

## CLINICAL STUDIES

## Interventional Cardiology

# Bradykinin-Induced Preconditioning in Patients Undergoing Coronary Angioplasty

Massoud A. Leesar, MD, FACC, Marcus F. Stoddard, MD, FACC, Srinivas Manchikalapudi, MD, Roberto Bolli, MD, FACC

Louisville, Kentucky

- OBJECTIVES** The purpose of this study was to determine whether administration of bradykinin reproduces the cardioprotective effects of ischemic preconditioning (PC) in patients undergoing percutaneous transluminal coronary angioplasty (PTCA).
- BACKGROUND** Experimental studies suggest that activation of the bradykinin B<sub>2</sub> receptor is an important trigger of ischemic PC. However, it is unknown whether bradykinin can precondition human myocardium against ischemia in vivo. Multicenter clinical trials have demonstrated an anti-ischemic effect of angiotensin-converting enzyme inhibitors, which has been postulated to result from potentiation of bradykinin; however, direct evidence for an anti-ischemic action of bradykinin in patients is lacking.
- METHODS** Thirty patients were randomized to receive a 10-min intracoronary infusion of bradykinin (2.5 µg/min) or normal saline. Ten minutes later they underwent PTCA (three 2-min balloon inflations 5 min apart).
- RESULTS** In control patients, the ST-segment shift on the intracoronary and surface electrocardiogram was significantly greater during the first inflation than during the second and third inflations, consistent with ischemic PC. In bradykinin-treated patients, the ST-segment shift during the first inflation was significantly smaller than in the control group, and there were no appreciable differences in ST-segment shift during the three inflations. Measurements of chest pain score and regional wall motion during inflation (quantitative two-dimensional echocardiography) paralleled those of ST-segment shift. Infusion of bradykinin had no hemodynamic effects and no significant adverse effects. Thus, intracoronary infusion of bradykinin before PTCA rendered the myocardium relatively resistant to subsequent ischemia, and the degree of this cardioprotective effect was comparable to that afforded by the ischemia associated with the first balloon inflation in control subjects. In a separate cohort of seven patients given the same dose of bradykinin, coronary hyperemia resolved completely within 10 min after the end of the infusion, indicating that bradykinin-induced vasodilation cannot account for the protective effects observed during the first balloon inflation.
- CONCLUSIONS** Bradykinin preconditions human myocardium against ischemia in vivo in the absence of systemic hemodynamic changes. Pretreatment with bradykinin appears to be just as effective as ischemic PC and could be used prophylactically to attenuate ischemia in selected patients undergoing PTCA. (J Am Coll Cardiol 1999;34:639-50) © 1999 by the American College of Cardiology

Ischemic preconditioning (PC) is a powerful cardioprotective mechanism whereby brief episodes of ischemia enhance the tolerance of the heart to subsequent ischemic insults (1-4). Although considerable evidence supports the occurrence of ischemic PC in patients with coronary artery

disease (5), the mechanisms responsible for the development of this mechanism in humans remain poorly understood. Experimental studies suggest that an important trigger of ischemic PC is the activation of bradykinin B<sub>2</sub> receptors (6-13). The involvement of bradykinin in ischemic PC is supported by a number of experimental studies that have demonstrated that local infusion of this peptide mimics the cardioprotective effects of ischemic PC (6,7,10-12,14), whereas the administration of bradykinin B<sub>2</sub> receptor antagonists prevents it (8-13). It was proposed that bradykinin triggers ischemic PC by activating the endothelial cell B<sub>2</sub> receptor, which couples with protein kinase C and initiates a signal transduction pathway analogous to that initiated by adenosine (11). The bradykinin hypothesis of

From the Division of Cardiology, University of Louisville, and the Jewish Hospital Heart and Lung Institute, Louisville, Kentucky 40292. This work was supported in part by NIH R01 Grants HL-43151 and HL-55757 (R.B.), by AHA Kentucky Affiliate Grant KY-96-GS-39 (M.A.L.) by an Alliant Community Trust Grant (M.A.L.) and by the Medical Research Grant Program of the Jewish Hospital Research Foundation, Louisville, Kentucky. Presented at the 47th Annual Scientific Session of the American College of Cardiology, Atlanta, Georgia, April 1998.

Manuscript received December 23, 1998; revised manuscript received April 14, 1999, accepted June 3, 1999.

**Abbreviations and Acronyms**

ACE	=	angiotensin-converting enzyme
DCI	=	digital cardiac imaging
ECG	=	electrocardiogram
LV	=	left ventricular
PC	=	preconditioning
PKC	=	protein kinase C
PTCA	=	percutaneous transluminal coronary angioplasty
SAVE	=	Survival and Ventricular Enlargement
SOLVD	=	Studies of Left Ventricular Dysfunction
TRACE	=	Trandolapril Cardiac Evaluation
LAD	=	left anterior descending coronary artery

ischemic PC is further supported by the demonstration that intracardiac production of this peptide increases during myocardial ischemia (15,16).

Furthermore, it has been shown that direct intracoronary infusion of bradykinin reduces infarct size in dogs (17). In addition, the cardioprotective effects of inhibitors of the angiotensin-converting enzyme (ACE) (kininase II) appear to be due to inhibition of the breakdown of bradykinin, because bradykinin antagonists can reverse them (17,18). Although pharmacologic activation of either adenosine A<sub>1</sub> (2,19,20) or bradykinin B<sub>2</sub> (6,7,10-12,14) receptors can induce a PC-like effect, at least under certain conditions the activation of both receptors appears to be necessary to trigger the protective effects of ischemic PC (11).

To date, evidence suggesting a role of bradykinin in ischemic PC has been obtained in rabbits (10,11), rats (12) and dogs (6-9). A study of isolated atrial trabeculae subjected to substrate-free hypoxia with rapid pacing (21) supports a role of bradykinin in triggering ischemic PC in the human heart. However, because there are numerous fundamental differences between substrate-free hypoxia of isolated atrial trabeculae in vitro and ischemia of the intact ventricle in vivo, data obtained in the former preparation cannot necessarily be extrapolated to the latter setting. Specifically, it remains unknown whether bradykinin can precondition the intact human heart in vivo and, if so, whether it can protect ventricular myocardium (as opposed to atrium myocardium) against ischemia (as opposed to substrate-free hypoxia in vitro). Accordingly, it is necessary to explore the bradykinin hypothesis in the clinical setting. To date, there is no report that bradykinin can precondition human myocardium in vivo.

