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Impact of Coronary Risk Factors on Contribution of Nitric Oxide and Adenosine to Metabolic Coronary Vasodilation in Humans

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Objectives. **The contribution of nitric oxide (NO) and adenosine to the increase in coronary blood flow (CBF) induced by cardiac pacing was investigated in 28 subjects with angiographically normal coronary arteries with and without one or more risk factors for atherosclerosis.**

Background. **NO and adenosine are important in the regulation of coronary circulation, and the inhibition of NO synthesis increases adenosine production during cardiac pacing in experimental models.**

Methods. **Coronary artery diameters and CBF were assessed by quantitative coronary arteriography and Doppler flow velocity measurement. Plasma levels of nitrites and nitrates (NOx) (stable end products of NO), adenosine and lactate were measured, and blood gas analysis was performed.**

Results. **The extent of CBF response to cardiac pacing did not differ between the 14 subjects with and the 8 subjects without risk factors for atherosclerosis. NOx (12.0 ± 0.9 vs. 14.9 ± 1.1 μmol/liter [mean ± SD], $p < 0.05$), but not adenosine (50.8 ± 7.2 vs. 50.8 ± 6.5 nmol/liter), levels in coronary sinus blood increased in the subjects without risk factors. In contrast, adenosine (58.9 ± 7.5 vs. 77.4 ± 9.8 nmol/liter, $p < 0.05$), but not NOx (11.1 ± 1.1 vs. 12.2 ± 1.1 μmol/liter), levels increased in subjects with risk factors. Aminophylline, an antagonist of adenosine receptors, blunted CBF response to cardiac pacing in six subjects with risk factors. The number of risk factors showed a negative correlation ($p < 0.05$) with NOx production and a positive correlation ($p < 0.05$) with adenosine production during cardiac pacing, respectively.**

Conclusions. **NO and adenosine are increased during metabolic coronary vasodilation induced by cardiac pacing. Adenosine production may be a compensatory mechanism when NO production is reduced.**

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Coronary blood flow (CBF) increases in response to an increased metabolic demand of the myocardium (1). Although the precise mechanism remains unknown, it is likely that metabolic, myogenic and endothelial factors are involved in the regulation of the tone of coronary smooth muscle (2). The vascular endothelium plays an important role in the modulation of smooth muscle function by releasing vasoactive substances (3,4). Nitric oxide (NO) is an important endothelium-derived relaxing factor (5,6), the release of which can be stimulated by a variety of pharmacologic agents (7) and by physiologic stimuli that increase shear stress in blood vessels (8,9). Although several studies have shown that an inhibitor of NO synthase reduces the CBF response to increased myocardial oxygen consumption (MVO_2) induced by rapid pacing in dogs (10) and humans (11), others have detected no such effects in these models (12,13). Also, it has not been clarified whether NO levels indeed increase in response to increased MVO_2.

The development of risk factors for atherosclerosis, such as hypercholesterolemia (HC), hypertension (HTN) or diabetes mellitus (DM), may reduce the vasodilator response to pharmacologic agents, indicative of endothelial dysfunction (11,14–16). Recently, Quyyumi et al. (11) showed that both the extent of the CBF response to cardiac pacing and the contribution of NO to this response are reduced in subjects with risk factors for coronary atherosclerosis. Adenosine is a potent vasodilator that participates in the regulation of the coronary circulation (1,17). The inhibition of NO synthesis increases adenosine production under baseline conditions (18,19), during the increase in CBF after brief periods of myocardial ischemia (20) and during increased MVO_2 induced by pacing (21), suggesting that adenosine production increases when NO production is impaired. However, it is not clear whether risk factors affect

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Abbreviations and Acronyms

ANOVA = analysis of variance
CBF = coronary blood flow
CVR = coronary vascular resistance
DM = diabetes mellitus
HC = hypercholesterolemia
HTN = hypertension
MVO_2 = myocardial oxygen consumption
NO = nitric oxide
NOx = nitrites and nitrates

the production of NO or adenosine, or both, during stress and whether adenosine compensates for the CBF response to cardiac pacing when NO production is impaired in humans.

