The endothelium regulates vascular smooth muscle tone by releasing a variety of dilating and constricting factors in response to neural, humoral and autocrine stimulation. Important endothelium-dependent relaxing agents include nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor. In the human coronary circulation, NO contributes to resting tone and to flow-mediated dilation, but these functions are perturbed in patients with atherosclerosis or in the presence of risk factors such as hypercholesterolemia, hypertension, diabetes mellitus and smoking (1–3). The resulting endothelial dysfunction promotes paradoxical epicardial constriction and depressed microvascular dilation that may accentuate myocardial ischemia. However, the precise mechanisms leading to defective endothelial function and vasomotion remain unclear.

Recent data indicate an important role for the vascular kallikrein-kinin system, in particular of bradykinin (BK) in the regulation of vascular function (4). Bradykinin is a nonapeptide generated from kininogen by the action of kallikreins and is rapidly degraded by kininases such as angiotensin-converting enzyme (ACE) (5). Endothelial and vascular smooth muscle cells tonically generate BK that has important autocrine and paracrine functions (5). At least two types of kinin receptors, termed B1 and B2, have been identified in vascular tissue, though the vasodilator action of BK is largely believed to be mediated by endothelial kinin receptors (6) via promotion of NO, prostacyclin and hyperpolarizing factor release (7–9).

Because BK regulates resting tone and flow-mediated epicardial vasodilation in human coronary arteries (4,10), we hypothesized that abnormal flow-mediated vasomotion in patients with atherosclerosis and its risk factors results from an abnormality in kinin receptor function or in its signal transduction pathways. Furthermore, because BK is metabolized by ACE and tissue ACE activity is at least partially regulated genetically by the ACE insertion/deletion (I/D) polymorphism, it is possible that the action of BK on the vascular wall will be influenced by this genetic footprint (11).

Thus, the aim of our study was to investigate whether coronary vascular kinin receptor function: a) correlates with endothelial muscarinic receptor function in patients with atherosclerosis or its risk factors, thus reflecting generalized impairment of receptor-mediated NO release, b) correlates with flow-mediated epicardial vasomotion, and c) is influenced by serum ACE levels or the ACE I/D gene polymorphism. We also assessed whether BK-mediated coronary vasodilation is NO-mediated.
Abbreviations and Acronyms

ACE  = angiotensin-converting enzyme inhibitor
ACH  = acetylcholine
ANOVA = analysis of variance
BK   = bradykinin
BP   = blood pressure
I/D  = insertion/deletion
L-NMMA = L-N\textsuperscript{G} monomethyl arginine
NO   = nitric oxide
SNP  = sodium nitroprusside

METHODS

Patients. We studied 53 patients—34 with coronary atherosclerosis and 19 with normal coronary arteries and risk factors for atherosclerosis who were undergoing diagnostic cardiac catheterization for investigation of chest pain or abnormal noninvasive tests. The control group included nine patients with angiographically normal coronary arteries without risk factors who had noncardiac chest pain (that is, normal angiogram and noninvasive tests). Risk factors predisposing to endothelial dysfunction were defined as the presence of hypertension (blood pressure > 140/90 mm Hg), hypercholesterolemia (low-density lipoprotein > 160 mg/dL), diabetes, current smoking or smoking in the previous year and angiographic evidence of atherosclerosis (Table 1). There were 41 (66%) men. Patients with recent myocardial infarction, valvular heart disease, symptomatic heart failure or those treated with ACE inhibitors in the previous two weeks were excluded. The study was approved by the National Heart, Lung, and Blood Institute Investigational Review Board, and informed consent was obtained from all patients.

Protocol. All cardiac medications were withdrawn at least 48 h before the study, and aspirin or other cyclooxygenase inhibitors were discontinued seven days before. After diagnostic coronary angiography was performed, a 6F guide catheter was introduced into the proximal segment of a coronary artery, and blood flow velocity was measured using a 0.018 inch (0.014 inch with infusion catheters) wire employed the 62.5 ng/min and 4 mg/min dose of BK in atherosclerotic patients followed by infusion at 30 \( \mu \)g/min for 2 min each. Normal controls were given the 30 \( \mu \)g/min dose of ACH. This regimen avoided excessive constriction that may occur at higher doses of ACH in atherosclerotic coronary arteries.