Elucidation of the effects of bradykinin on myocardial ischemia in humans may also help to explain the mechanism for the apparent cardioprotective effects of ACE inhibitors in patients with coronary artery disease. Several multicenter trials have demonstrated a beneficial effect of ACE inhibitors in patients with coronary artery disease (22,23) and a number of studies, such as SOLVD (Studies of Left Ventricular Dysfunction) (24), SAVE (Survival and Ventricular Enlargement) (25), and TRACE (Trandolapril

Cardiac Evaluation) (26), have shown that these agents significantly diminish the occurrence of myocardial infarction and other ischemic end points. The mechanism for this salutary action is unclear, but conceivably it may involve attenuation of the degradation of bradykinin by ACE (10,18,23,27-29). To our knowledge, no published studies have directly assessed the effect of bradykinin on myocardial ischemia in humans.

As a first step toward elucidating the role of bradykinin in ischemic PC in humans, we tested the hypothesis that pretreatment with this peptide, in the absence of ischemia, induces a cardioprotective effect comparable to that induced by ischemic PC in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). To this end, we administered intracoronary bradykinin 10 min before PTCA and determined whether the severity of ischemia during the subsequent balloon inflations was attenuated as compared with untreated control patients. In addition, we determined whether the expected PC response (i.e., the progressive decrease in ST-segment shift and chest pain during subsequent balloon inflations) was abrogated by pretreatment with bradykinin. We selected the setting of PTCA because 1) considerable evidence supports the occurrence of ischemic PC during subsequent balloon inflations (30-39); 2) this manifestation of PC has been characterized more extensively than any other clinical manifestation of PC (30-39); 3) unlike other clinical settings, during PTCA the duration of ischemia can be standardized and several clinical variables can be relatively well controlled, and 4) patients can be pretreated. The results demonstrate, for the first time, that bradykinin protects the human heart against ischemia in vivo.

## METHODS

**Study population.** The patient population consisted of 30 subjects referred for PTCA of an isolated obstructive lesion (internal diameter reduction >70% by visual assessment) in the proximal two-thirds of a major coronary artery. Patients were prospectively selected on the basis of the following criteria: 1) no angiographically visible collateral vessels; 2) no history or electrocardiographic (ECG) evidence of prior myocardial infarction in the territory supplied by the vessel undergoing PTCA; 3) no wall-motion abnormalities in the region subserved by the artery undergoing PTCA; 4) normal left ventricular (LV) ejection fraction; 5) no conduction defects on the ECG; 6) no evidence of LV hypertrophy on the echocardiogram; and 7) no baseline ST-segment abnormalities on the surface or intracoronary ECG. Fifteen patients were admitted with a diagnosis of unstable angina, and the remaining 15 had clinically stable angina pectoris (Table 1). The average interval between the last episode of angina and PTCA was  $6.2 \pm 1.1$  days in control subjects and  $5.4 \pm 0.7$  days in bradykinin-treated patients. No patient had angina pectoris in the 48 h before PTCA. On the LV angiogram, the ejection fraction was

**Table 1.** Clinical Features of the Two Groups of Patients

	Control Group (n = 15)	Bradykinin-Treated Group (n = 15)
Age (yr)	60 ± 3	59 ± 3
Gender (M/F)	12/3	11/4
Hypertension	10	13
Smoking	9	8
Diabetes mellitus	3	4
Previous CABG	0	0
Previous PTCA	2	5
Previous myocardial infarction in non-PTCA territory	3	2
LV ejection fraction (%)	55 ± 2	54 ± 3
Anginal syndrome on admission		
CCS Class 1-2	8	7
CCS Class 3-4	7	8
Interval between last episode of angina and PTCA (days)	6.2 ± 1.1	5.4 ± 0.7
Intracoronary nitroglycerin administration		
Before first inflation	1	0
After first inflation	3	0
Antianginal medications		
Intravenous nitroglycerin	2	5
Long-acting nitrates	13	9
Calcium channel-blocking agents	5	4
Beta-blocking agents	7	8

CABG = coronary artery bypass graft surgery; PTCA = percutaneous transluminal coronary angioplasty; LV = left ventricular; CCS = Canadian Cardiovascular Society.

55 ± 2% in control patients and 54 ± 3% in bradykinin-treated patients (Table 1). This study was approved by the Institutional Review Board on July 17, 1996; informed consent, via an institutionally approved human investigation form, was obtained in all patients.

**Experimental protocol.** In this single-blind study, patients were randomly allocated either to a control or a bradykinin-treated group. The control group consisted of 15 patients (12 men and 3 women, ranging in age from 40 to 77 years; mean age, 60 ± 3 years); the bradykinin-treated group consisted of 15 patients (11 men and 4 women, ranging in age from 41 to 76 years; mean age, 59 ± 3 years) (Table 1). All patients were being treated with aspirin (325 mg/d) for ≥48 h before PTCA; 22 patients (13 controls and 9 bradykinin-treated) were receiving long-acting nitrates, 15 (7 controls and 8 bradykinin-treated) were receiving beta-blockers and 9 (5 controls and 4 bradykinin-treated) were receiving calcium channel antagonists for ≥48 h before PTCA (Table 1). Antianginal medications were not discontinued before the procedure. Seven patients (two controls and five bradykinin-treated) received IV nitroglycerin before and throughout PTCA, and four patients (controls) received intracoronary nitroglycerin (Table 1). All patients

were studied after an overnight fast and were premedicated with midazolam (1 mg IV 10 min before the procedure).

The PTCA procedure was performed by a standard technique using the femoral approach. After placement of the guiding catheter and performance of baseline coronary angiography, an IV bolus of 10,000 IU of heparin was administered; additional boluses of heparin were given during the procedure to achieve an activated clotting time of >300 s. Nonionic contrast medium (Iopamidol, Bracco Diagnostics, New Brunswick, New Jersey; 796 mOsm/kg) was used in all patients. After venous cannulation, a 5F bipolar temporary transvenous pacemaker was advanced under fluoroscopic guidance to the right ventricular apex and set to demand mode for heart rate backup. A 2.2F Tracker coronary-infusion catheter (Boston Scientific, Inc., Maple Grove, Minnesota) was advanced over a 0.014-inch (0.036 cm) guide wire (Traverse wire, Advanced Cardiovascular Systems, Santa Clara, California) into the proximal portion of the coronary artery for selective intracoronary infusion of bradykinin or saline. (Bradykinin was obtained from Sigma F and D Division, St. Louis, Missouri.) A stock solution was prepared in normal saline at a concentration of 12.5 µg/ml. The solution was sterilized by the School of Pharmacy at the University of Kentucky.

On the day of the study, a sample of stock solution was diluted in normal saline to a concentration of 25 µg in 50 ml and infused at a rate of 2.5 µg/min over 10 min. This dose of bradykinin was selected on the basis of pilot studies in which an infusion of 5.0 µg/min was found to cause arterial hypotension. The control group received an equivalent volume of vehicle (normal saline). After infusion of either bradykinin or vehicle, the Tracker catheter was removed. After a 10-min drug-free period, the lesion was crossed with a 0.014-inch (0.036 cm) guide wire. The PTCA was performed with Boston Scientific, Inc. or Cordis balloon dilation catheters ranging in diameter from 2.5 to 4.0 mm. Balloon sizes were determined by examining normal regions of the coronary artery adjacent to the stenosis. After the balloon was positioned across the lesion, patients underwent three balloon inflations, each lasting 120 s, interspersed with 5-min periods of reperfusion during which the balloon was deflated and withdrawn proximal to the lesion with the guide wire remaining across the lesion. Balloon inflation pressures ranged from 4.0 to 10.0 atmospheres (atm). Five minutes after the end of the third inflation, the study protocol was terminated and decisions regarding further inflations or other interventional procedures were made on an individual basis.

