Effects of Catecholamines, Histamine, and Nitroglycerin on Flow, Oxygen Utilization, and Adenosine Production in the Perfused Guinea Pig Heart

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SUMMARY Adenosine release by the myocardium has been linked to a decrease in myocardial oxygen content and has been implicated in producing the concomitant increase in coronary flow. Epinephrine, norepinephrine, and histamine increase myocardial oxygen consumption and coronary flow. Nitroglycerin on the other hand, increases coronary flow but does not increase oxygen consumption. The purpose of the present study was to determine whether changes in coronary sinus oxygen levels induced by these drugs are closely associated with changes in adenosine release. Isolated guinea pig hearts were perfused with Krebs-Henseleit solution (pH 7.4, 37°C) aerated with 95% O₂-5% CO₂. Coronary flow, contractile force, coronary sinus oxygen levels, and adenosine and its degradative products in perfusates were measured before and during the infusion of varying doses of epinephrine, norepinephrine, histamine, or nitroglycerin. All four compounds produced significant increases in coronary flow. The catecholamines and histamine had a positive inotropic effect, increased myocardial oxygen consumption, and decreased coronary sinus oxygen levels. The decrease in coronary sinus oxygen was accompanied by increased levels of adenosine in the perfusates. Nitroglycerin, on the other hand, did not change contractile force, increased coronary sinus oxygen levels, and did not increase the rate of adenosine release. Changes in inosine and hypoxanthine, degradative products of adenosine metabolism, paralleled those of adenosine in all experiments. These findings indicate that adenosine release is intimately associated with a reduction of coronary sinus oxygen levels and, further, that adenosine is not a mediator in the vasodilation induced by nitroglycerin.

CHANGES IN coronary blood flow are linked closely to the oxygen needs of the myocardium. When coronary sinus oxygen levels (a reflection of myocardial cell oxygen tension) fall below a critical point, myocardial demand exceeds supply and coronary blood flow increases.1 The elevated flow provides additional oxygen to the myocardium to reestablish the necessary balance between supply and demand.

Adenosine, a potent coronary vasodilator, is a degradative product of adenine nucleotide metabolism and has been proposed as a mediator in the metabolic regulation of coronary blood flow.2-3 In support of this concept, adenosine has been detected in the normal, well-oxygenated dog heart,4 and adenosine levels have increased following periods of anoxia and hypoxia in isolated perfused hearts. In addition, adenosine levels increase following brief periods of coronary artery occlusion in dogs.5-7 Finally, the amount of adenosine released and the increase in coronary flow in the isolated nonworking perfused heart are closely related.8

There is, therefore, considerable evidence that adenosine production is increased in response to a reduction in the supply of oxygen to the myocardium. On the other hand, little data have been obtained relating an increased production of adenosine to increased myocardial oxygen demand. Katori and Berne3 showed that, in the presence of 8-azaguanine, an inhibitor of adenosine deaminase, epinephrine increased adenosine release from the perfused cat heart. More recently, angina pectoris induced by rapid atrial pacing has been shown to be accompanied by elevated adenosine levels in coronary sinus blood.9

Because there is a paucity of data relating increased myocardial oxygen demand to increased adenosine release we attempted to determine whether the relationship between increased adenosine release and increased coronary flow exists when changes in flow are induced by increasing the myocardial oxygen demand.

Epinephrine, norepinephrine, histamine, and nitroglycerin were selected because all produce an increase in coronary flow. The catecholamines and histamine are positive inotropic and chronotropic agents in the guinea pig and are associated with increased oxygen consumption. Nitroglycerin, on the other hand, has no effect on contractile force and reduces oxygen consumption.10

Methods

After guinea pigs were stunned by a blow on the head, their hearts were rapidly excised and immersed in ice-cold Krebs-Henseleit solution. The aortas were cannulated and the coronary vessels perfused with nonrecirculating Krebs-Henseleit solution (37°C, pH 7.4). The perfusion fluid was bubbled with 95% O₂-5% CO₂ and perfusion pressure was maintained constant at 60 cm H₂O with a modified Langendorff apparatus. Coronary flow was measured with a Statham electromagnetic flow probe placed in the perfu-
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sion circuit. A catheter was placed in the coronary sinus and a portion of the coronary venous effluent diverted through a small chamber in which the oxygen content of the effluent was measured by means of a calibrated polarographic electrode (Yellow Springs Instruments). Contractile force and rate were measured with a force transducer (Grass FT .03) attached via a string and clip to the apex of the heart. All parameters were recorded continuously (Beckman Dynograph).