Ten minutes after ACH testing and repeat baseline measurements ensuring that the blood flow velocity had returned to the previous baseline, responses to BK were assessed. In a preliminary dose-finding study, we infused BK at 62.5 ng/min, 0.25 \( \mu \)g/min, 1 \( \mu \)g/min and 4 \( \mu \)g/min for 2 min each to demonstrate that there was progressive vasodilation at all these doses with greater change at 4 \( \mu \)g/min compared with the 1 \( \mu \)g/min dose. Coronary blood flow increase at the 4 \( \mu \)g/min dose of BK was in the range of that observed with ACH. Therefore, for the study we employed the 62.5 ng/min and 4 \( \mu \)g/min. The sequence in which ACH and BK were infused was randomized.

Once blood flow velocity had returned to its baseline value, endothelium-independent function was estimated.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9</td>
<td>1, 2, 3 or More</td>
</tr>
<tr>
<td>Men</td>
<td>4</td>
<td>17, 13, 10</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45 ± 3</td>
<td>53 ± 2, 54 ± 3, 51 ± 2</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>132 ± 7</td>
<td>130 ± 7, 170 ± 12*, 173 ± 10†</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>44 ± 5</td>
<td>41 ± 3, 41 ± 4, 40 ± 3</td>
</tr>
<tr>
<td>Hypertension (n [%])</td>
<td>0</td>
<td>6 (29), 9 (53), 13 (87)</td>
</tr>
<tr>
<td>Diabetes (n)</td>
<td>0</td>
<td>1, 1</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>0</td>
<td>2, 2</td>
</tr>
<tr>
<td>Atherosclerosis (n [%])</td>
<td>0</td>
<td>7 (33), 12 (71), 15 (100)</td>
</tr>
<tr>
<td>Plasma ACE (U/L)</td>
<td>10.8 ± 1.7</td>
<td>9.9 ± 1, 10.4 ± 1.2, 10.8 ± 1.2</td>
</tr>
</tbody>
</table>

*p < 0.05; †p < 0.01 compared with controls.

ACE = angiotensin-converting enzyme inhibitor; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
with intracoronary sodium nitroprusside (SNP) given at 40 μg/min for 3 min, and flow reserve was measured with intracoronary adenosine administered at 2.2 mg/min for 2 min.

**EFFECTS OF L-NMMA.** To compare the contribution of NO to the effects of ACH and BK, repeat baseline measurements were made in 20 patients after a 10-min interval while continuing dextrose 5% infusion. This was followed by a 10-min infusion of L-N<sup>5</sup> monomethyl arginine (L-NMMA, Clinalfa AG, Switzerland), a specific inhibitor of NO synthesis from L-arginine, at 64 μmol/min (1 ml/min). This dose has previously been shown to significantly inhibit NO-dependent responses to pharmacological and physiological stimuli in humans (2,3). While continuing the infusion of L-NMMA at 64 μmol/min, ACH (30 μg/min) and, 10 min later, BK (62.5 ng/min and 4 μg/min) were readministered for 2 min each.

**Determination of ACE genotypes.** Angiotensin-converting enzyme genotyping was performed by laboratory personnel blinded to the endothelial function data. Angiotensin-converting enzyme genotypes were determined by use of polymerase chain reaction according to previously published protocols (12,13).

**Statistical analysis.** Data are expressed as mean ± SEM. Differences between means were compared by using paired or unpaired Student t test, as appropriate. All p values were two-tailed, and a value <0.05 was considered to be of statistical significance. Dose response curves were compared by analysis of variance (ANOVA) using the SAS software (SAS Institute; Cary, North Carolina). If the F value was significant, a Bonferroni multiple comparison test was performed. Correlation analysis was performed using Pearson’s correlation coefficient.

**RESULTS**

**Hemodynamic changes.** The 30 μg/min dose of ACH was tolerated by all patients, did not alter systemic blood pressure (BP), and was, therefore, used for comparing vasomotor responses between groups. Bradykinin did not alter systemic BP at the 62.5 ng/min dose, but at the 4 μg/min dose, a mild decrease was observed: 110 ± 2 to 107 ± 15 mm Hg, p = 0.01. Intracoronary SNP significantly reduced BP from 110 ± 2 to 102 ± 2 mm Hg, p < 0.001.

