Adenosine and Metabolic Regulation of Coronary Blood Flow in Dogs with Renal Hypertension

Stephen W. Ely, Ph.D., Chi-Wen Sun, M.D., Robert M. Knabb, Ph.D., Jeffrey M. Gidday, B.S., Rafael Rubio, Ph.D., and Robert M. Berne, M.D.

SUMMARY It has been demonstrated that resting coronary vascular resistance is elevated with chronic hypertension and concomitant cardiac hypertrophy. The present study employed a model of 6-week, one-kidney, one-wrapped Page hypertension to determine if the ability of the heart to match an increase in oxygen demand with an increase in oxygen supply (coronary blood flow) is impaired, and to determine if these vasoregulatory abnormalities are attributable to inadequate adenosine release. Studies were performed in a pentobarbital anesthetized, open-chest canine preparation using a pericardial infusate method to determine adenosine release. Results showed that dobutamine (a beta-receptor agonist) induced increases in myocardial oxygen consumption (MVO₂) over a physiological range (8-30 ml O₂/min⁻¹·100 g⁻¹) that were accompanied by an increase in coronary blood flow (CBF) with no change in oxygen extraction. The relationship between MVO₂ and CBF was not different between the normotensive (NTC) and hypertensive (RHT) animals. Pericardial infusate adenosine (PI ADO) concentrations were not different for the same MVO₂ and CBF, and the relationships for MVO₂ vs PI ADO as well as PI ADO vs CBF were unaltered by hypertension. However, the relationship between PI ADO and coronary vascular resistance (CVR) was altered in the RHT group such that a given PI ADO concentration was associated with a significantly higher CVR. These data suggest that, over the range of MVO₂ studied, there are no limitations in metabolic regulation of the coronary circulation of RHT animals, and that the higher CVR encountered in the RHT group is not the result of a reduced release of the endogenous vasodilator, adenosine.

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Key Words: myocardial metabolism • local blood flow regulation • adenosine nucleosides • pathophysiology • dobutamine

CARDIAC hypertrophy induced by hypertension has been shown to adversely affect the coronary circulation. Such effects include elevated coronary vascular resistance at rest, elevated minimal coronary vascular resistance, and reduced capillary density. The limited coronary vasodilatory reserve may render the hypertrophied myocardium more vulnerable to ischemia. It is unclear, however, whether this limitation has any effect on the ability of the heart to meet a change in oxygen demand over the physiological range with a change in oxygen supply (coronary blood flow).

Experimental evidence suggests that the limited coronary reserve in the hypertensive-hypertrophied heart may be the result of the failure of new vessel growth to keep pace with the rate of hypertrophy, a reduction of vascular cross-sectional area due to hypertension-induced hypertrophy of vascular smooth muscle, or extravascular compression of the intramural coronary vessels by the hypertrophied myocardium. Other factors critical to the regulation of coronary vascular resistance include metabolic factors. The coronary circulation is controlled primarily by a metabolically linked mechanism, and work from our laboratory as well as others suggests that the vasoactive metabolite adenosine, may be an important feature of this mechanism.

In view of the fact that resting coronary vascular resistance is elevated in hypertension, we proposed to determine if the ability of the heart to increase coronary blood flow in response to an increase in myocardial metabolism is impaired, and to determine if these vasoregulatory abnormalities are attributable to inadequate adenosine release.
Methods

Hypertensive Model

We employed a one-kidney, one wrapped model of page hypertension. Male dogs weighing 20 to 30 kg were anesthetized with sodium pentobarbital (30 mg/kg). A right flank incision was made under aseptic conditions, and the right kidney was wrapped in silk and then Saran Wrap. The incision was closed, and antibiotics were administered (Combicotic, 5000 units/lb/day) for 2 weeks. One week following the wrapping procedure, the animals were again anesthetized, a left flank incision was made, and the left kidney removed. The animals were then housed for 6 weeks and maintained on a standard diet with water ad libitum.