**Assessment of myocardial ischemia.** Lead V<sub>5</sub> of the electrocardiographer (Hewlett-Packard, Model M1700 A) was connected to the coronary guide wire. The intracoronary ECG (derived from the guide wire) along with the remaining 11 standard surface leads were recorded continuously at a paper speed of 50 mm/s during the three balloon inflations and at selected times after deflation. At all

time-points, the ST-segment shift was measured 80 ms after the J point on a minimum of three complexes. The sums of the absolute values of the ST-segment shifts from baseline on the surface ECGs and on the intracoronary ECGs were calculated separately and expressed in millimeters (1 mm = 0.1 mV). All ECG recordings were analyzed by a cardiologist who had no knowledge of the study protocol.

As elaborated elsewhere (39), there is considerable evidence that changes in the ST-segment shift are a valid marker of changes in the severity of myocardial ischemia. For example, recent investigations in experimental animals have demonstrated that the magnitude of the ST-segment shift accurately reflects the presence and magnitude of the infarct size limitation afforded by either ischemic (40) or pharmacologic (41) PC. Furthermore, studies in patients undergoing PTCA have shown that the ST-segment shift correlates with both metabolic and contractile parameters of myocardial ischemia—that is, with the magnitude of lactate production (42) and regional wall-motion abnormalities (43).

**Assessment of chest pain.** At the beginning of the procedure, patients were informed that they may develop chest pain during balloon inflations. At the end of each inflation, the intensity of the cardiac pain was assessed using a visual-analog scale (44). Patients were asked to put a mark on a 100-mm scale marked from no symptoms [0] to the most severe symptoms [100]. The intensity of the chest pain was measured in millimeters from 0 to the subject's mark.

**Assessment of coronary blood flow and coronary collaterals.** To determine whether the cardioprotective effects of bradykinin could have been due to coronary vasodilation, coronary blood flow was measured in an additional group of seven patients. A 0.014-inch (0.036 cm) Doppler guide wire (Flowire; Cardiometrics, Mountain View, California) was advanced into the coronary artery and positioned distal to the Tracker infusion catheter. Particular care was taken to avoid placement into a side branch and to avoid post-stenotic velocity jets. Baseline average peak velocity was recorded. Once a stable, maximal Doppler signal correlated with a crisp gray-scale Doppler envelope, as previously described (45,46), was obtained, bradykinin was infused at a rate of 2.5  $\mu\text{g}/\text{min}$  for 10 min, and average peak velocity was recorded again immediately after the end of infusion and 10 min after the end of infusion. Coronary angiograms were performed at baseline, immediately after the infusion of bradykinin and 10 min after the infusion of bradykinin using an 8F guiding catheter. A single "working view" that permitted clear visualization of the coronary segment without foreshortening was selected for quantitative analysis. The angiograms were recorded using a cineangiographic system (Philips, Cincinnati, Ohio), and on-line quantitative angiography was performed with the Philips Digital Cardiac Imaging (DCI) Automated Coronary Analysis System, as previously described (47). Coronary artery diameter was

measured in a 5-mm segment of vessel beginning 2.5 mm beyond the tip of the flow wire. Coronary blood flow was derived from the average peak velocity and diameter measurements using the formula

$$Q_d = \frac{\pi D^2}{4} (0.5 \times \text{APV}),$$

where  $Q_d$  = Doppler-derived time-average flow,  $D$  = vessel diameter,  $\text{APV}$  = time average of the spectral peak velocity and  $\pi = 3.14$  (45). The use of the Doppler guide wire for intravascular measurement of coronary flow velocity has been previously validated (45,46).

After measurement of coronary blood flow, the Doppler coronary flow wire was withdrawn, mounted on the balloon angioplasty catheter, and then readvanced into the coronary artery. The wire was passed across the lesion and positioned in a distal segment of the vessel undergoing PTCA. After the balloon was positioned across the lesion, the patients underwent three 2-min balloon inflations separated by 5-min intervals of reperfusion (same protocol used in the other cohort of 30 patients). Average peak velocity was recorded continuously before, during and for 5 min after each balloon inflation. Collateral blood flow was defined as retrograde or persistent antegrade flow during balloon occlusion, as previously reported (48).

**Echocardiographic studies.** Quantitative two-dimensional echocardiograms were performed serially in 18 patients (9 control and 9 bradykinin-treated participants) at baseline, after infusion of bradykinin or saline, at the end of each balloon inflation (i.e., 110 to 120 s into the inflation) and at 5 min after each balloon deflation. The methods have been previously described in detail (39,49). Briefly, two-dimensional images of the left ventricle were obtained from the apical four- and two-chamber views with a phased-array echocardiographic machine (SONOS 1500 or 2500, Hewlett-Packard) and a 2.5-MHz transducer. The images were recorded on 1/2-inch videotape for subsequent review and analysis. The echocardiograms were analyzed quantitatively for regional LV wall-motion abnormalities with the use of a commercially available microcomputer system (GTI, Freeland, Indianapolis, Indiana). Quantitative analysis of regional wall motion was performed from the apical four- and two-chamber views using a centerline method that corrects for ventricular translation. The method constructs 100 equidistant chords perpendicular to a line centered between digitized LV end-diastolic and end-systolic endocardial borders (49). One hundred equidistant chords perpendicular to the centerline were constructed between boundaries, which represented motion of corresponding points of the LV endocardium. Chords from the anterior wall, apex, mid septum and apical septum were considered to be in the distribution of the left anterior descending coronary artery (LAD). Chords in the inferior and lateral walls were considered to be in the distribution of the right and circumflex coronary arteries, respectively.

These assumptions were verified by noting the distribution of the wall-motion abnormalities during balloon inflation. The average shortening of the chords in the distribution of the coronary artery undergoing PTCA was determined serially at baseline, after bradykinin or saline infusion, during balloon inflation and 5 min into each recovery period. The LV ejection fraction was calculated by the biplane modified Simpson's method (50). The echocardiographic studies were analyzed by an echocardiographer (M.F.S.) who had no knowledge of the treatment.