After a 30-minute equilibration period, perfusates were collected for 10 minutes to establish basal levels of metabolite release. Contractile force, coronary sinus oxygen tension, and coronary flow also were monitored during this period to establish baseline levels. This period is referred to as "control," and subsequent changes in coronary flow and coronary sinus oxygen tension are expressed in terms of percent of control (control values = 100%).

Dose-response relationships were determined for the catecholamines and histamine as follows. At the end of the control period, infusion into the aortic cannula of one of these agents diluted in Krebs-Henseleit solution to a final concentration of 10 μg/ml (expressed as the salt) was begun and continued at a constant rate for 11 minutes. The coronary effluent was discarded during the first minute and collected during the subsequent 10-minute period. The infusion rate then was increased for 11-minute periods and the perfusate was collected as described. Dose-response curves were not obtained with nitroglycerin since constant infusion appeared to result in tachyphylaxis. The average control coronary flow in hearts used in this study approximated 5 ml/min and drug infusion rates of 0.010, 0.025, 0.050, and 0.20 ml/min resulted in calculated concentrations of 0.02, 0.05, 0.1, and 0.4 μg/ml of coronary perfusate. Since the infusion rate was constant during any one perfusion period, the perfusate concentration of drug decreased as coronary flow increased. Nucleosides and base (hypoxanthine) from perfusates collected during the control and experimental periods were adsorbed with activated charcoal (Norit A), eluted with 10% pyridine in 50% ethanol, air dried, then redissolved in 0.05 m phosphate buffer. Adenosine, inosine, and hypoxanthine were quantitated enzymatically7, 11, 12 with a dual-beam spectrophotometer (Acta C-III, Beckman Instruments). Adenosine, inosine, or hypoxanthine added to perfusion fluid with subsequent titration as described resulted in recoveries ranging from 80% to 85% of the added substrate, with an average recovery of 82%.

To determine whether changes in heart rate alone were associated with adenosine release, five guinea pig hearts were paced electrically using a pulse (3 V, 2 msec) from a Grass SM6 stimulator delivered via electrodes attached to the atria. After 30 minutes of equilibration, the spontaneous heart rate was recorded. The hearts then were paced sequentially at rates of 300, 350, 400, and 450 beats/min for 10 minutes. Per fusates were collected during each 10-minute period and adenosine was quantified as previously described.

Statistical analysis of the data was performed using Student's paired t-test, and differences were considered significant only if P < 0.05.

Drugs used were: histamine diphosphate (Eli Lilly), nitroglycerin (Parke, Davis) /-epinephrine hydrochloride (Parke, Davis) /-norepinephrine bitartrate (Winthrop Labs).

Results

All drugs produced coronary vasodilation. Figure 1 shows the time course and directional changes in selected

![Figure 1](http://circres.ahajournals.org/figs/)
DRUG-INDUCED DILATION AND ADENOSINE PRODUCTION/Wiedmeier and Spell 505

The data presented in Figure 2 show changes in coronary flow and coronary sinus oxygen levels attained at equilibrium during the infusion of 2.0 μg/min of the various agents used in this study. At this dose (2.0 μg/min), norepinephrine increased coronary flow to 250% of preinfusion levels (Fig. 2), whereas the same dose of histamine, epinephrine, or nitroglycerin induced smaller increases in flow to 160%, 145%, and 146% of control, respectively. The catecholamines and histamine caused a concomitant decrease in the oxygen content of the coronary sinus effluent (Fig. 2B). In contrast, coronary sinus oxygen tension was increased by nitroglycerin.

As shown in Figure 3, catecholamine- and histamine-induced coronary sinus oxygen tension and adenosine release are dose-dependent. Increased flow coupled with decreased coronary sinus oxygen levels observed during the infusion of these three agents is indicative of increased myocardial oxygen consumption. A progressive increase in the infused dose resulted in increased oxygen consumption, as shown in Table 1. At an infused dose of 2.0 μg/min, norepinephrine was the most potent stimulant of oxygen consumption. Linear regression analysis of the relationship between increased adenosine release and increased myocardial oxygen consumption induced by the catecholamines and histamine indicates a high positive correlation, as shown in Figure 4.

