

Bradykinin-Induced Vasodilation of Human Coronary Arteries In Vivo: Role of Nitric Oxide and Angiotensin-Converting Enzyme

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Objectives. The present study aimed to determine the role of nitric oxide (NO) and angiotensin-converting enzyme (ACE) in bradykinin (BK)-induced dilation of human coronary arteries in vivo.

Background. BK, produced by way of the kinin-kallikrein system, causes endothelium-dependent vasodilation. However, little is known about the mechanism of BK-induced dilation of coronary arteries in humans in vivo.

Methods. The effects of an inhibitor of NO synthesis and of an ACE inhibitor on BK-induced coronary vasodilation were examined in 20 patients who had no significant atherosclerotic stenosis in the artery under study. Lumen diameters of the large epicardial coronary arteries and coronary blood flow (CBF) were measured by quantitative coronary arteriography and intracoronary Doppler technique.

Results. Intracoronary infusion of BK (0.6 and 2.0 $\mu\text{g}/\text{min}$) increased coronary artery diameter and CBF with no change in arterial pressure or heart rate. The BK-induced increases in coronary artery diameter and CBF were significantly reduced ($p < 0.01$) after pretreatment with N^{G} -monomethyl-L-arginine (200 μmol) and were significantly increased ($p < 0.01$) after pretreatment with enalaprilat (50 μg).

Conclusions. BK-induced dilation of human large epicardial and resistance coronary arteries is mediated by NO and increased by prior ACE inhibition.

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The vascular endothelium is involved in the control of vascular tone by the production of a variety of substances (1,2). Bradykinin (BK), an endogenous substance produced by way of the vascular kinin-kallikrein system (3,4), induces endothelium-dependent vasodilation (5-11). Groves et al. (12) demonstrated that intracoronary infusion of a selective BK antagonist reduces the diameter of coronary arteries, coronary blood flow (CBF) and flow-mediated endothelium-dependent vasodilation in humans in vivo. These findings suggest a role of endogenous BK in the control of coronary circulation. However, little is known concerning the mechanisms of the BK-induced dilation of human coronary arteries in vivo.

There are inconsistent findings in the published data concerning the contribution of nitric oxide (NO) to BK-induced coronary vasodilation. Some studies (7-9) show that inhibition

of NO synthesis reduces BK-induced dilation of canine coronary arteries, whereas others (5,6) demonstrate that inhibitors of NO synthesis have few or only minor effects on BK-induced dilation of porcine coronary arteries. BK is rapidly inactivated by kininase II (3,4,13), which is identical to angiotensin-converting enzyme (ACE). The effects of ACE inhibitors on BK-induced coronary vasodilation differ among species (8-10). The difference in the vascular ACE activities to degrade BK may explain the differing actions of ACE inhibitors among species. It is therefore important to evaluate the mechanism of BK-induced coronary dilation of human coronary arteries in vivo.

The present study aimed to determine the role of NO and ACE in BK-induced dilation of coronary arteries in humans in vivo by investigating the effects of an inhibitor of NO synthesis and of an ACE inhibitor on BK-induced coronary vasodilation in patients with no significant coronary artery disease.

Methods

Study patients. Twenty patients (13 men, 7 women; mean age 58 ± 11 years) who were undergoing diagnostic coronary arteriography were studied. All patients had no significant coronary artery stenosis, as assessed by angiography, in the artery under study. Patients with significant coronary artery

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Abbreviations and Acronyms

ACE	=	angiotensin-converting enzyme
BK	=	bradykinin
CBF	=	coronary blood flow
ECG	=	electrocardiogram
L-NMMA	=	N ^G -monomethyl-L-arginine
NO	=	nitric oxide

disease (diameter stenosis <50%), previous myocardial infarction or evidence of left ventricular dysfunction were excluded. None of the study patients had any ST segment abnormalities in the rest electrocardiogram (ECG). Five patients had hypercholesterolemia (total cholesterol >220 mg/dl), six had essential hypertension (systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg) and six were smokers. No patient had diabetes mellitus (fasting blood glucose >140 mg/dl or a positive result on glucose tolerance testing).