**Statistical analysis.** All data are reported as mean value  $\pm$  SEM. The ST-segment shifts, chest pain score, chordal shortening, LV ejection fraction, coronary blood flow and vessel diameter were analyzed with a one-way or two-way repeated-measures ANOVA, as appropriate. Post hoc contrasts between groups at various time-points or between time-points within one group were performed with the Student *t* test for unpaired or paired data, as appropriate, using the Bonferroni correction (51). The remaining continuous or dichotomous variables were compared between the two groups using unpaired Student *t* tests or chi-square tests, respectively. The echocardiographic data were analyzed with the SPSS program, version 6.1; the remaining data were analyzed with Microsoft Excel HP, version 7.0. A *p* value  $<0.05$  was considered statistically significant.

## RESULTS

Fifteen patients in the control group and 15 in the bradykinin-treated group met the criteria detailed under "Methods" and had technically adequate intracoronary and surface ECGs associated with complete resolution of ischemia between balloon inflations. Complete resolution of ischemia was defined as chest pain resolution and return of the ST-segment on the intracoronary and surface ECGs to within 1 mm of baseline during the 5 min that elapsed between the first, second and third balloon inflations. The clinical features of the control and bradykinin-treated patients are outlined in Table 1. There were no significant differences between the two groups.

**Coronary angioplasty.** The anatomic and hemodynamic features of the study population are summarized in Table 2. The PTCA was successfully performed in all 30 patients; coronary stenosis was reduced from  $81 \pm 3\%$  to  $20 \pm 1\%$  in the control group and from  $84 \pm 3\%$  to  $17 \pm 1\%$  in the bradykinin-treated group. The balloon pressure was similar in the control and bradykinin-treated groups (Table 2). The infusion of bradykinin had no appreciable effect on heart rate or arterial blood pressure; these two variables did not differ between the two groups during the three inflations (data not shown). The rate-pressure product was also similar (Table 2). There was no ECG or enzymatic evidence of myocardial injury in any patient.

**Table 2.** Anatomic and Hemodynamic Features of the Two Groups of Patients

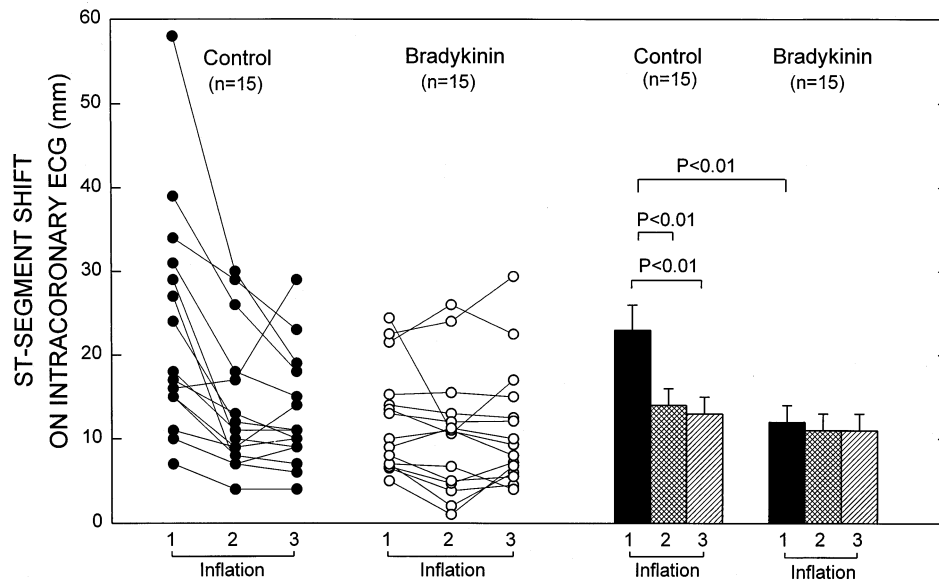
	Control Group (n = 15)	Bradykinin-Treated Group (n = 15)	P Value
Vessel undergoing PTCA			
LAD	10	6	NS
LCx	4	6	NS
RCA	1	3	NS
Site of PTCA			
Proximal third	7	7	NS
Mid-third	8	8	NS
Diameter stenosis, %			
Before PTCA	$81 \pm 3$	$84 \pm 3$	NS
After PTCA	$20 \pm 1$	$17 \pm 1$	NS
Inflation pressure, atm			
Inflation 1	$5.8 \pm 0.2$	$5.6 \pm 0.4$	NS
Inflation 2	$5.6 \pm 0.2$	$5.7 \pm 0.4$	NS
Inflation 3	$5.9 \pm 0.2$	$5.6 \pm 0.4$	NS
RPP ( $\times 10^3$ )			
Inflation 1	$10.7 \pm 0.7$	$10.2 \pm 0.5$	NS
Inflation 2	$9.5 \pm 0.8$	$10.5 \pm 0.5$	NS
Inflation 3	$9.8 \pm 0.7$	$10.6 \pm 0.7$	NS

LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; RCA = right coronary artery; PTCA = percutaneous transluminal coronary angioplasty; RPP = rate-pressure product (heart rate  $\times$  systolic blood pressure; atm = atmosphere).

**Electrocardiographic manifestations of myocardial ischemia.** All patients exhibited ST-segment elevation during balloon inflation. In the control group, the ST-segment shift was significantly greater during the first balloon inflation than during the second and third inflations on both the intracoronary ECG ( $23 \pm 3$  vs.  $14 \pm 2$  and  $13 \pm 2$  mm, respectively; Fig. 1) and the surface ECG ( $16 \pm 3$  vs.  $10 \pm 2$  and  $9 \pm 2$  mm, respectively; Fig. 2). In contrast, in the bradykinin-treated group, no differences existed in the ST-segment shift during the first, second and third balloon inflations, on either the intracoronary ECG ( $12 \pm 2$ ,  $11 \pm 2$  and  $11 \pm 2$  mm, respectively; Fig. 1) or the surface ECG ( $7 \pm 1$ ,  $7 \pm 1$  and  $7 \pm 1$  mm, respectively; Fig. 2).

The ST-segment shift recorded on the intracoronary ECG was significantly smaller in the bradykinin-treated group than in the control group during the first balloon inflation ( $12 \pm 2$  vs.  $23 \pm 3$  mm [ $-48\%$ ],  $p < 0.01$ ) (Fig. 1), but did not differ significantly between the two groups during the second and third inflations ( $11 \pm 2$  vs.  $14 \pm 2$  mm and  $11 \pm 2$  vs.  $13 \pm 2$  mm, respectively;  $p = \text{NS}$ ) (Fig. 1). Similarly, the ST-segment shift recorded on the surface ECG was significantly smaller in the bradykinin-treated group than in the control group during the first inflation ( $7 \pm 1$  vs.  $16 \pm 3$  mm, respectively;  $p < 0.01$ ), but did not differ significantly between the two groups during the second and third inflations ( $7 \pm 1$  vs.  $10 \pm 2$  mm and  $7 \pm 1$  vs.  $9 \pm 2$  mm, respectively;  $p = \text{NS}$ ) (Fig. 2).