In the present study, we measured plasma levels of nitrites and nitrates (NOx), stable end products of NO (22) and adenosine in coronary sinus blood during cardiac pacing in subjects with angiographically normal coronary arteries with and without risk factors for atherosclerosis. We also examined the role of increased adenosine during cardiac pacing in the subjects with risk factors by means of aminophylline, an antagonist of adenosine receptors (23). We further determined whether the presence of risk factors affects production of NOx and adenosine during cardiac pacing.

Methods

Subjects. We studied 28 subjects with angiographically normal or nearly normal (<5% narrowing) coronary arteries undergoing diagnostic cardiac catheterization for evaluation of chest pain (Table 1). Subjects with previous myocardial infarction, valvular heart disease or evidence of left ventricular dysfunction were excluded. Eight subjects (two men, six women; mean [±SD] age 61 ± 2 years) had none of the following risk factors for atherosclerosis: HTN (blood pressure >140/90 mm Hg), HC (serum total cholesterol >220 mg/dl) or DM (fasting blood glucose >110 mg/dl). The remaining 22

Table 1. Patient Characteristics

Subject No./Gender	Age (yr)	Cholesterol Level (mg/dl)	HTN	DM	Medical Therapy			
					Nitrates	Calcium Channel Blocker	Beta-Blocker	ACEI
No risk factors								
1/F	44	164	−	−	−	+	−	−
2/F	66	192	−	−	+	+	−	−
3/F	63	214	−	−	−	−	−	−
4/M	60	183	−	−	−	−	−	−
5/M	67	215	−	−	−	−	−	−
6/F	52	170	−	−	+	−	−	−
7/F	74	146	−	−	+	−	−	−
8/F	53	218	−	−	−	+	−	−
Risk factors								
9/F	67	174	+	−	+	−	−	+
10/M	68	138	−	+	+	+	−	−
11/M	74	180	+	+	−	−	+	−
12/M	72	227	−	−	+	−	−	−
13/M	59	226	+	−	−	+	−	−
14/F	63	266	−	−	+	−	−	−
15/M	66	253	+	+	+	+	−	−
16/M	57	218	+	−	+	−	+	−
17/M	66	187	+	−	+	+	−	+
18/F	71	241	+	−	−	+	−	−
19/M	62	194	+	+	−	−	−	−
20/M	68	252	+	+	−	+	−	−
21/F	58	243	−	+	+	−	−	−
22/F	71	226	+	−	+	−	−	−
Risk factors + aminophylline								
23/M	56	204	+	−	−	+	−	−
24/M	67	114	+	+	−	+	−	−
25/F	81	230	−	−	−	−	−	−
26/F	62	200	+	−	−	+	−	−
27/F	53	173	+	−	−	+	−	−
28/M	51	215	+	+	−	−	−	+

ACEI = angiotensin-converting enzyme inhibitor; DM = diabetes mellitus; F = female; HTN = hypertension; M = male; + = yes; − = no.

subjects with one or more of these risk factors were classified into two groups: 14 subjects without (9 men, 5 women; mean age 66 ± 1 years) and 6 subjects with (3 men, 3 women; mean age 62 ± 5 years) aminophylline treatment before pacing.

Protocol. Cardiac catheterization was performed with subjects in the fasting state. Antianginal and antihypertensive medications were discontinued at least 12 h before the study. A 6F bipolar pacing catheter was positioned at the right atrium. After baseline hemodynamic variables at steady state were recorded, the pacing rate was increased by 20 beats/min every 3 min to the highest rate attainable without inducing second-degree atrioventricular block. CBF velocity was measured with a Doppler flow wire, and coronary arteriography was performed at rest and immediately after the end of the highest pacing rate. Coronary sinus blood was sampled at each steady state. In six subjects with risk factors, the effects of adenosine antagonism with aminophylline on the CBF response to pacing were assessed (23). After aminophylline was infused intravenously at the dose of 6 mg/kg body weight over 10 min, the previous protocol was repeated. The study protocol was approved by the institutional review committees on human research of Osaka University School of Medicine and Ishinkai Yao General Hospital. All subjects gave written informed consent.