Quantitative coronary arteriography. We evaluated the changes in lumen diameter of the large epicardial coronary arteries at a segment 2 mm distal to the tip of the Doppler wire that lacked lumen irregularity or atherosclerotic stenosis. We selected a view that would permit clear visualization of the coronary segment under study without overlapping branches. The distance from the X-ray focus to the object and that from the object to the image intensifier were kept constant during the study. Coronary angiograms were recorded on 35-mm cinefilm with use of an angiographic system (Bicor and Hicor, Siemens-Asahi Inc., Tokyo, Japan). A nonionic contrast medium (Iomeprol, Eisai Co., Tokyo, Japan) was used.

An end-diastolic frame of the angiogram was selected, and the lumen diameter was determined quantitatively by a densitometric analysis system. The diameter of the segment of interest was measured three times, and the mean value of these measurements was used for later analysis. A Judkins catheter was used to calibrate the lumen diameter in millimeters. The accuracy and precision of the measurements were as reported previously (11,14).

Measurement of CBF velocity and CBF. A 6F Judkins catheter was placed at the orifice of the left main coronary artery and a 0.018-in. (0.046-cm) or 0.014-in. (0.036-cm) Doppler-tipped guide wire (FloWire, Cardiometrics Inc.) was advanced into the proximal portion of the left anterior descending (n = 16) or the left circumflex (n = 4) coronary artery through the Judkins catheter. CBF velocity was measured continuously and a peak flow velocity was determined by use of an on-line spectral analyzer (FlowMap, Cardiometrics Inc.). Peak blood flow velocity spectral signals and their mean values were monitored continuously. Mean CBF was calculated as follows (15):

$$\text{CBF (ml/min)} = 0.5 \times \text{Average peak flow velocity (cm/min)} \\ \times \text{Cross-sectional area (cm}^2\text{)}.$$

The cross-sectional area was determined by quantitative coronary arteriography. Coronary vascular conductance (ml/

min·mm Hg) was estimated from the division of CBF (ml/min) by mean arterial pressure (mm Hg).

Drugs. BK and N^G-monomethyl-L-arginine (L-NMMA) were purchased from Clinalfa AG, Basel, Switzerland. Enalaprilat (Vasotec) was a gift of Merck Sharpe & Dohme. Papaverine was purchased from Dainihon-Seiyaku Co. Each drug was diluted with physiologic saline solution immediately before administration.

Study protocol. The study protocol was approved by the Institutional Review Committee on Human Research, Kyushu University Faculty of Medicine. Written informed consent was obtained from each patient.

Cardiac catheterization was performed in the fasting state after oral premedication with diazepam (5 mg). Three patients with essential hypertension were taking an ACE inhibitor, which was discontinued ≥ 5 days before the study. Ten other patients were taking antianginal medications such as calcium channel blockers and nitrate that were discontinued ≥ 24 h before the study. We examined acetylcholine-induced coronary vasomotion before L-NMMA administration in 10 of the 20 patients (4 in protocol 1, 5 in protocol 2 and 1 in protocol 3). In these 10 patients intracoronary infusion of acetylcholine at 3 $\mu\text{g/min}$ increased coronary artery diameter by $11 \pm 13\%$ and CBF by $59 \pm 45\%$.

Protocol 1. We examined the effect of L-NMMA on BK-induced coronary vasodilation in six patients. After steady state baseline systemic arterial pressure, heart rate and blood flow velocity were recorded, coronary arteriography was performed to obtain the baseline diameter of the epicardial coronary artery. BK was then infused at 0.6 $\mu\text{g/min}$ for 1 min into the left main coronary artery through a Judkins catheter with use of an infusion pump (1 ml/min). We confirmed in preliminary studies that intracoronary infusion of saline solution at 1 ml/min did not affect any of those variables. One minute after initiation of BK infusion, CBF velocity was measured and coronary arteriography performed. We waited 5 min before administration of the next higher dose of BK (2.0 $\mu\text{g/min}$). Before the infusion of BK, contrast medium in the catheter was withdrawn to avoid its vasodilating action. During BK infusion, CBF velocity peaked at about 30 s and reached a plateau by 50 s.