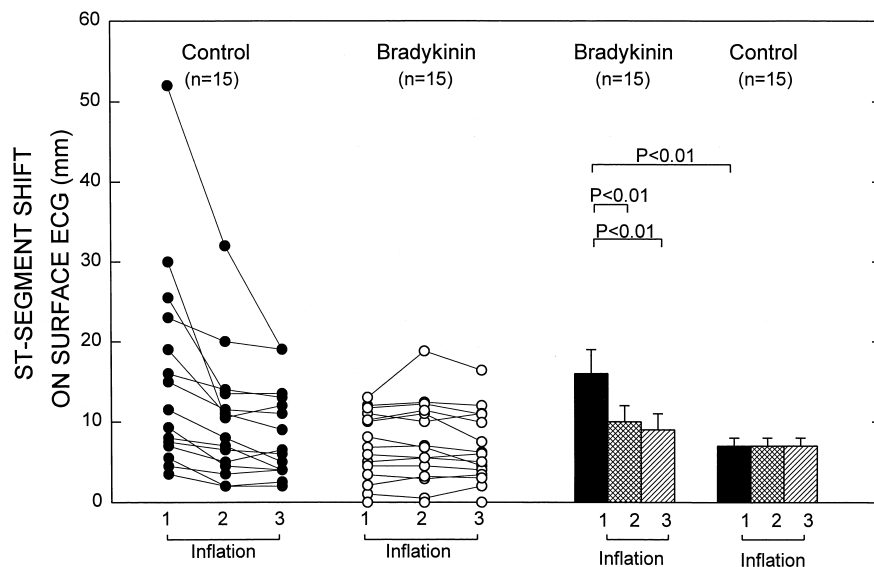
The effect of bradykinin on the ST-segment shifts was



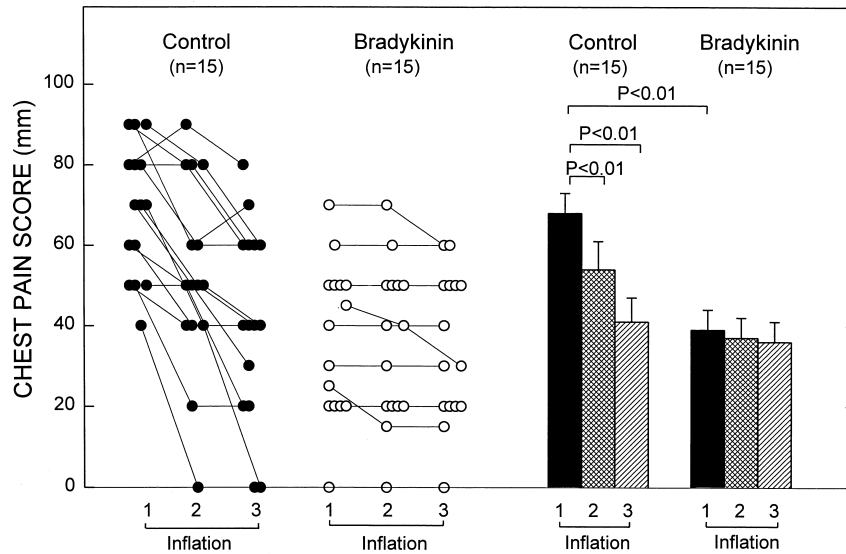
**Figure 1.** Individual (left panel) and average (right panel) values of ST-segment shifts on the intracoronary ECG at the end of the first, second and third balloon inflations in control and bradykinin-treated patients. In control patients, the ST-segment shifts decreased during the second and third inflations compared with the first. In contrast, in bradykinin-treated patients the ST-segment shifts were similar during all three inflations. During the first inflation, the ST-segment shift was less in bradykinin-treated compared with control patients. Values are means  $\pm$  SEM.

independent of the presence of a history of unstable angina. Indeed, when the analysis was restricted to the 15 patients with stable angina pectoris, the results were similar to those obtained in the entire cohort. For example, during the first, second and third balloon inflations, the intracoronary ST-segment shift averaged  $26 \pm 5$ ,  $14 \pm 3$  and  $14 \pm 3$  mm,

respectively, in the eight control patients with stable angina pectoris and  $10 \pm 2$ ,  $9 \pm 3$  and  $10 \pm 2$  mm, respectively, in the seven bradykinin-treated patients with stable angina pectoris. During the first inflation, the values in the treated patients were significantly ( $p < 0.05$ ) less than the corresponding values in the control patients.



**Figure 2.** Individual (left panel) and average (right panel) values of ST-segment shifts on the surface ECG at the end of the first, second and third balloon inflations in control and bradykinin-treated patients. In control patients, the ST-segment shifts decreased during the second and third inflations compared with the first. In contrast, in bradykinin-treated patients the ST-segment shifts were similar during all three inflations. During the first inflation, the ST-segment shift was less in bradykinin-treated than in control patients. Values are means  $\pm$  SEM.



**Figure 3.** Individual (left panel) and average (right panel) values of chest pain score during the first, second and third balloon inflations in control and bradykinin-treated patients. In control patients, the chest pain score decreased progressively from the first to the third inflation. In contrast, in bradykinin-treated patients the chest pain score did not change significantly during the three inflations. During all three inflations, the chest pain score was significantly less in bradykinin-treated compared with control patients. Values are means  $\pm$  SEM.

**Chest pain.** In the control group, the severity of chest pain was significantly greater during the first inflation than during the second and third inflations ( $68 \pm 5$  vs.  $54 \pm 7$  and  $41 \pm 6$  mm, respectively; Fig. 3). In contrast, in the bradykinin-treated group, the chest pain score did not differ significantly during the first, second and third inflations ( $39 \pm 5$ ,  $37 \pm 5$  and  $36 \pm 5$  mm, respectively; Fig. 3). The chest pain score was significantly smaller in the bradykinin-treated group than in the control group during the first balloon inflation ( $-43\%$  [ $p < 0.01$ ]) but not during the second and third inflations (Fig. 3).