**Effect of risk factors on the responses to ACH, BK, SNP and adenosine.** **CORONARY MICROVASCULAR FUNCTION.** There was a correlation between the response to ACH and BK at the higher, but not the lower, dose of BK (Fig. 1) (r = 0.33, p = 0.007 for % change in flow ACH vs. BK, 4 μg/min). Patients with ACH-mediated microvascular dilation in the lowest tertile also had reduced BK-mediated dilation, and vice versa. To further investigate endothelium-dependent and independent responses, we compared patients without (control group) to those with risk factors. Baseline heart rate, coronary blood flow and resistance in the control group were similar to patients with risk factors. However, there was progressive impairment in ACH-mediated microvascular dilation with increasing numbers of risk factors (p = 0.025, ANOVA, Fig. 2). By contrast, BK-mediated microvascular dilation was similar at both doses in controls and in patients with risk factors (p = 0.5, ANOVA, Fig. 3). Similarly, endothelium-independent dilation with SNP was not significantly altered in patients with, compared to those without, multiple risk factors (p > 0.1, ANOVA, Fig. 2).

We also corrected the vasodilation in response to the two endothelium-dependent dilators with the response to SNP. The ratio of ACH:SNP-induced dilation in controls was 106 ± 9%, compared with 73 ± 6% in patients with risk...
factors (p = 0.04). By contrast, the ratio of BK:SNP-induced dilation in controls (106 ± 9%) was similar to patients with risk factors (104 ± 5%, p = NS). Flow reserve with adenosine was similar in controls and patients with multiple risk factors: reduction in coronary vascular resistance of 77 ± 3% and 73 ± 1%, respectively, p = 0.24.

CORONARY EPICARDIAL FUNCTION. Bradykinin-mediated epicardial vasodilation was similar in controls and patients with risk factors (p = 0.3 by ANOVA between groups [Fig. 3]). There was no correlation between the epicardial coronary dilator responses to ACH and BK (r = 0.06, p > 0.5 for both doses of BK). Acetylcholine-induced coronary epicardial vasomotion was heterogenous even within the same vessel. Therefore, epicardial responses were compared by segments rather than by individual patients. Seventy-six segments constricted (by 10.4 ± 0.9% compared with baseline), and an equal number of segments dilated (7.3 ± 0.8%), with ACH. Both doses of BK produced similar dilation in segments that constricted and those that dilated with ACH. At the 4 μg/min dose, BK induced 13.1 ± 1.4% dilation in the segment that constricted and 11.2 ± 1.3% dilation in the segments that dilated with ACH (p = 0.3). Sodium nitroprusside-mediated epicardial vasodilation was also similar between the two groups; 18.9 ± 1.8% and 19.5 ± 1.5%, respectively (p = 0.8).

Effect of L-NMMA on the responses to ACH and BK. L-N^G monomethyl arginine significantly increased mean BP from 104 ± 3 to 110 ± 4 mm Hg, p < 0.001. At rest, L-NMMA produced epicardial and microvascular constriction; coronary vascular resistance was 14 ± 3.6% higher (p = 0.02), and epicardial diameter was reduced by 3.9 ± 1% (p < 0.001, Fig. 4). L-N^G monomethyl arginine also suppressed epicardial and coronary microvascular responses.

![Figure 3](image-url)  
**Figure 3.** Effect of risk factors on bradykinin-induced coronary epicardial dilation (measured as % change in diameter) and microvascular dilation (measured as % change in resistance). No difference in responses between risk groups by analysis of variance.

![Figure 4](image-url)  
**Figure 4.** Bradykinin- (left) and acetylcholine- (right) mediated microvascular (top) and epicardial (bottom) vasomotion before (control solid circles with solid lines) and after L-NMMA (open circles with dashed lines, n = 20). *p = 0.03, **p = 0.003, ***p < 0.001 comparing control with L-NMMA. L-NMMA = L-N^G monomethyl arginine.
to ACH and to the low dose of BK (62.5 ng/min). However, at the 4 μg/min dose of BK, epicardial and microvascular responses were not inhibited by L-NMMA (Fig. 4).

**Flow-mediated epicardial vasomotion during pacing.** During pacing 20 epicardial coronary artery segments constricted (25.1 ± 1%, abnormal response) and 30 dilated (9.6 ± 1%, normal response). Bradykinin-mediated epicardial dilation was diminished in segments that constricted abnormally with pacing compared with segments that dilated normally during pacing (p = 0.027, Fig. 5). By contrast, ACH-mediated epicardial changes were similar in segments that constricted (0.2 ± 2%) compared with those that dilated (−0.5 ± 3%, p = 0.8) with pacing. Similarly, epicardial dilation with SNP was similar in the constricting and dilating segments: 19 ± 3% and 20 ± 3%, respectively (p = 0.9).