After a 10-min recovery period during which all hemodynamic variables returned to the baseline level, L-NMMA (200 μmol) was infused into the left coronary artery over 12 min, as described elsewhere (15). A second infusion of BK was then administered at the same dose used in the first infusion. Coronary arteriography was performed as in the first infusion. We selected this 200- μmol dose of L-NMMA because it significantly attenuated the acetylcholine-induced increase in diameter of a large epicardial coronary artery and CBF (15).

Two doses of BK (0.6 and 2.0 $\mu\text{g/min}$) were administered in the present study, as preliminary studies had shown that an intracoronary dose of 0.2 $\mu\text{g/min}$ of BK did not cause significant coronary vasodilation, whereas a dose of 6.0 $\mu\text{g/min}$ caused hypotension.

Protocol 2. We examined the effect of an ACE inhibitor on BK-induced coronary vasodilation in eight patients. After

Table 1. Effect of Bradykinin on Systemic Arterial Pressure, Heart Rate, Coronary Artery Diameter and Coronary Blood Flow

	Baseline Value	After Bradykinin ($\mu\text{g}/\text{min}$)	
		0.6	2.0
Mean arterial pressure (mm Hg)	97 ± 13	97 ± 13	94 ± 14
Heart rate (beats/min)	61 ± 13	60 ± 14	62 ± 14
Coronary artery diameter (mm)	2.6 ± 0.8	$2.8 \pm 0.8^*$	$3.0 \pm 0.8^*$
Coronary blood flow (ml/min)	41 ± 27	$79 \pm 46^*$	$107 \pm 65^*$

* $p < 0.01$ versus baseline value. Data shown are mean value \pm SD (n = 20).

baseline systemic arterial pressure, heart rate, coronary artery diameter and CBF were recorded, BK (0.6 $\mu\text{g}/\text{min}$) was infused into a Judkins catheter in the left coronary artery as in protocol 1. Enalaprilat (50 μg over 2 min) was then infused into the same artery. BK was then infused at the same dose used in the initial infusion. Coronary arteriography was performed before and after BK. We selected this dose of enalaprilat because it had no effect on basal systemic arterial pressure and heart rate but significantly augmented BK-induced coronary vasodilation. Because BK (0.6 $\mu\text{g}/\text{min}$) after enalaprilat significantly decreased systemic arterial pressure, we did not use the 2.0- $\mu\text{g}/\text{min}$ dose of BK after enalaprilat.

Protocol 3. We investigated the possibility of time-related changes in BK-induced coronary vasodilation in six patients. Intracoronary infusion of BK at the same dose was repeated twice in a time sequence similar to that of protocol 1.

Papaverine administration. In all 20 patients, at the end of each protocol we assessed maximal vasodilator capacity by administering papaverine (10 mg/5 ml) into the left coronary artery for 1 min. The papaverine-induced increase in CBF was recorded as we (14) have previously described.

Statistical analysis. Data are expressed as mean value \pm SD. Effects of L-NMMA on BK-induced coronary vasodilation were compared by using two-way analysis of variance, followed by Bonferroni's multiple comparison tests. Effects of enalaprilat on BK-induced coronary vasodilation were compared by Student *t* test. A *p* value < 0.05 was considered statistically significant.

Results

BK-induced coronary vasodilation. Table 1 shows the effect of BK on mean systemic arterial pressure, heart rate, lumen diameter of a large epicardial coronary artery and CBF in all 20 patients. BK increased ($p < 0.01$) the epicardial coronary artery diameter and CBF in a dose-dependent manner, but it did not alter systemic arterial pressure or heart rate.