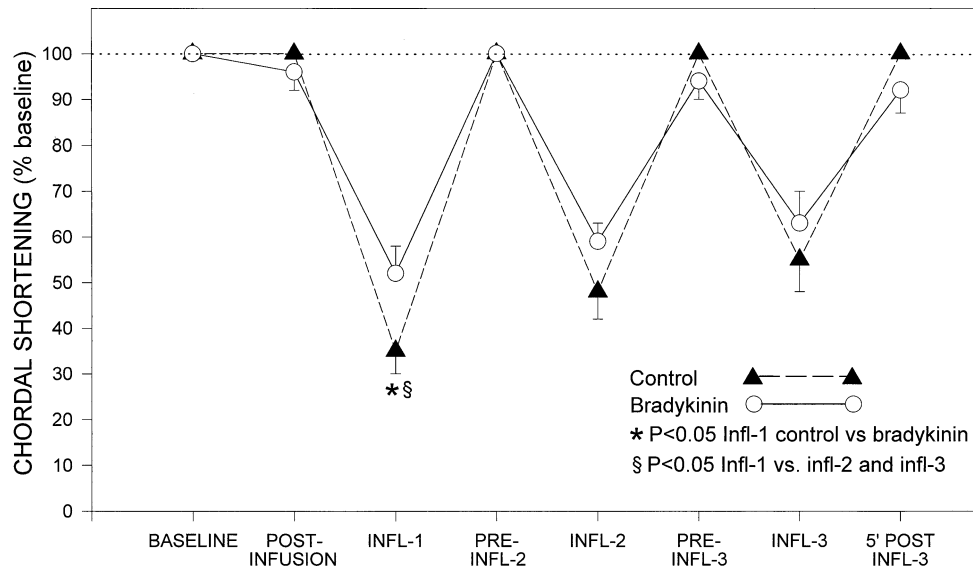
The effect of bradykinin on the severity of chest pain was independent of the presence of unstable angina. Indeed, in the 15 patients with stable angina pectoris, the chest pain score was significantly less in the bradykinin-treated group than in the control group during the first ( $-47\%$  [ $p < 0.01$ ]) and second ( $-40\%$  [ $p < 0.05$ ]) inflation.

**Echocardiographic data.** In the control group, chordal shortening in the distribution of the artery undergoing PTCA averaged  $7.9 \pm 0.3$  mm before the infusion of normal saline and  $8.0 \pm 0.3$  mm after the infusion. In the bradykinin-treated group, chordal shortening averaged  $7.9 \pm 0.4$  mm before the infusion and  $7.6 \pm 0.3$  mm after the infusion. Thus, administration of bradykinin had no appreciable effect on regional LV wall motion. In the control group, chordal shortening in the territory subserved by the occluded artery decreased markedly (by  $65 \pm 5\%$ ) during the first balloon inflation and recovered 5 min after deflation (Fig. 4). During the second and third inflations, the decrease in chordal shortening was significantly less than

during the first inflation ( $-52 \pm 6\%$  and  $-45 \pm 7\%$ , respectively; each  $p < 0.05$  vs. first inflation) (Fig. 4). In the bradykinin-treated group, the reduction in chordal shortening during the first inflation was significantly smaller than in the control group ( $-48 \pm 6\%$  vs.  $-65 \pm 5\%$ ,  $p < 0.05$  [Fig. 4]). Furthermore, in contrast to the control group, in the bradykinin-treated group there were no significant differences in chordal shortening during the first, second and third balloon inflations ( $-48 \pm 6\%$ ,  $-41 \pm 4\%$  and  $-37 \pm 7\%$ , respectively) (Fig. 4).

Left ventricular ejection fraction did not change significantly before and after the intracoronary infusion of normal saline ( $65 \pm 2\%$  and  $68 \pm 2\%$ , respectively) or bradykinin ( $61 \pm 2\%$  and  $62 \pm 2\%$ , respectively). In the control group, LV ejection fraction decreased to  $40 \pm 1\%$ ,  $43 \pm 1\%$  and  $47 \pm 1\%$  during the first, second and third inflations, respectively ( $p = \text{NS}$ ). In the bradykinin-treated group, LV ejection fraction fell to  $45 \pm 2\%$ ,  $48 \pm 3\%$  and  $47 \pm 2\%$  during the first, second and third inflations, respectively ( $p = \text{NS}$ ).

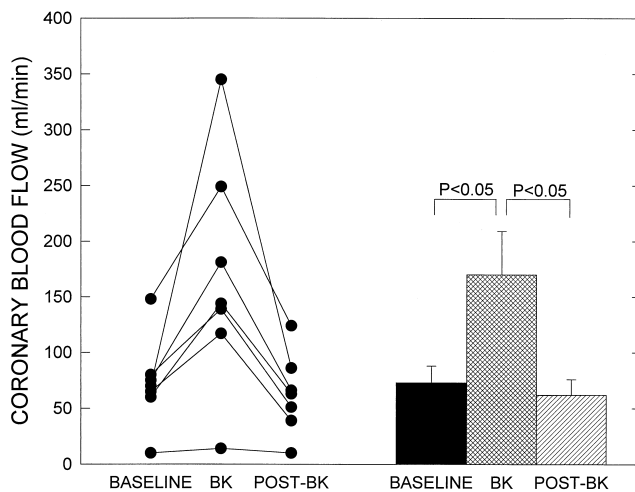
**Coronary blood flow.** In a separate cohort of seven patients not included in the studies of ST-segment shifts and chest pain, selective infusion of bradykinin into the artery undergoing PTCA resulted in a significant increase in coronary blood flow and coronary diameter, but these changes were short-lived and subsided completely within 10 min from the discontinuation of the infusion. Average coronary artery blood flow increased from  $73 \pm 15$  ml/min at baseline to  $170 \pm 39$  ml/min immediately after bradyki-



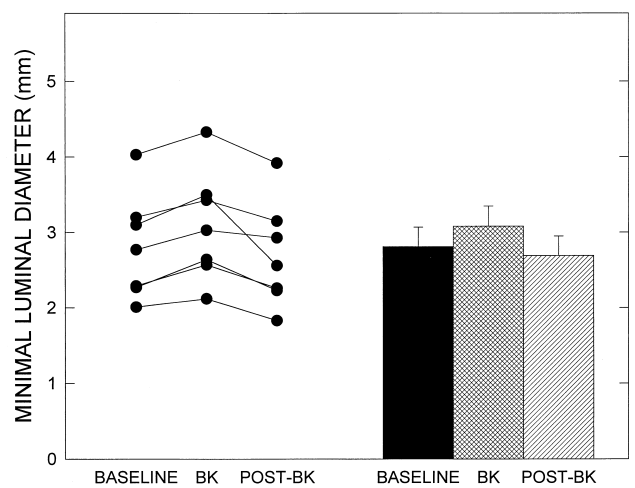
**Figure 4.** Chordal shortening in the ischemic/reperfused LV region at baseline (before bradykinin or saline infusion), after infusion, at the end of the first, second and third balloon inflations (INFL-1, INFL-2 and INFL-3), immediately before the second and third inflations (PRE-INFL-2 and PRE-INFL-3) and 5 min after the third inflation in control and bradykinin-treated patients (n = 9 in each group). Chordal shortening was determined by quantitative two-dimensional echocardiography using the centerline method (see “Methods”) and expressed as a percent of baseline values. In control patients, the decrease in chordal shortening was significantly less during the second inflation and third inflation compared with the first inflation. In contrast, in bradykinin-treated patients, chordal shortening did not change significantly during the three inflations; furthermore, during the first inflation, chordal shortenings were less in bradykinin-treated patients than in control patients. Values are means ± SEM.

nin infusion and returned to baseline levels ( $62 \pm 14$  ml/min) 10 min after the infusion of bradykinin (Fig. 5). Similarly, coronary artery diameter increased from  $2.81 \pm 0.26$  mm at baseline to  $3.08 \pm 0.27$  mm at the end of the bradykinin infusion and returned to baseline levels ( $2.69 \pm$

$0.26$  mm) 10 min after the infusion of bradykinin (Fig. 6). Collateral blood flow during balloon inflation (evidenced by retrograde flow in the occluded artery) was noted only in one patient who had subtotal stenosis of the LAD. In the other patients, no appreciable antegrade or retrograde flow



**Figure 5.** Individual (left panel) and average (right panel) values of coronary artery blood flow before the infusion of bradykinin (baseline), immediately after the infusion of bradykinin (BK) and 10 min after the infusion of bradykinin (post-BK). Coronary flow increased significantly during the infusion of bradykinin but returned to baseline values within 10 min after the infusion. Values are means ± SEM.