Experimental Protocol

Renal hypertensive (RHT) and normotensive control (NTC) dogs (20–30 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The animals were intubated and ventilated with a Harvard (607) positive pressure respirator (Harvard Instruments, Harvard, Massachusetts) on room air enriched with 95% O₂, 5% CO₂) with the following millimolar composition: NaCl, 121.4; KCl, 4.7; CaCl₂, 2.5; NaHCO₃, 21.9; MgSO₄, 1.2; KH₂PO₄, 1.2; glucose 11.1.

Following cannulation of the left coronary artery, the animals were allowed to stabilize for 45 minutes before samples were taken. Samples were taken during steady state conditions as evidenced by a stable heart rate, mean arterial blood pressure, and coronary blood flow. The effects of a 10 μg/kg/min i.v. infusion of dobutamine (a beta-receptor agonist that stimulates cardiac contractile force), on heart rate, blood pressure, coronary blood flow, myocardial oxygen consumption, and adenosine release were compared with control conditions. Dobutamine (Eli Lilly and Company, Indianapolis, Indiana) was infused (0.8 ml/min) until steady-state elevations in heart rate, blood pressure, and coronary blood flow were observed (5 to 10 minutes). Upon reaching a steady state, the pericardial space was flushed 5 times with 25 ml aliquots of the Krebs-Henseleit solution. Then a 25 ml aliquot was infused into the pericardial space and withdrawn following a 4.5-minute contact time. This aliquot was placed in a 125 ml Ehrlenmeyer flask immersed in boiling water bath for 10 minutes. At the halfway point during the 4.5-minute sampling period, paired arterial and coronary sinus blood samples were withdrawn anaerobically into heparinized glass syringes, capped, and kept on ice until analyzed for oxygen content (Lex-O₂-con, Lexington Instruments, Lexington, Massachusetts).

At the termination of the experiment, the heart was quickly arrested with potassium chloride solution (25 mEq/liter, 4°C) perfused through the aortic root for 2 minutes, until the coronary vessels were cleared of blood. The hearts were then perfused with a 2% glutaraldehyde in 0.2 M sodium phosphate buffer (pH 7.4) for 15 minutes. The hearts were weighed and tissue samples from the left ventricle subepicardium, midwall, and subendocardium were taken and placed in the primary fixative. The tissues were processed for electron and light microscopy. Electron microscopy
showed adequately fixed tissue with open, clear capillaries. Samples for light microscopy were cut in cross section and stained with toluidine blue. Muscle fiber diameters were determined using a Zeiss Videoplan system (Carl Zeiss, Inc., New York, New York) which performed computerized planimetry on the 5 µm tissue sections. The cell circumference was digitized, and the minimum diameter (µm) was determined and used as an indication of fiber size. Only those cells with a centrally located nucleus were analyzed. In each animal, 100 fibers from subepicardial, midwall, and subendocardial regions were analyzed.

Sample Processing and Analysis

After immersion in the boiling water bath for 10 minutes, pericardial infusate samples were kept on ice until the end of the experiment when further processing was complete. The samples were centrifuged to remove calcium precipitates and denatured protein, and evaporated. The dried residue was reconstituted in distilled water, filtered (Millipore, 0.22 µm, Millipore Filter Corporation, Bedford, Massachusetts) and analyzed by high performance liquid chromatography. We employed reverse-phase liquid chromatography (Waters Associates, M-6000-A pump, Model 44 absorbance detector) in which 100 µl of processed sample was injected onto a 1 cm spherisorb-5-RP18 guard column and ultrasphere-5-C18 analytical column with a single mobile phase (isocratic elution method) consisting of a 4 mM KH2PO4 buffer, pH 4.6 with 5% methanol, and a flow rate of 1.5 ml/min. The ultraviolet absorbance of the sample was monitored at 254 nm and recorded on a strip recorder.