There was no correlation between pacing-induced coronary microvascular vasodilation and the responses to either ACH, BK or SNP.

**Serum ACE levels, ACE gene polymorphism and the response to ACH and BK.** The median serum ACE level in our group was 9.9 U/ml. There was a correlation between serum ACE levels and the epicardial vasodilator response to BK (4 μg/min); r = −0.3, p < 0.001. We, therefore, divided patients into those with a high ACE level (> median value, mean 14.4 ± 0.6 U/ml, n = 30) and the remaining patients with low ACE levels (≤ median, mean 6.7 ± 0.4 U/ml, n = 32, Fig. 6). Epicardial vasodilation with BK, especially at the higher dose, was greater in patients with lower ACE levels, and vice versa (p = 0.016,ANOVA, Fig. 6).

Because serum ACE levels are at least partly determined by the ACE I/D polymorphism (r = −0.5, p = 0.0001, ACE level vs. I/D genotype) and there was a weak but significant correlation between the epicardial response to BK (4 μg/min) and ACE genotype (r = 0.2, p = 0.02), we investigated the impact of this genotype on the vascular responses to BK. Vasodilation with BK at the 4 μg/min dose was lower in patients with the DD genotype (7.4 ± 1.9%) compared with those with the II genotype (14.9 ± 2.9%, p = 0.03), with the ID genotype subset having an intermediate response (13.1 ± 1.3%). The dose-response curve with BK demonstrated reduced vasodilation in the DD patients compared with the rest, with the difference approaching statistical significance (p = 0.06, ANOVA, Fig. 6).

There was no relationship between coronary epicardial and microvascular vasodilation with ACH, or the microvascular dilation with BK and either the serum ACE levels or the ACE I/D genotype.

**DISCUSSION**

The first major finding of the study was that the presence of risk factors for atherosclerosis was not accompanied by impairment of BK-dependent epicardial or microvascular dilation. Although there was a correlation between microvascular responses with the two agonists, there was no correlation between the epicardial responses to ACH and BK; segments with a constrictor response to ACH had vasodilation in response to BK that was similar to segments that dilated with ACH. These findings indicate that injury to the vascular endothelium by conventional risk factors, or
by atherosclerosis itself, may be receptor-specific, involving the muscarinic receptor and its regulatory pathways, with relative sparing of the kinin receptor and its pathways.

Second, abnormal epicardial vasomotion during physiologic stress, measured as constriction during pacing, is associated with a depressed response to BK. By contrast, ACH responses were similar in segments that dilated compared with those that constricted with pacing, suggesting that abnormal reactivity of epicardial coronary arteries during physiologic stress is better represented by a pharmacologic probe such as BK.

Third, ACH and low-dose BK-mediated vasodilation was inhibited by L-NMMA, indicating the contribution of NO to these responses. However, high-dose BK responses were unchanged with L-NMMA, demonstrating the contribution of factors independent of NO to BK-mediated coronary vasodilation at higher doses.

Finally, our data illustrates the crucial role of ACE, the enzyme involved in the metabolism of BK, in determining the responses to BK; patients with higher vascular ACE activity, segregated either by serum ACE levels or by the presence of the ACE DD genotype, had impaired epicardial responses to BK.

**Impact of risk factors for endothelial dysfunction on vascular responses to BK.** Blood vessels from hypercholesterolemic animals and atherosclerotic human subjects have reduced NO activity under basal conditions and impaired reactivity to some endothelium-dependent vasodilators, including ACH, 5-hydroxytryptamine and substance P, but not to others such as BK, adenosine diphosphate, norepinephrine and A23187 (14–16). Similarly, ACH-mediated, but not BK-mediated, vasodilation is depressed in hypercholesterolemia (17). In patients with variant angina, intact coronary epicardial responses to BK were reported in segments that constricted with ACH (18). Our finding that coronary epicardial and microvascular dilation in response to BK is preserved in patients with risk factors for atherosclerosis or mild atherosclerosis is consistent with these studies. However, in significantly stenosed segments of coronary arteries, BK-mediated vasodilation appears to be reduced, indicating that kinin receptor function becomes impaired only in advanced atherosclerosis (18).

