has been demonstrated in oxygenated heart muscle, the amount found increasing rapidly during myocardial ischemia. However, the adenosine content of heart muscle and coronary venous blood have not been related to coronary blood flow rate during reactive hyperemia, so that these findings must be considered as only circumstantial support for the adenosine hypothesis. Indeed, reports that doses of aminophylline which strongly antagonize the coronary vasodilatory effect of adenosine in oxygenated hearts have the following properties: (1) it is present in oxygenated as well as ischemic heart muscle; (2) it accumulates proportionately smaller effect on myocardial reactive hyperemia suggests that adenosine may have no role at all in the hyperemia response.

The experiments designed to answer the first question were based on a preliminary study from this laboratory which showed that if one assumes that coronary blood flow rate during myocardial reactive hyperemia is regulated by a vasodilatory metabolite which accumulates during ischemia and that the log (dose-) response approximation describes the relationship between flow and the concentration of this metabolite, the metabolite should have the following properties: (1) it is present in oxygenated as well as ischemic heart muscle; (2) it accumulates...
in the myocardium during coronary occlusion in proportion to the length of occlusion; and (3) its concentration falls exponentially during reactive hyperemia.

Previous work\textsuperscript{2,3} has demonstrated that adenosine meets the first two criteria. The present study shows that cardiac adenosine levels decrease exponentially during reactive hyperemia at a rate predicted by the kinetics of coronary flow rate, but that the relationship between coronary flow rate and myocardial adenosine levels during postischemic reactive hyperemia is different from the relationship between coronary flow rate and coronary plasma adenosine levels during intracoronary infusions of this nucleoside in normally perfused hearts.

\textbf{Methods}\textsuperscript{*}

The experimental preparation was that described previously.\textsuperscript{3} Briefly, 78 beagles of either sex weighing 12-18 kg were anesthetized with pentobarbital (approximately 30 mg/kg, iv), the chest was opened, and the left coronary artery was cannulated with a Gregg cannula and perfused from the left carotid artery. Coronary blood flow rate was monitored with an electromagnetic flowmeter interposed in the perfusion line, and perfusion pressure was measured with a Statham P23Gb strain gauge connected to the perfusion line by a side arm. Heparin (200 mg, iv) was administered prior to coronary cannulation.

\textbf{Myocardial Adenosine Levels during Reactive Hyperemia}

Control observations included records of coronary blood flow and perfusion pressure and the hyperemic response to 15 seconds of coronary occlusion. After a 10-minute wait to allow for complete metabolic recovery from coronary occlusion,\textsuperscript{4} the perfusion line was again clamped for 15 seconds and myocardium was sampled 10, 20, 30, 40, 50, or 60 seconds later with tongs cooled in liquid nitrogen. Because the sample and adherent tissue were large (1-2 g) and often was transmural, blood pressure and coronary flow rapidly fell after biopsy. Accordingly, each heart could be sampled only once. A second series of experiments examined tissue adenosine levels 5, 10, 15, and 20 seconds after the beginning of reactive hyperemia following 5 seconds of coronary occlusion. The adenosine content of perchloric acid extracts of these tissue samples was estimated by an enzymatic spectrophotometric technique.\textsuperscript{3}

The data tabulated include coronary blood flow and perfusion pressure at the moment of biopsy, left ventricular weight, and adenosine content. Analysis of the record of the control reactive hyperemia response\textsuperscript{5} provided an estimate of the rate constant for the disappearance of a vasodilatory metabolite for comparison with the rate of change of tissue adenosine levels.