Effect of L-NMMA on BK-induced coronary vasodilation. Intracoronary administration of L-NMMA (200 μmol) did not significantly alter basal arterial pressure (85 ± 11 and 85 ± 12 mm Hg, respectively, before and after L-NMMA), heart rate (60 ± 14 and 58 ± 13 beats/min), coronary artery diameter (2.3 ± 0.7 and 2.4 ± 0.6 mm) or CBF (32 ± 19 and 27 ± 15 ml/min). Pretreatment with L-NMMA significantly inhibited the BK-induced increase in coronary artery diameter and CBF. Percent

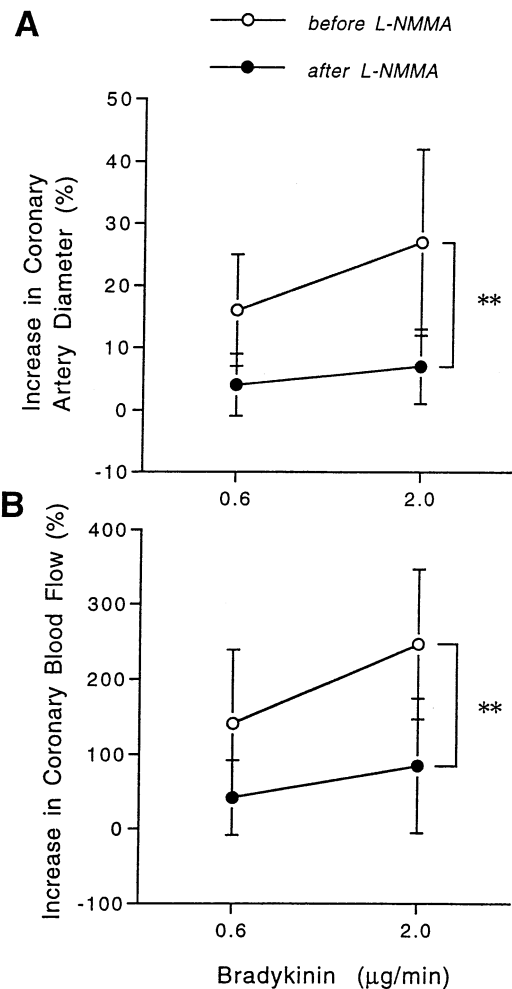


Figure 1. BK-induced increases in coronary artery diameter (A) and CBF (B) before and after L-NMMA in six patients. BK did not affect systemic arterial pressure or heart rate during the study. Data are shown as mean value \pm SD (n = 6). ** $p < 0.01$ before and after L-NMMA by two-way analysis of variance.

changes in BK-induced increases in coronary artery diameter and CBF were significantly smaller ($p < 0.01$) after than before L-NMMA (Fig. 1). BK administration did not alter systemic arterial pressure or heart rate before or after L-NMMA.

Effect of enalaprilat on BK-induced coronary vasodilation. Intracoronary administration of enalaprilat (50 μg) did not alter basal systemic arterial pressure (98 ± 13 and 97 ± 13 mm Hg, respectively, before and after enalaprilat), heart rate (58 ± 12 and 59 ± 13 beats/min), coronary artery diameter (2.9 ± 0.9 and 2.7 ± 0.7 mm) or CBF (44 ± 16 and 36 ± 13 ml/min). Enalaprilat significantly augmented the BK-induced increase in coronary artery diameter, CBF and coronary vascular conductance, whereas systemic arterial pressure decreased significantly during BK infusion after enalaprilat administration. The percent changes in BK-induced increases in coronary artery diameter, CBF and coronary vascular conductance were significantly greater after enalaprilat (Fig. 2).

Reproducibility of BK-induced coronary vasodilation. The increase in coronary diameter and CBF did not differ