The precision of the assay was determined by repetitive assay of individual samples. The results indicated that the variance of the assay was 10% in the range of adenosine concentrations encountered. Recoveries averaged 85%, and reported adenosine values were not corrected for these losses during processing and analysis.

Adenosine peaks were identified by comparing the retention time of the sample to known standards, and confirmed by the disappearance of the adenosine peak when treated with adenosine deaminase (Sigma Chemical Company, St. Louis, Missouri). The peaks were quantified with peak height and external standards. This method was linear over the range of 0.01 to 1.0 µM. Linear regression analysis for peak height vs adenosine concentration yielded \( r = 0.99 \) (n = 8).

Statistical Analysis

Student’s \( t \) test was used to determine statistical differences between groups (NTC vs RHT), and Student’s paired \( t \) test was used to determine differences within groups. Linear regression analysis was used to determine the correlation between two variables. All results are expressed as means ± standard error, and a \( p \) value of \(< 0.05\) was considered significant.

Results

The anatomical data for hearts obtained are shown in table 1. Six weeks of hypertension resulted in a significant increase in left ventricle (LV) weight (29%), LV to body weight ratio (38%), and a significant increase in muscle fiber diameters in the subendocardial (24%), midwall (36%), and subepicardial (29%) regions. The capillary fiber ratio was 1:1 in both the NTC and RHT.

Differences between the NTC and RHT groups as well as the effects of dobutamine within each group on each of the measured variables are shown in table 2. During control conditions, the RHT group had a significantly higher mean arterial blood pressure (29%) and coronary vascular resistance (27%). Dobutamine infusion (10 \( \mu \)g·kg\(^{-1}\)·min\(^{-1}\)) produced similar increases in both groups (NTC and RHT) in heart rate, blood pressure, myocardial oxygen consumption (MVO\(_2\)), coronary blood flow (CBF), and pericardial adenosine (PI ADO) release. Coronary resistance was decreased in both groups to a similar extent.

Linear regression analysis was performed to determine the relationships between MVO\(_2\) and CBF, MVO\(_2\) and PI ADO, and PI ADO and CBF. These data are plotted in figures 1, 2, and 3, respectively. Regression analysis demonstrated that, in the NTC animals, a significant relationship existed between MVO\(_2\) and PI ADO as well as PI ADO and CBF. These data support the previous work from our laboratory.\(^{23, 24}\) They also demonstrate that, as oxygen consumption was increased by dobutamine to levels that even exceed those obtained in the conscious dog,\(^{24}\) running on a treadmill (4 mph, 10% grade), there was a parallel increase in adenosine release, and the increase in adenosine release is associated with a parallel increase in coronary blood flow. It is important to note that oxygen extraction did not change significantly during dobutamine infusion (table 2). Significant linear relationships also existed between MVO\(_2\) and CBF, MVO\(_2\) and PI ADO, and for PI ADO and CBF in the animals with 6-week

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Anatomical Data for Control and Hypertrophied Dog Hearts</th>
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<tbody>
<tr>
<td></td>
<td>Muscle fiber diameters</td>
</tr>
<tr>
<td></td>
<td>Endo (µm)</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>LV wt (g)</td>
</tr>
<tr>
<td>NTC (n = 6)</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>RHT (n = 6)</td>
<td>23 ± 1</td>
</tr>
</tbody>
</table>

Results expressed as means ± SEM. RHT = renal hypertensives; BW = body weight; LV = left ventricle; Endo = subendocardium; Mid = midwall, Epi = subepicardium; wt = weight. *\( p < 0.05\) from normotensive controls (NTC).
Table 2. Effects of Dobutamine in Normotensive Control (NTC) and Renal Hypertensive (RHT) Dogs