\textsuperscript{*} In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

\textbf{Efflux of Purines from the Heart during Reactive Hyperemia}

Four dogs were prepared as described above and, in addition, a metal-tipped plastic catheter for blood sampling was placed in the coronary sinus via the right atrium.\textsuperscript{4} This catheter, which was kept as short as possible (about 15 cm) to minimize purine degradation during transit, was connected to a two-port stopcock fitted with a syringe to flush the catheter by withdrawing stagnant blood from it. Control 1-ml samples of arterial and coronary venous blood were drawn and quickly mixed with 2 ml of ice-cold 0.6 N HClO\textsubscript{4} in a tared tube. Serial arterial and venous samples were then obtained during reactive hyperemia following a 15-second coronary occlusion. The beginning and end of each sampling period were timed by a pedal-actuated marker beam on the oscillographic recording of blood pressure and flow. The amount of blood added to each tube was estimated gravimetrically, denatured protein was separated by centrifugation, and a sample of the supernatant fluid was neutralized with 5 N KOH. The neutralized blood extracts of three dogs were assayed for individual adenosine, inosine, and hypoxanthine levels and, in one dog, for the sum of these purines, by simultaneously adding all three enzymes to the assay curve.

\textbf{Relation between Adenosine Concentration and Coronary Flow Rate}

After recording control blood flow rate and perfusion pressure, the coronary perfusion line was clamped for 15-20 seconds while recording continued through the ensuing reactive hyperemia. Peak reactive hyperemia flow was used as an index of maximum coronary vasodilation. After a 10-minute recovery period, a spectrophotometrically standardized solution of adenosine (approximately 2.7 mm) was infused with a Harvard pump via a needle inserted in the perfusion line at a point just above the Gregg cannula. The rate of infusion was increased stepwise over a range between 7 and 360 \textmu l/min, each infusion continuing until coronary flow was stable for 30-60 seconds. Hematocrit was estimated at the beginning and end of each experiment and these data were averaged.

Data tabulated included coronary flow rate and perfusion pressure during the stable response to adenosine infusion, left ventricular weight, and adenosine concentration in coronary plasma water.

\textbf{Calculations}

Coronary vascular conductance was used to normalize blood flow data from dogs having different heart weights and perfusion pressures. This index of coronary vascular tone was calculated as the quotient of coronary flow rate (ml·min\textsuperscript{-1}·g LV\textsuperscript{-1}) divided by perfusion pressure (mm Hg). Tissue adenosine concentration was calculated as the quotient of adenosine content divided by the volume of the extracellular space. The latter is taken to be the difference between the "5-minute" sucrose space\textsuperscript{1} and vascular volume, and has been estimated as 0.28 ml/g LV in the dog (Olsson, unpublished). The concentration of
Results

Myocardial Adenosine Levels during Reactive Hyperemia

Data on myocardial adenosine levels during reactive hyperemia are shown in Figure 1. The data at zero time, i.e., at the onset of reactive hyperemia, and the control levels were reported previously. Because the present reactive hyperemia experiments were done at the same time as and were interspersed with these experiments, the use of these data here is believed valid. The average adenosine content after 15 seconds of coronary occlusion was 1.82 nmol/g LV and fell to control levels by 50 seconds of reactive hyperemia. Adenosine levels after 5 seconds of coronary occlusion were 0.72 nmol/g LV and returned to the normal range at 20 seconds of reactive hyperemia.

The data in Figure 2 are used to examine the hypothesis that adenosine levels decrease exponentially during reactive hyperemia to a steady state level greater than zero. The expression describing this process is $-\frac{dx}{dt} = k(x - x_0)$, in which $x$ and $x_0$ represent adenosine concentration at time $t$ and during the control state, respectively, and $k$ is a rate constant. Accordingly, the difference between the adenosine concentration during reactive hyperemia and the control value are plotted on a logarithmic axis as a function of time. These plots are linear, so these experimental data are compatible with this hypothesis. The apparent rate constant for the disappearance of adenosine after a 5-second occlusion was $-0.080 \pm 0.011$ sec$^{-1}$ and after a 15-second occlusion was $-0.034 \pm 0.007$ sec$^{-1}$. The estimates of these rate constants obtained by analyzing the control reactive hyperemia flow curves in each dog were $-0.069 \pm 0.009$ sec$^{-1}$ after 5 seconds of occlusion and $-0.041 \pm 0.009$ sec$^{-1}$ after a 15-second occlusion. Neither differs significantly from the rates of disappearance estimated from tissue adenosine data.