**Figure 6.** Individual (left panel) and average (right panel) values of minimal luminal diameter before the infusion of bradykinin (baseline), immediately after the infusion of bradykinin (BK) and 10 min after the infusion of bradykinin (post-BK). Minimal luminal diameter increased significantly during the infusion of bradykinin but returned to baseline values within 10 min after the infusion. Values are means ± SEM.

was observed during the three balloon inflations. The fact that the protective effect of bradykinin (Figs. 1 to 4) was still manifest after the hyperemic response had disappeared (Fig. 5) indicates that it was not due to recruitment of collateral vessels.

**Adverse effects of bradykinin.** The infusion of bradykinin was well tolerated by the patients, and no significant adverse effects were noted. All patients developed mild chest pain during the infusion of bradykinin, which resolved promptly after the end of the infusion. Bradykinin had no effect on heart rate and blood pressure. Therefore, it appears that this dose of bradykinin can be safely infused in patients before PTCA.

## DISCUSSION

Experimental evidence indicates that ischemic PC is a multifactorial cellular response initiated by the activation of various membrane receptors, which appear to act via a common signal transduction pathway (3,4). Studies in patients undergoing PTCA have shown that ischemic PC can be triggered by the activation of adenosine A<sub>1</sub> (34,35,39) and alpha<sub>1</sub> adrenergic (38) receptors and is mediated by the opening of K<sub>ATP</sub> channels (33). However, one important mechanism that has not yet been evaluated in humans *in vivo* is the activation of the bradykinin B<sub>2</sub> receptor.

**Salient findings.** The present study demonstrates that intracoronary infusion of bradykinin before PTCA enhances the tolerance of the heart to subsequent ischemia in a manner analogous to that observed during ischemic PC. Specifically, pretreatment with bradykinin resulted in a 48% decrease in the intracoronary ST-segment shift and a 43% decrease in the chest pain score during the first balloon inflation, indicating that the severity of ischemic injury was significantly attenuated. The ST-segment shift and the chest pain score noted during the first balloon inflation in bradykinin-treated patients were indistinguishable from those observed during the third inflation in control patients, indicating that the degree of protection afforded by bradykinin was comparable to that afforded by prior exposure to brief ischemia in the control group. This conclusion is further corroborated by the observation that in control patients the ST-segment shift, the severity of chest pain and the magnitude of regional LV wall-motion abnormalities decreased after the first balloon inflation, whereas in bradykinin-treated patients no significant decrease occurred in any of these variables during the second or third inflation compared with the first (that is, the ischemic PC effect associated with the first and second inflations failed to enhance the protection induced by pretreatment with bradykinin). Thus, it appears that in bradykinin-treated patients, the myocardium was already "maximally" preconditioned during the first balloon inflation.

The protection afforded by bradykinin represents a form

of pharmacologic PC rather than simply an anti-ischemic effect of the drug, because it was observed 10 min after the end of the infusion, at a time when bradykinin was no longer present and the coronary vasodilator effects of this peptide had completely resolved. Furthermore, the protective effects of bradykinin cannot be ascribed to a negative inotropic effect (possibly secondary to increased NO production [7,9,10]), because the infusion of bradykinin produced no changes in regional LV wall motion.

The finding that pretreatment with bradykinin elicits a PC-like effect in patients undergoing PTCA is compatible with the hypothesis that endogenous release of this substance may contribute to ischemic PC in humans. Previous studies have demonstrated that bradykinin plays a role in ischemic PC in various experimental models (6-13). However, to the best of our knowledge, the present study is the first evidence that bradykinin preconditions human myocardium against ischemia *in vivo*.

**Roles of adenosine and bradykinin in preconditioning.** We (39) have previously reported that intracoronary infusion of adenosine mimics the cardioprotective effects of ischemic PC in patients undergoing PTCA—the same clinical setting utilized in this investigation. The present finding that a different agonist elicits cardioprotective effects comparable to those of adenosine (39) has important pathophysiologic implications for the mechanism of PC in humans. Experimental studies have demonstrated that several G-protein-coupled membrane receptors can trigger a signaling cascade that culminates in enhanced resistance to ischemic injury, presumably via a common effector pathway, which appears to involve the activation of protein kinase C and the opening of K<sub>ATP</sub> channels (3,4). Our observations that a comparable degree of protection can be achieved with activation of either adenosine or bradykinin receptors supports the existence of a similar paradigm in the intact human heart and suggests that ischemic PC in patients involves multiple membrane receptors.

It has been proposed (11) that this multifactorial nature of the PC response serves to enhance its effectiveness, because brief ischemia is associated with the release of several ligands potentially capable of evoking the response. Depending on the intensity of the ischemic stimulus, these ligands may or may not be released in sufficient quantity to achieve the "threshold" stimulation necessary to trigger ischemic PC. If the PC stimulus is relatively mild, stimulation of multiple membrane receptors will be necessary to achieve the threshold. Conversely, a more robust PC stimulus may release greater quantities of ligands, such that each (or some) of them is sufficient to trigger ischemic PC.

**Possible role of collateral flow.** It could be argued that, in control patients, the alleviation of ischemia observed during subsequent balloon inflations was due to collateral recruitment rather than ischemic PC. Considerable evidence, however, suggests that this was not the case (52). For example, it has been found that the development of toler-

ance to ischemia during PTCA does not correlate with collateral function, as assessed by myocardial contrast echocardiography (37) and by measurements of peak flow velocity in the contralateral artery (38). Using a pressure-derived collateral flow index, only 30% of the observed variation in intracoronary ECG ST-segment shifts could be accounted for by collateral recruitment (53). That investigation (53) differed from most studies of PTCA because there was no evidence of enhanced tolerance to ischemia on the second inflation versus the first.