Estimation of coronary artery diameter and blood flow. Coronary angiograms were recorded with a cineangiographic system (Toshiba). Quantitative angiography was performed with ARTEK software (CCIP-310, Cathex, Tokyo, Japan) (24). An appropriate view that permitted clear visualization of the target artery was selected. The view angle, the distance from the X-ray focus to the object and the distance from the object to the image intensifier were maintained constant during the study. An end-diastolic frame of the arteriogram was selected, and the lumen diameter of the segment of the artery distal to the Doppler wire was determined with a validated densitometric analysis system. The diameters were measured by examiners who had no knowledge of the clinical characteristics of the subjects. The size of the Judkins catheter was used to calibrate the arterial diameter. A 6F guide catheter was introduced into the left main coronary artery, and blood flow velocity was measured with an 0.018-in. wire equipped with a Doppler crystal at its tip (Cardiometrics FloWire) (16). The Doppler flow wire was advanced into either the left main coronary artery or the proximal segment of a major epicardial coronary artery. CBF was calculated from $\pi \times$ Average peak flow velocity $\times 0.125 \times$ (Arterial diameter)^2 (16). Coronary vascular resistance (CVR) was calculated as the mean arterial pressure divided by CBF. A 7F multipurpose catheter was inserted through the right cubitus vein into the mid-coronary sinus for blood sampling.

Chemical analysis. Plasma levels of lactate in coronary sinus venous blood were determined enzymatically (25). The lactate extraction ratio was calculated by the following formula: (Arterial lactate level − Venous lactate level)/Arterial lactate level × 100 (26). Blood gas analysis was performed with an ABL300 blood gas analyzer (Radiometer, Copenhagen, Denmark). MVO_2 was calculated as the product of CBF and the difference in oxygen content of coronary artery and venous blood. Plasma levels of adenosine were measured by radioimmunoassay, as previously described (26,27). Adenosine is thought to be degraded rapidly (1,17). To minimize this degradation, we sampled blood with a syringe containing dipyridamole, an inhibitor of adenosine uptake, and added the samples to the solution that contained an inhibitor of adenosine deaminase and a chelator of calcium. The sampling time was <10 s for all subjects. Although some amount of adenosine may have been degraded during the sampling period, the sampling times at rest and at the end of cardiac pacing were similar. Thus, the ratio of the measured to absolute levels of adenosine should have been similar at rest and at the end of cardiac pacing. Plasma NOx levels were assayed with the Griess reagent, as previously described (28).

Statistical analysis. Results are expressed as mean value ± SD. Serial changes in plasma NOx and adenosine levels and hemodynamic variables during cardiac pacing in subjects with and without risk factors were compared using two-way repeated measures analysis of variance (ANOVA). Hemodynamic and angiographic variables under baseline conditions among the subjects with and without risk factors and those treated with aminophylline were compared with one-factorial ANOVA followed by the Bonferroni multiple comparison test. The total number of risk factors was considered a continuous variable and examined by linear regression analysis. Finally, the effects of risk factors on NOx and adenosine production were examined by multiple linear regression analysis. A p value <0.05 was considered statistically significant.