<table>
<thead>
<tr>
<th></th>
<th>NTC</th>
<th>RHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>144 ± 14</td>
<td>189 ± 7*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>104 ± 6</td>
<td>130 ± 6*</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min-1100 g-1)</td>
<td>53 ± 3</td>
<td>108 ± 10*</td>
</tr>
<tr>
<td>A-V O₂ (extraction) (ml O₂/100 ml-1)</td>
<td>15.9 ± 0.6</td>
<td>16.8 ± 0.8</td>
</tr>
<tr>
<td>MVO₂ (ml/min-1100 g-1)</td>
<td>8.6 ± 0.7</td>
<td>17.9 ± 1.4*</td>
</tr>
<tr>
<td>Coronary vascular resistance (mm Hg/ml/min-1100 g-1)</td>
<td>1.95 ± 0.13</td>
<td>1.25 ± 0.13*</td>
</tr>
<tr>
<td>Pericardial adenosine (pmol/ml-1)</td>
<td>56 ± 3</td>
<td>120 ± 12*</td>
</tr>
</tbody>
</table>

Results expressed as means ± SEM. NTC: n = 6; RHT: n = 6; Dobut = dobutamine, 10 μg·kg⁻¹·min⁻¹ i.v.; MVO₂ = myocardial oxygen consumption; \*p < 0.05 from paired control.

Figure 4 demonstrates that, at the same level of MVO₂ and CBF, the concentration of adenosine in the pericardial infusate in the NTC was not different from that obtained in the renal hypertensive dogs during control conditions. With dobutamine infusion, MVO₂, CBF, and PI ADO were elevated significantly, but no difference between groups was observed.

Figure 5 depicts the relationship between PI ADO and coronary vascular resistance during control and dobutamine infusion conditions in the NTC and RHT groups. A significant linear relationship between these variables was determined for both the NTC and RHT animals. In the RHT group, however, a given concentration of PI ADO corresponded to a higher coronary vascular resistance during control conditions and during dobutamine infusion.

Discussion

Our present study demonstrates that there is no apparent impairment in the ability of the hypertensive-hypertrophied heart to metabolically regulate coronary blood flow over a range of myocardial oxygen consumption considered to be physiological. Previous studies have documented an impaired coronary vascular reserve in hypertension.\(^{1,4,8}\) In our present study, however, as oxygen consumption was increased from 8 to 30 ml O₂/min/100 g LV, a concomitant increase in renal hypertension (RHT) (figures 1, 2, and 3 respectively), and there was no difference in these relationships between the NTC and RHT groups.

Figure 1

Regression analyses of myocardial oxygen consumption (MVO₂) vs mean coronary blood flow (CBF). NTC = normotensive control; RHT = renal hypertensive. C = control data points; D = dobutamine (10 μg·kg⁻¹·min⁻¹ i.v.) data points. Circled letters indicate data points from RHT animals. All r values are significant at p < 0.01.
**Figure 2.** Regression analysis of myocardial oxygen consumption (MVO₂) vs pericardial infusate adenosine (PI ADO) concentration. See figure 1 for abbreviations.

**Figure 3.** Regression analysis of pericardial infusate adenosine (PI ADO) concentration vs mean coronary blood flow (CBF). See figure 1 for abbreviations.

**Figure 4.** Pericardial infusate adenosine (PI ADO) concentrations for normotensive controls (NTC, n = 6) and renal hypertensive (RHT, n = 6) dogs are shown for control and dobutamine infusion. Values are means ± SEM. Coronary blood flow (ml/min/100g⁻¹) (CBF) for control NTC was 53 ± 3; for control RHT, 55 ± 3; for dobutamine-treated NTC, 108 ± 10,* and for dobutamine-treated RHT, 111 ± 14.* Myocardial oxygen consumption (MVO₂) (ml/min/100g⁻¹) for control NTC was 8.6 ± 0.7; for control RHT, 8.3 ± 0.2; for dobutamine-treated NTC, 17.9 ± 1.4,* and for dobutamine-treated RHT, 18.7 ± 2.7.* Asterisk indicates p < 0.05 from paired control.
coronary blood flow and hence oxygen delivery occurred without a significant change in oxygen extraction. The relationship between myocardial oxygen consumption and coronary blood flow was similar in the NTC and RHT groups. Although we observed a normal relationship between MVO₂ and CBF, the detrimental effects of a limited coronary vasodilatory reserve in the hypertensive heart may become manifest with higher levels of oxygen consumption, a more severe degree of ventricular hypertrophy, or perhaps most important, with superimposed coronary artery stenosis.