A comparison of adenosine changes observed in response to 2.0 μg/min of each of the catecholamines, histamine, and nitroglycerin is shown in Figure 5. Parallel changes in the perfusate levels of the nonvasoactive degradative products of adenosine metabolism were also observed at a dose of 2.0 μg/min (Fig. 6). Significant increases in inosine release were induced by histamine, epi-

experiments. Infusion of epinephrine or norepinephrine produced a prompt increase in contractile force accompanied by a decrease in the coronary sinus oxygen tension which was sustained during the duration of the infusion. The catecholamines also induced a transient decrease in coronary flow which lasted for approximately 30 seconds. This decrease was followed by a subsequent increase which was sustained for the duration of the drug infusion.

Biphasic flow and sinus oxygen tension responses were observed during the infusion of histamine. Coronary flow stabilized somewhat below the peak level but significantly above the preinfusion level (Fig. 1). A similar pattern was observed with changes in coronary sinus oxygen levels, i.e., a peak level followed by a decrease. The decrease in oxygen tension was more pronounced than the decrease in flow, however, resulting in a significant reduction of coronary sinus oxygen levels, compared with those observed during the control period.

Initial responses observed during the infusion of nitroglycerin (2.0 μg/min) were similar to those seen with histamine except that nitroglycerin had no inotropic effect. At equilibrium, the changes in coronary flow and coronary sinus oxygen levels were parallel, i.e., both increased and remained elevated throughout the nitroglycerin infusion period (Fig. 1).
neuropeptide, and norepinephrine, while only epinephrine and norepinephrine increased hypoxanthine levels above those observed during the control period.

Although catecholamines and histamine exert a positive chronotropic effect in the perfused guinea pig heart, changes in rate alone cannot account for the increased adenosine levels (Figs. 3-5). Table 2 is a comparison of adenosine release in spontaneously beating and paced hearts. Increasing the heart rate in 50-beat increments from 300 to 450 beats/min produced no significant increase in adenosine release or in contractile force. The maximal level of adenosine release in paced hearts in the absence of drugs was observed at a rate of 450/min (1.22 nmol/g per 10 min), whereas the maximal spontaneous rate (399/min with 0.5 μg/min norepinephrine) was associated with a release of 9.22 nmol/g per 10 min.

Discussion

These findings indicate that the increased oxygen demand induced by norepinephrine, epinephrine, and histamine results in increased adenosine production which is accompanied by an increase in coronary flow. The decreased coronary vascular resistance associated with the infusion of nitroglycerin, on the other hand, is not associated with the release of adenosine.

Increased coronary flow in response to catecholamines has been shown in numerous studies,13-16 and these drugs also have been shown to have a profound effect on oxygen consumption.13 Thus, myocardial oxygen imbalance can be induced not only by limiting the oxygen supply but also by interventions that increase myocardial oxygen demand. Changes in coronary sinus oxygen tension observed with both epinephrine and norepinephrine (Figs. 1, 2B, and 3) suggest that, initially, the increased myocardial oxygen requirement associated with the infusion of catecholamines is met by increased extraction of oxygen from the perfusion fluid. The amount of oxygen that can be supplied by increased extraction is limited, however, and flow must increase to meet the increased demand. The temporal relationship of changes in coronary flow and coronary sinus oxygen tension (Fig. 1) supports the view that catecholamines increase coronary flow indirectly by increasing myocardial metabolism and thus oxygen demand (Table 1). Increased levels of adenosine and its degradative products, inosine and hypoxanthine, in perfusates of hearts infused with catecholamines (Fig. 6) indicate that the observed increases in coronary flow are linked to myocardial metabolism and adenosine release. These changes are, moreover, dose-dependent (Fig. 3). Inosine and hypoxanthine, although not vasoactive, are metabolites of adenosine and probably reflect changes in tissue levels of this nucleoside.17

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** Linear regression analysis showing the relationship between adenosine release and myocardial oxygen consumption in response to increasing amounts of histamine (△), epinephrine (●), and norepinephrine (●). Y = the equation for the line; R = correlation coefficient. Data are mean values taken from Tables 1 and 2.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5** Adenosine production prior to (open bars) and during (hatched bars) the infusion of histamine, epinephrine, norepinephrine, or nitroglycerin, 2.0 μg/min. Bars represent the mean ± standard error of the mean, and filled circles indicate a significant change (P < 0.05).
Histamine has previously been reported to increase contractile force and coronary flow in the dog and guinea pig. Figure 1 indicates that the transient increase in coronary sinus oxygen tension induced by histamine may be the result of a direct dilating effect of this drug. The subsequent increased oxygen need associated with increased metabolism (Table 1) results in an increase in oxygen extraction and a subsequent fall in coronary sinus oxygen tension. The biphasic flow and coronary sinus oxygen response might be attributable to a component of direct vasodilation superimposed on metabolic dilation associated with decreased coronary sinus oxygen tension. These responses may be mediated via H₁ and H₂ receptors; H₁ receptor mediation producing direct vasodilation; and H₂ receptor mediation producing metabolic effects. The above findings suggest a similar mechanism of action for histamine and catecholamines during the steady state response.