Results

There were no significant differences in hemodynamic variables, such as heart rate, rate-pressure product, MVO_2, lactate extraction ratio, coronary artery diameter, CBF and CVR during baseline conditions between subjects with and without risk factors (Table 2). Cardiac pacing significantly ($p < 0.05$) increased rate-pressure product, MVO_2, coronary artery diameter and CBF and decreased CVR in subjects with and without risk factors, respectively (Table 2). NOx and adenosine levels in coronary sinus blood during baseline conditions did not differ significantly between subjects with and without risk factors (Fig. 1). In subjects without risk factors, NOx, but not adenosine, levels increased in response to cardiac pacing (Fig. 1). In contrast, in subjects with risk factors, adenosine, but not NOx, levels increased in response to cardiac pacing (Fig. 1). Intravenous infusion of aminophylline did not significantly change heart rate (70 ± 4 vs. 78 ± 4 beats/min), mean blood pressure (109 ± 6 vs. 114 ± 6 mm Hg) or rate-pressure product (76 ± 7 vs. 89 ± 7 mm Hg·beats/min·10^{-2}). Hemodynamic variables in subjects treated with aminophylline did not significantly differ from those at baseline in subjects with and without risk factors. Aminophylline blunted the increases in CBF and coronary artery diameter in response to cardiac pacing in subjects with risk factors (Table 2).

Table 2. Changes in Hemodynamic and Metabolic Variables During Cardiac Pacing

	No Risk Factors		Risk Factors		Risk Factors + Aminophylline	
	Baseline	Pacing	Baseline	Pacing	Baseline	Pacing
HR (beats/min)	70 ± 10	128 ± 5*	70 ± 10	131 ± 9*	78 ± 9	138 ± 21*
RPP (mm Hg·beats/min·10^{-2})	94 ± 11	173 ± 16*	98 ± 21	181 ± 26*	89 ± 18	163 ± 21*
MVO_2 (ml/min)	5.6 ± 2.9	8.1 ± 3.6*	4.5 ± 4.1	9.1 ± 4.5*	—	—
Lactate extraction ratio (%)	30 ± 23	36 ± 25	35 ± 11	32 ± 10	—	—
Coronary artery diameter (mm)	2.3 ± 0.7	2.5 ± 0.7*	2.1 ± 0.4	2.3 ± 0.5*	2.7 ± 0.3	2.7 ± 0.2
CBF (ml/min)	62 ± 30	98 ± 40*	54 ± 35	82 ± 43*	48 ± 18	57 ± 17
CVR (mm Hg·ml^{-1} min)	3.3 ± 2.0	1.8 ± 1.2*	3.5 ± 2.0	2.2 ± 1.7*	2.6 ± 0.8	2.2 ± 0.5

*$p < 0.05$ versus baseline value. Data presented are mean value ± SD. CBF = coronary blood flow; CVR = coronary vascular resistance; HR = heart rate; MVO_2 = myocardial oxygen uptake; RPP = rate-pressure product.

The presence of HC, HTN or DM did not affect baseline NOx or adenosine levels. Multiple linear regression analysis revealed that NOx production negatively correlated with the presence of DM ($p < 0.05$) and adenosine production positively correlated with the presence of HTN ($p < 0.05$). The correlation between NOx production and risk factors other than DM and between adenosine production and risk factors other than HTN was not significant. The number of risk factors showed a negative correlation with NOx production (Fig. 2A) and a positive correlation with adenosine production (Fig. 2B) during cardiac pacing.

Discussion

Contribution of NO and adenosine to metabolic coronary vasodilation in subjects without risk factors. CBF increases in response to increased metabolic demands of the myocardium (1). The contribution of NO to the CBF response to increased MVO_2 remains controversial (10–13). In previous experimental and clinical studies (10–13), the role of NO in the CBF response to increased MVO_2 was evaluated with the use of an inhibitor of NO synthase. NO rapidly decomposes to form NOx in the body (22), and endogenous production of NO can be detected by assaying the plasma NOx levels (29). We showed that plasma NOx levels in coronary sinus blood were increased in response to the increase in MVO_2 induced by cardiac pacing in subjects with angiographically normal coronary arteries without risk factors for atherosclerosis. This observation suggests that increased NO release may contribute to the CBF response to increased MVO_2 in subjects without risk factors.