Efflux of Purines from the Heart during Reactive Hyperemia

Data on coronary arteriovenous difference of (adenosine + inosine + hypoxanthine) are summarized in Figure 3. In the first three dogs, adenosine was not detected in coronary venous blood at any time, nor did the inosine and hypoxanthine levels show any trends. Accordingly, only the sum of these purines was estimated in the fourth dog, and data on the sum of all three purines are presented here. There was a small but significant efflux of purine in the control state ($P < 0.05$) which increased during reactive hyperemia and returned to near control levels after an average of 64 seconds. Because coronary blood flow was elevated during at least the first through third sampling periods, the net efflux was even larger than these arteriovenous differences indicate. However, since...
coronary flow rate was not in a steady state, efflux was not calculated.

**Relationship between Adenosine Levels and Coronary Flow Rate**

Figure 4 compares the relationship between coronary flow rate and extracellular adenosine concentration during reactive hyperemia with this relationship during the intra-coronary infusion of adenosine. The least square regression of coronary conductance (\(\phi\)) on log[adenosine] during infusions of this nucleoside is described by the equation \(\phi = 1.22 + 1.54 \log[\text{adenosine}]\). The corresponding equations for reactive hyperemia following 5- and 15-second occlusions were, respectively, \(\phi = 0.83 + 3.58 \log[\text{adenosine}]\) and \(\phi = 0.93 + 2.76 \log[\text{adenosine}]\). The slopes of the two curves describing the reactive hyperemia experiments are significantly steeper than the one describing adenosine infusions (0.05 > \(P > 0.025\)), but they are not significantly different from each other (\(P > 0.3\)).

**Discussion**

This study supports the hypothesis that adenosine regulates coronary blood flow during myocardial reactive hyperemia. Following coronary occlusions of 5 or 15 seconds, adenosine levels appear to return to control values exponentially. Moreover, these changes in adenosine level with time, i.e., the apparent rate constant of its disappearance, predict the changes in reactive hyperemia flow rate reasonably well. Although the relationship between endogenous adenosine concentration and coronary flow rate during reactive hyperemia differs from that characterizing the response to exogenous adenosine in oxygenated hearts, this does not exclude adenosine as a determinant of reactive hyperemia flow.

The model for reactive hyperemia flow changes derived from analysis of flow curves proposed a single agent which was dissipated by a process having second order kinetics. Subsequent experimental work requires revision of this model. Adenosine is taken up and incorporated into the nucleotide pool of cardiac cells, even during hypoxia. Cellular uptake appears to be the dominant pathway in oxygenated myocardium, but whether this is also true during reactive hyperemia is unknown. Adenosine is found in coronary venous blood during reactive hyperemia along with increased amounts of inosine and hypoxanthine, its degradation products, indicating that washout and deamination also influence tissue levels. The present study confirms a modest increase in coronary venous (inosine + hypoxanthine) levels, but adenosine, if present, was below the limits of detection. These factors which tend to reduce cardiac adenosine levels are opposed by the accelerated rate of adenosine production which probably persists for some time during reactive hyperemia. The magnitude and time course of these changes in adenosine production rate during reactive hyperemia also are unknown. Thus, the apparent rate constant of adenosine dissipation is the resultant of several processes, and the present study offers no clue as to which predominates. That this rate decreases as the duration of occlusion increases is probably not, as originally proposed, evidence for second order kinetics. A more reasonable explanation is that the individual contributions made by the factors enumerated above differ in magnitude according to the duration of coronary occlusion.