A progressive impairment of microvascular dilation in response to ACH compared with controls was observed with increasing exposure to conventional risk factors, a finding previously noted in the epicardial coronary circulation (19). There was a weak significant correlation between microvascular responses to BK and ACH, though unlike ACH, the microvascular responses with BK did not correlate with the presence of risk factors. Furthermore, segments that constricted with ACH, a response that is considered to be a hallmark of conductance vessel endothelial dysfunction, did not respond differently with BK when compared with segments that dilated with ACH. Thus, our findings suggest that mild atherosclerosis or its risk factors impair muscarinic receptor function with an uneven effect on endothelial kinin receptors, a finding that was not observed in a recent study of patients with hypercholesterolemia and hypertension (20). We analyzed coronary vascular responses in subsets of patients with either hypertension (n = 10) or hypercholesterolemia (n = 10) without any other risk factors and found no differences in epicardial vasomotion with BK compared with controls.

**Potential mechanisms.** First, ACH and BK act not only through different receptors but also through distinct intracellular second messenger pathways. Pertussis toxin, a specific inhibitor of the intracellular membrane-bound G protein, selectively impairs the response to muscarinic agonists without influencing responses to BK, indicating that the B2 receptor is not coupled with the Gq protein (21,22). Studies have suggested that BK responses are probably mediated, at least in part, by the pertussis toxin insensitive Gq protein-dependent signal transduction pathway. Experimentally, endothelial dysfunction is associated: 1) with selective impairment of Gi but not Gq protein function (23 and 2) with decreased expression with Gq proteins in atherosclerotic human epicardial coronary arteries (24).

Second, a significant component of BK-mediated dilation is from non–NO endothelium-derived relaxing factors, such as prostacyclin and endothelium-derived hyperpolarizing factor (25). This is supported by our observation that BK-mediated, but not ACH-mediated, dilation was not inhibited by L-NMMA, suggesting a greater release of non–NO endothelium-derived relaxing factors with BK. Furthermore, it is possible that BK-dependent release of prostacyclin and endothelium-derived hyperpolarizing factor may be preserved or even upregulated in atherosclerosis (26).

**Flow-mediated epicardial vasomotion.** Directionally similar coronary vasomotor changes have been reported with physiologic stress and ACH in previous studies (27). However, most of these investigations have involved segments of coronary arteries with significant atherosclerotic narrowing. These segments uniformly constricted with ACH and also with either mental stress or exercise. In this study most segments were either angiographically normal or had minimal irregularity. We have previously found a weak correlation between atrial pacing-induced microvascular changes and the response to ACH in patients with relatively normal epicardial coronary arteries (28). As in this study, that investigation failed to show a correlation between epicardial responses during pacing and with ACH.

It is appreciated that shear-induced epicardial coronary artery vasodilation is endothelium-dependent (3) and may, in part, be due to stimulation of endogenous BK because it can be attenuated by the B2 receptor antagonist, icatibant (4,29). Our finding of reduced BK-mediated vasodilation in segments that constricted with pacing is, therefore, consistent with these observations. Whether this represents downregulation of kinin receptors or a defect in their second messenger pathways needs to be further investigated.

**ACE activity and vascular responses.** Angiotensin-converting enzyme is an integral component of the renin-
angiotensin and kallikrein-kinin systems promoting the synthesis of angiotensin II and the metabolism of BK. The ACE I/D polymorphism accounts significantly for interindividual variability in plasma and tissue ACE levels (21). Our findings indicate that ACE activity determines BK vascular responses in the epicardial circulation, particularly at the higher dose (Fig. 6), with the responses lower in patients with higher circulating ACE levels and those with the ACE DD genotype. By contrast, ACH responses and microvascular responses to BK were not influenced by ACE levels or the ACE I/D polymorphism, a finding also confirmed in a larger population (30) (ref. 30 published in this issue of the Journal).

**Implications.** Bradykinin is an important regulator of human coronary vascular tone at rest and during conditions of increased flow (9). In this study we have demonstrated that BK responses are relatively preserved in patients with risk factors for atherosclerosis or mild angiographic atherosclerosis. Because effective inhibitors of BK degradation are available in the form of ACE inhibitors and neutral endopeptidase inhibitors, it is possible that enhancing endogenous BK activity with these drugs may reverse the abnormalities of coronary reactivity and endothelial dysfunction observed in patients with atherosclerosis and its risk factors.

**Acknowledgment**

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