In contrast, the contribution of adenosine to the CBF response to increased MVO_2 is not well understood. Several studies (30,31) have suggested that adenosine plays an important role in CBF response to increased MVO_2, however, Rossen et al. (23) detected no change in CBF response to increased MVO_2 after antagonism of adenosine receptors. We showed that adenosine levels in coronary sinus blood did not change during coronary vasodilation in subjects with angiographically normal coronary arteries without risk factors, suggesting that adenosine may not contribute to the CBF response to increased MVO_2 in these subjects.

Influence of risk factors. We demonstrated that plasma NOx levels in coronary sinus blood did not increase during cardiac pacing in subjects with angiographically normal coronary arteries with risk factors, consistent with the results of Quyyumi et al. (11) that the contribution of NO to CBF response to cardiac pacing is reduced in these subjects. The

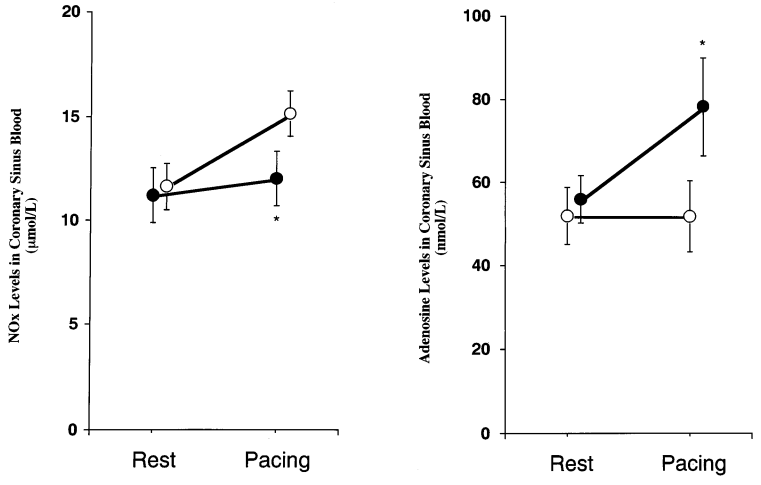

Figure 1. Response of NOx and adenosine levels in coronary sinus blood to cardiac pacing in subjects with **(solid circles)** and without risk factors **(open circles)**. Data shown mean value ± SD. *$p < 0.05$ versus subjects without risk factors.

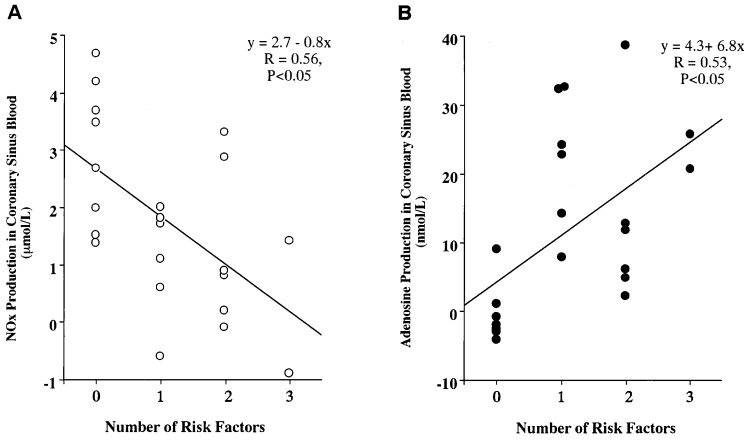

Figure 2. Scatterplots of the relation between number of risk factors of subjects and NOx **(A)** and adenosine **(B)** production in coronary sinus blood in response to cardiac pacing.

present study demonstrated that the CBF response to cardiac pacing was not impaired in subjects with risk factors. Because NOx release was depressed in these subjects, another vasodilatory substance may increase to compensate for the reduced coronary vasodilation caused by NO. Indeed, adenosine levels in coronary sinus blood increased in response to the increase in MVO_2 in subjects with angiographically normal coronary arteries with risk factors. Adenosine, which is a potent coronary vasodilator (1,17), may compensate for the reduced function of NO to maintain the CBF response to increased MVO_2 in these subjects. Indeed, we demonstrated that increases in CBF in response to cardiac pacing were blunted by aminophylline, an antagonist of adenosine receptors. These findings strongly suggest that adenosine may compensate for the reduced function of NO to maintain the CBF response to increased MVO_2 in subjects with risk factors for coronary atherosclerosis.