If low myocardial oxygen tension stimulates adenosine release, there should be increased release of adenosine accompanying the fall in coronary sinus oxygen tension and the increase in coronary flow produced by infusion of histamine and catecholamines. That this relationship does exist is shown by the data presented in Figures 3 and 4. The high correlation (r = 0.90) between adenosine release and oxygen consumption over a range of drug doses (Fig. 4) provides further support for this concept.

The decrease in coronary vascular resistance induced by nitroglycerin appears to be mediated through a different mechanism, i.e., flow and oxygen utilization are dissociated. At no time during the infusion of nitroglycerin was there a decrease in coronary sinus oxygen tension observed (Fig. 1). On the contrary, the oxygen level increased sharply, as did coronary flow, indicating that nitroglycerin does not depend upon decreased myocardial oxygen levels to produce its vasodilating effect. If adenosine release is linked to decreased myocardial oxygen tension, levels of adenosine in the perfusate would not be expected to increase during the infusion of nitroglycerin. As shown in Figure 5, this was indeed the case. These findings are supported by previous reports suggesting an "oxygen sparing" effect of nitroglycerin.

The positive chronotropic effect associated with catecholamines and histamine is not a major factor in the release of adenosine observed in the studies since, at paced rates of from 300 to 450 beats/min, adenosine release is increased above control levels in unpaced hearts (Table 2) but is not rate-dependent. When the increased rate is accompanied by an increase in contractile force, adenosine release is well correlated with the increased oxygen consumption.

These findings extend and support the concept that adenosine may play a major role in the regulation of coronary blood flow.

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Cholinergic Intervention on Myocardial Dynamics and Metabolism in the Nonworking Dog Heart

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SUMMARY In electrically paced hearts of dogs subjected to cardiopulmonary bypass, intracoronary infusion of carbamylcholine chloride (carbachol; 1 µg/kg per min) caused, in the absence of external cardiac work, an increase in coronary blood flow (46%) and a decrease in both myocardial force of contraction (53%) and systemic perfusion pressure (20%). Pretreatment with atropine effectively blocked these responses to carbachol. Previous treatment with reserpine indicated that adrenergic and cholinergic interactions did occur, as observed from the more significant changes in these parameters in the absence of catecholamines. Compared with control, carbachol significantly lowered both myocardial extraction ratio (50-25%) and uptake of oxygen (4.2-3.1 ml/min per 100 g). The decrease in oxygen metabolism accompanied a significant increase in both myocardial extraction (6-18%) and myocardial uptake (1.3-5.2 ng/min per 100 g) of plasma triglycerides. The increase in triglyceride uptake was accompanied by a decline in intracellular triglyceride hydrolysis, as verified by more than a 2-fold increase in percent of "3"C-tripalmitin incorporation. This change was concurrent with a 3-fold increase in intracellular triglyceride synthesis from "3"H-sodium palmitate substrate. While triglyceride uptake increased partly because of an increase in coronary blood flow, it was postulated that carbachol decreased myocardial cyclic adenosine 3',5'-monophosphate (cyclic AMP) by a mechanism similar to that previously reported for acetylcholine and prostaglandin E,

THE EFFECTS OF cholinergic substances on myocardial metabolism have been investigated extensively since Szekeres et al. reported that acetylcholine (ACh) prevented a fall in cardiac muscle stores of glycogen in rats exposed to hypoxia. Since this work was reported, ACh has been shown to reduce formation of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in particulate preparations of adenylate cyclase from dog hearts. This study was followed by the observation that ACh caused a rise of cyclic guanosine 3',5'-monophosphate (cyclic GMP) in the isolated perfused rat heart. One of the most significant actions of the cholinergic neurotransmitter has been its regulation of cardiac dynamics and metabolism through its interaction with adrenergic substances in heart muscle. Blukoo-Allotey et al. showed cholinergic-adrenergic interaction when they found that simultaneous administration of epinephrine and ACh inhibited glycolysis by epinephrine. Glaviano et al. reported that ACh antagonism of norepinephrine stimulation of lipid uptake and utilization in the intact dog heart was mediated through
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