Several mechanisms have been implicated in the elevated resting and minimal vascular resistances seen in hypertension. They include myogenic constriction, structural changes in the vasculature resulting in an increased wall/lumen ratio, augmented reactivity to vasoconstrictor agents, decreased reactivity to vasodilator agents, or extravascular compression of the intramural coronary vessels by the hypertrophied myocardium. We sought to examine the possibility that a reduced release of an endogenous vasodilator (adenosine) is involved in the elevated coronary vascular resistances seen with hypertension. We assessed adenosine release using a pericardial infusate technique in which subepicardial interstitial adenosine diffuses across the epicardium into the infusate during a 4.5-minute contact time. Detailed discussions concerning the advantages and limitations of this method have been reported previously. Infusate concentrations of adenosine may not equilibrate with interstitial concentrations and therefore cannot be used as a quantitative measure of interstitial adenosine concentration. However, this method is believed to be useful as a proportional index of changes in interstitial adenosine concentration as pericardial infusate values reflect changes in tissue and coronary sinus plasma adenosine concentrations. At present, the pericardial infusate method appears to be the best method of estimating interstitial adenosine concentration due to the fact that tissue measurements are complicated by the presence of an intracellular pool of adenosine. Adenosine release into coronary sinus plasma is also used as an index of myocardial adenosine release. A preliminary report from our laboratory using the coronary plasma adenosine release method in the hypertensive dog confirmed the results of our present study utilizing the pericardial infusate method. However, we have observed a large variability using the coronary plasma method whereas, by comparison, the pericardial infusate method is relatively invariant.

Data from our present study utilizing the pericardial infusate method suggest that the relationships among oxygen demand, adenosine release, and oxygen sup-
ply are not altered in the hypertensive heart. Although coronary vascular resistance is elevated in the RHT group at rest and during dobutamine infusion, the concentration of adenosine in the pericardial infusate is not different between the two groups (RHT vs NTC) during the two conditions studied. Thus, it appears that a reduced release of adenosine, as judged by the pericardial infusate technique and by coronary venous washout, may not be responsible for the elevated resting coronary vascular resistance seen in the hypertensive heart. We cannot rule out the possibility of an altered sensitivity of coronary vascular smooth muscle to adenosine. It is difficult to interpret dose-response relationships to exogenous adenosine applied in vivo due to the immense ability of the vascular endothelial cells to take up adenosine. However, in vitro studies by Cohen and Berkowitz have shown that the sensitivity to adenosine is reduced in vascular strips obtained from hypertensive animals. Also, sensitivity to nitroglycerin and isoproterenol was reduced in vessel strips from hypertensive animals, suggesting that there may be a primary defect in the ability of vascular smooth muscle from hypertensive vessels to relax.

In summary, we employed a one-kidney, one wrapped model of Page hypertension in the dog to determine the relationships between myocardial oxygen consumption, adenosine release, coronary blood flow, and coronary vascular resistance. Oxygen consumption manipulated by dobutamine infusion resulted in no impairment in metabolic blood flow regulation. The elevated coronary resistance seen in hypertension at rest and during dobutamine infusion was not associated with a reduced release of adenosine. Other factors such as impaired sensitivity of vascular smooth muscle to endogenous vasodilators, inability of smooth muscle to relax normally, or increased wall/lumen ratio may be responsible for the vascular abnormalities seen in hypertension, and require further investigation.

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