Several investigators (11,32) reported that subjects with risk factors for atherosclerosis showed a depressed vasodilator response to the increase in MVO_2. However, we demonstrated that the CBF response to cardiac pacing at nearly identical cardiac metabolic status, as evidenced by MVO_2 and lactate extraction ratio, was similar between subjects with and without risk factors. The discrepancy between our and other studies might be attributable to the differences of the definition of risk factors and the extent of stress. Risk factors in our subjects were lower and the level of stress milder than that in previous studies (11,32). It is possible that increased adenosine cannot compensate totally for reduced NO production in the presence of higher risk factors and greater stress.

We found no differences in plasma NOx levels during baseline conditions between subjects with and without risk factors. If risk factors are associated with a decrease in NO production in response to cardiac pacing, why is NOx production not decreased at rest? One possible explanation is that only the capacity to produce NO in response to stress was depressed in subjects with risk factors. Some coronary risk factors may produce superoxide, which can chemically neutralize NO by forming peroxynitrate (33). Because our assay for NOx may also detect peroxynitrate, we may have overestimated NO production under baseline conditions in subjects with risk factors by using NOx levels as an indicator of NO levels.

Interaction between NO and adenosine. Myocardial ischemia increases adenosine production (17,26), suggesting that cardiac pacing may have induced myocardial ischemia in subjects with risk factors in the present study. However, the lactate extraction ratio, an index of myocardial ischemia, did not differ at rest and at the end of cardiac pacing in these subjects, suggesting that myocardial ischemia is not responsible for the pacing-induced adenosine production observed in subjects with risk factors.

Inhibition of NO synthesis increases adenosine production under baseline conditions (18,19), during the increase in CBF after brief periods of myocardial ischemia (20) and during increased MVO_2 induced by pacing (21). We recently showed (19) that the inhibition of NO synthesis induced an increase in ecto-5′-nucleotidase activity and a consequent increase in adenosine levels in open chest dog hearts. These observations suggest that activation of ecto-5′-nucleotidase might be one possible mechanism for increased adenosine levels when NO bioavailability is reduced. It is possible that the risk factors themselves directly increase adenosine production under pathophysiologic conditions.

The contribution of NO during coronary vasodilation induced by cardiac pacing in clinical studies using an NO synthase inhibitor remains undetermined (11,13). Because inhibition of NO during cardiac pacing may affect production of adenosine, which modifies coronary circulation (18–21), we must interpret the results of those study using an NO synthase inhibitor with care, and direct measurements of NOx and adenosine in coronary venous blood may be useful for evaluating the contribution of NO and adenosine to the coronary circulation in humans.

Recently, Egashira et al. (32) and Zeiher et al. (34) reported that endothelial dysfunction in highly selected patients with angiographically normal coronary arteries is associated with noninvasive evidence for myocardial ischemia during exercise stress. However, the present study suggests that adenosine may compensate for the reduced NO production that is indicative of endothelial dysfunction. One possible

explanation for this discrepancy is that adenosine as well as NO production may be impaired in the highly selected subjects in the studies of Egashira et al. (32) and Zeiher et al. (34). Another possibility is that we assessed the CBF response to relatively modest stress in our subjects compared with the subjects of Egashira et al. (32) and Zeiher et al. (34). Thus, it is possible that increased adenosine cannot compensate for reduced NO production when the degree of stress is greater.

Clinical implications. Our findings suggest that reduced NO production during stress may be compensated for by an increase in adenosine production. Substitution of adenosine may provide a strategy for preventing myocardial ischemia during stress in patients with endothelial dysfunction.

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