Transforming reactive hyperemia flow rate data into an index of the concentration of a hypothetical vasodilator facilitates testing whether changes in adenosine levels can account for the changes in reactive hyperemia flow. Such an analysis assumes as an approximation that coronary conductance, \(\phi\), is proportional to the logarithm of the concentration of adenosine, i.e., \(\phi = \alpha \log[\text{adenosine}]\), in which \(\alpha\) is a scaling factor. Using this relationship, one may analyze reactive hyperemia flow curves to determine whether the changes in vasodilator concentration with time can account for the time course of flow changes. Such an analysis of reactive hyperemia flow in conscious dogs indicates that, following 15-second occlusions, the vasodilator disappears at a rate between -0.03 and -0.05 sec\(^{-1}\), and after 5-second occlusions at a rate of about -0.01 sec\(^{-1}\). In the present study the rates estimated from tissue adenosine data were, respectively, -0.034 and -0.08 sec\(^{-1}\), similar to the values obtained from analysis of flow curves, -0.041 and -0.074 sec\(^{-1}\). Thus, the changes in tissue adenosine concentration can account for the time course of reactive hyperemia flow.

In addition, it is necessary that the scaling factor, \(\alpha\), be of the appropriate magnitude. This was evaluated by constructing dose-response curves from the tissue adenosine concentration and coronary conductance data during reactive hyperemia and comparing these curves to one constructed from experiments in which adenosine was infused into the coronary circulation. The vasodilatory potency of adenosine was different in the two situations; a half maximal coronary flow was associated with a tissue adenosine concentration of 2.0 \(\mu\)M during reactive hyperemia and a concentration of 4.0 \(\mu\)M during adenosine infusions. Moreover, the slopes of the dose-response curves of reactive hyperemia were steeper. One interpretation of this result is that adenosine is not the substance responsible for reactive hyperemia. If this is the case, whatever substance regulates reactive hyperemia flow must be present in concentrations which are similar in effect to these concentrations of adenosine, and the time

![Figure 4: Relationship between coronary conductance and tissue adenosine concentration during reactive hyperemia following 5 seconds (+) or 15 seconds (○) of coronary occlusion. Shown also is the relationship between coronary conductance and adenosine concentration during intracoronary infusions of this nucleoside (●). Vertical and horizontal bars represent ± 1 SEM.](image-url)
course of the changes in its concentration must be very similar to those of adenosine. An equally plausible explanation recognizes critical differences in the two types of experimental preparation. During an intracoronary infusion of adenosine, this nucleoside is metabolized in the blood and by cells in the vascular wall, so that the effective concentration at the coronary myocyte is undoubtedly lower than that estimated from the rates of adenosine infusion and coronary blood flow. It is also possible that the coronary resistance vessels do not respond instantaneously to the falling concentration of adenosine. Because of such a retarded response, coronary tone at any moment would reflect the effect of a higher adenosine concentration at an earlier time. Each of these factors would tend to bring the two dose-response curves closer but would not explain the difference in their slopes. During reactive hyperemia, the environment of the coronary resistance vessels is probably quite different from that in a normally perfused heart. Local oxygen tension is probably lower, a factor known to augment the vasoactivity of adenosine in isolated strips of coronary artery. In intact, beating hearts, arterial hypoxemia also augments the vasoactivity of adenosine; the dose-response curve becomes steeper and is shifted toward a lower concentration range. Local acidosis and/or elevated Pco2 may also promote the coronary vasodilatory effects of adenosine during reactive hyperemia. Changes in arterial Pco2 produce directionally similar changes in coronary vascular resistance, and hypercapnic acidosis enhances both the vasodilatory potency of intracoronary adenosine and the magnitude of the reactive hyperemia response. The participation of other modifiers such as cyclic nucleotides or prostaglandins, while lacking detailed experimental support, cannot be excluded.

The apparent enhancement of adenosine vasoactivity during reactive hyperemia could explain why aminophylline antagonizes the effect of an intracoronary infusion of adenosine, yet has a relatively small effect on reactive hyperemia during reactive hyperemia of the dog heart. Am J Physiol 216: 56-62, 1969


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