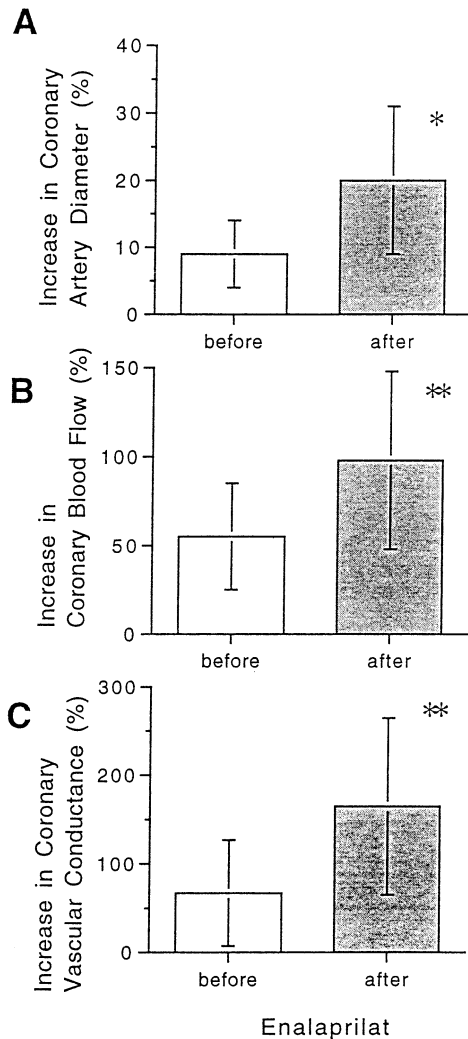


Figure 2. BK-induced increases in coronary artery diameter (A), CBF (B) and coronary vascular conductance (C) before (open bars) and after (closed bars) the administration of enalaprilat in eight patients. BK (0.6 $\mu\text{g}/\text{min}$) did not affect systemic arterial pressure before enalaprilat but decreased it significantly from 98 ± 13 to 80 ± 12 mm Hg ($p < 0.05$) after enalaprilat. BK did not alter heart rate before or after enalaprilat. Data shown are mean value \pm SD ($n = 8$). * $p < 0.05$, ** $p < 0.01$ versus before enalaprilat by paired t test.

significantly between the first and second administration of BK (Fig. 3).

Papaverine-induced coronary vasodilation. There was no significant difference in percent increases in CBF among patients of protocol 1 ($285 \pm 29\%$), protocol 2 ($302 \pm 65\%$) and protocol 3 ($296 \pm 52\%$).

Discussion

Role of NO and ACE in BK-induced human coronary arteries in vivo. BK that is produced by way of the vascular kinin-kallikrein system (3,4,13) stimulates the release of NO, prostacyclin or endothelium-derived hyperpolarizing factor, alone or in combination, from vascular endothelial cells and,

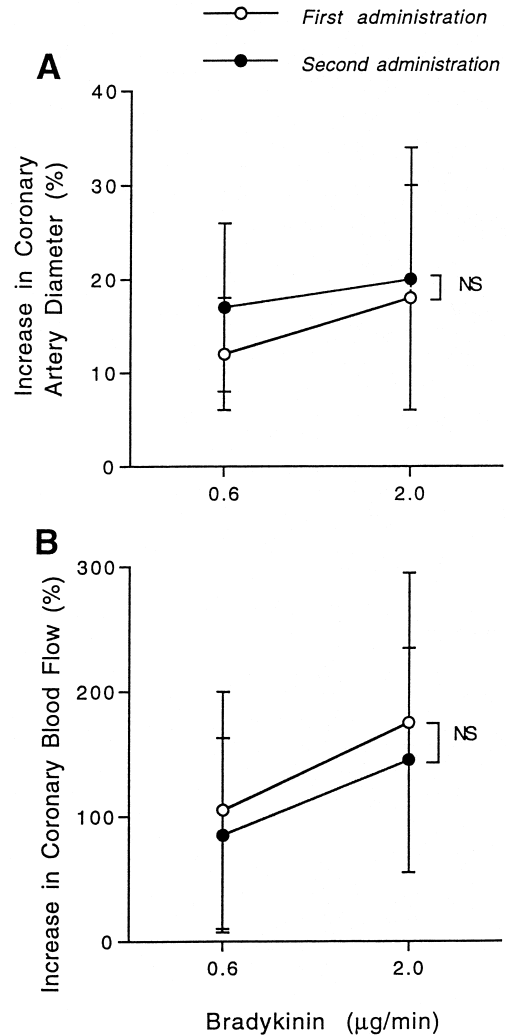


Figure 3. Increases in coronary artery diameter (A) and CBF (B) in six patients after the first and second administration of BK. BK did not affect systemic arterial pressure or heart rate during the study. Data shown are mean value \pm SD ($n = 6$). NS = difference not significant.

thus, causes endothelium-dependent coronary vasodilation (3-11). The relative contribution of these endothelium-derived substances to BK-induced coronary vasodilation differs among species and with the specific vascular beds being investigated. Little is known about the role of NO in BK-induced dilation of human coronary arteries in vivo. In the present study, BK increased coronary artery diameter and CBF without affecting systemic arterial pressure or heart rate, indicating that BK caused the dilation of large epicardial and resistance coronary arteries. Pretreatment with L-NMMA markedly reduced the BK-induced increases in arterial diameter and CBF. We (15) previously reported that L-NMMA, at the dose used in the present study, inhibited the acetylcholine-induced increases in coronary artery diameter and CBF in patients with normal coronary arteriograms. Quyyumi et al. (16) reported similar findings. These findings suggest that the dilation of large epicardial and resistance coronary arteries induced by BK is mediated mainly by endothelium-derived NO in humans in vivo.

Because ACE is identical to kininase II and present on the vascular endothelium, BK is inactivated by ACE (3,4,13). It is thus assumed that ACE inhibitors may act on kininase II and increase the tissue concentrations of BK which, in turn, augment the vasodilation induced by BK, leading to an increase in NO synthesis. Sudhir et al. (17) showed that in dogs vasodilation of resistance coronary arteries induced by an ACE inhibitor was totally inhibited by the bradykinin receptor antagonist whereas ACE inhibitor-induced vasodilation of large epicardial coronary arteries was reduced by L-NMMA. However, the effect of ACE inhibition on BK-induced dilation of human coronary arteries had not been investigated. Pretreatment with enalaprilat in the present study significantly augmented BK-induced increases in arterial diameter and CBF. Because systemic arterial pressure decreased significantly after enalaprilat, we calculated the coronary vascular conductance and found that the BK-induced increase in coronary vascular conductance was significantly greater after enalaprilat. The hypotension induced by BK after enalaprilat may be attributable to an unmasking of the BK-induced vasodilation of the systemic vasculature after ACE inhibition. These findings suggest that BK-induced dilation of human large epicardial and resistance coronary arteries is augmented after enalaprilat. This effect of enalaprilat appeared to result from an inhibition of BK breakdown induced by the inhibition of vascular ACE.

We evaluated the reproducibility of the BK-induced vasodilation to determine whether the vasodilator effect of BK would be smaller at its second administration than at its first because of tachyphylaxis (18). Our results indicate that the effects of L-NMMA and enalaprilat were not related to time.

Limitations of the study. A major limitation of the present study is the small number of patients studied. Thus, we could not determine whether the BK-induced vasodilation was impaired in the presence of certain cardiovascular disorders. Further studies are needed to elucidate a possible relation between BK-induced vasodilation and disorders such as hypercholesterolemia, atherosclerosis, hypertension, left ventricular hypertrophy, and heart failure.

Summary and clinical implications. The present study showed that BK-induced dilation of human large epicardial and resistance coronary arteries was inhibited by L-NMMA, an inhibitor of NO synthesis, and was increased by enalaprilat, an ACE inhibitor. Our findings have several implications 1) the results may contribute to wider use and better understanding of ACE inhibitors. 2) Our data indicate that NO is responsible for BK-induced dilation of human coronary arteries in vivo. We (11) and other investigators (19) showed that BK-induced dilation was impaired at the atherosclerotic site of human coronary arteries. Thus, it is likely that impaired BK-induced dilation at the atherosclerotic site is mediated by a reduction in the release of NO. 3) The potentiation of the BK-induced vasodilation by enalaprilat suggests that, in addition to inhibiting angiotensin II formation, ACE inhibitors increase the release of NO by an increased tissue concentration of BK. The TREND study by Mancini et al. (20) has shown that treatment

with an ACE inhibitor, quinapril, lessens endothelial dysfunction in patients with angiographically normal or minimally decreased coronary arteries. Thus, the TREND study might strengthen our present observation that an ACE inhibitor, enalaprilat, increased the BK-induced release of NO.

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