It could also be argued that bradykinin induced cardioprotection simply by improving collateral perfusion to the ischemic region. This possibility, however, is unlikely for several reasons. First, the coronary flow measurements demonstrate that the bradykinin-induced vasodilation subsided completely within 10 min of the end of the infusion (Figs. 5 and 6), so that any increase in collateral flow, even if it occurred, should have resolved before the first balloon inflation (which was performed 10 min after the end of bradykinin infusion).

Second, a hypothetical bradykinin-induced increase in collateral flow would require that the collateral vessels be dilated in their entire length, including the portion of these vessels that is outside of the ischemic coronary bed (i.e., the portion that is within the adjacent coronary beds not infused with bradykinin), because dilation of only a portion of a collateral vessel would not significantly reduce total resistance in that vessel. Because bradykinin was infused intracoronarily, however, only the portion of the collateral vessels contained within the ischemic vascular bed was exposed to this agent. Finally, evidence of collateral circulation during PTCA has been found only in a minority of patients (31,37,38,54), whereas the protective effects of bradykinin (as evidenced by the lack of decrease in ST-segment shift after the first inflation) were observed in most of the patients studied in the present investigation (Figs. 1 and 2).

**Previous studies.** The only study that has examined the role of bradykinin in ischemic PC in human myocardium has been performed in an *in vitro* model in which isolated right atrial trabeculae were submitted to simulated ischemia (substrate-free hypoxia with rapid pacing) followed by reoxygenation (21). It was found that the protection afforded by the combination of subthreshold ischemia and ACE inhibitors was abolished by the bradykinin B<sub>2</sub> receptor antagonist HOE 140, suggesting that ACE inhibitors augment ischemic PC via B<sub>2</sub> receptor activation (21). The present investigation expands upon this previous study by demonstrating 1) that bradykinin preconditions the intact human heart *in vivo*, 2) that it protects ventricular myocardium (as opposed to atrial myocardium) and 3) that it is effective in alleviating ischemia (as opposed to substrate-free hypoxia).

The exact cellular mechanism whereby bradykinin induces a PC-like state remains unclear. Goto et al. (11) have shown that the PC-like effect of bradykinin in rabbit hearts

could be abolished by the protein kinase C (PKC) inhibitors polymixin B and staurosporine, indicating that bradykinin induces protection via a PKC-mediated signaling pathway. This is the same signaling pathway that is activated by adenosine (3,4), which provides a plausible explanation for the fact that both adenosine and bradykinin can induce comparable protection in experimental animals (11) and in patients undergoing PTCA, as shown in our previous study (39) and in the current study.

**Implications.** In conclusion, this study demonstrates that in patients with coronary artery disease, pretreatment with bradykinin confers significant protection during a subsequent coronary occlusion, as evidenced by an attenuation of the electrocardiographic, mechanical and symptomatic manifestations of ischemia. To our knowledge, this is the first indication that bradykinin protects human myocardium against ischemia *in vivo*. Judging from the variables measured in the present investigation, the magnitude of bradykinin's cardioprotective actions appears to be equivalent to that of ischemic PC.

These results have pathophysiologic and therapeutic implications. From a pathophysiologic standpoint, they suggest that bradykinin may play a role in ischemic PC in humans, because this peptide has been shown to be produced during brief coronary occlusions in patients (55). It is important to note that the notion that bradykinin exerts cardioprotective effects provides a possible explanation for the known anti-ischemic actions of ACE inhibitors. It is well established that ACE is present in the vascular endothelium as well as in the parenchyma of many tissues, including the heart (28). Also, ACE-induced degradation of bradykinin appears to occur in many tissues, including the myocardium (23). Studies of ACE inhibitors (24-26) have demonstrated that these agents cause a reduction of ischemic events that cannot be explained solely by improved hemodynamics (29). The present finding that bradykinin attenuates the severity of ischemic injury is compatible with the hypothesis that the anti-ischemic effects of ACE inhibitors may be mediated, at least in part, by enhanced tissue kinin levels.

From a practical standpoint, pretreatment with bradykinin may be a useful prophylactic measure in patients undergoing PTCA who are at risk for complications. For example, patients with substantially impaired LV function or large regions of myocardium subtended by the target vessel can develop severe hemodynamic compromise or refractory arrhythmias in the event of abrupt vessel closure. Because such patients are likely to suffer irreversible myocardial or end-organ damage due to hypotension and ischemia and might not be expected to survive the typical 130-150-min time period before reperfusion by surgical revascularization, there is a need to develop strategies that can lessen the severity of ischemic myocardial damage. Although adenosine can induce a cardioprotective effect comparable to that of bradykinin (39), infusion of adenosine

into the right or a dominant left circumflex artery is hazardous because it may cause bradyarrhythmias or hypotension as a result of sinus bradycardia and/or atrioventricular block. Bradykinin is devoid of these side effects and therefore provides an alternative form of protection for those patients in whom intracoronary adenosine is contraindicated.

**Reprint requests and correspondence:** Dr. Roberto Bolli, Division of Cardiology, University of Louisville, Louisville, Kentucky 40292. E-mail: rbolli@louisville.edu.

## REFERENCES

- Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124-36.
- Auchampach JA, Gross GJ. Adenosine A<sub>1</sub> receptors, K<sub>ATP</sub> channels, and ischemic preconditioning in dogs. *Am J Physiol* 1993;264:H1327-36.
- Cohen MV, Downey JM. Preconditioning during ischemia. *Cardiol Rev* 1995;3:137-49.
- Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E. Medical and cellular implications of stunning, hibernation and preconditioning: an NHLBI workshop. *Circulation* 1998;97:1848-67.
- Kloner RA, Yellon D. Does ischemic preconditioning occur in patients? *J Am Coll Cardiol* 1994;24:1133-42.
- Vegh A, Szekeres L, Parratt JR. Local intracoronary infusions of bradykinin profoundly reduce the severity of ischaemia-induced arrhythmias in anaesthetized dogs. *Br J Pharmacol* 1991;104:294-5.
- Vegh A, Papp JG, Szekeres L, Parratt JR. Prevention by an inhibitor of the L-arginine-nitric oxide pathway of the antiarrhythmic effects of bradykinin in anaesthetized dogs. *Br J Pharmacol* 1993;110:18-9.
- Parratt JR. Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol Sci* 1994;15:19-25.
- Vegh A, Papp JG, Parratt JR. Attenuation of the antiarrhythmic of ischaemic preconditioning by blockade of bradykinin B<sub>2</sub> receptors. *Br J Pharmacol* 1994;113:1167-72.
- Wall TM, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. *J Pharmacol Exp Ther* 1994;270:681-9.
- Goto M, Liu Y, Yang X-M, Ardell JL, Cohen MV, Downey JM. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ Res* 1995;77:611-21.
- Brew EC, Mitchell MB, Rehiring TF, et al. Role of bradykinin in cardiac functional protection after global ischemia-reperfusion in rat heart. *Am J Physiol* 1995;269:H1370-8.
- Miki T, Miura T, Ura N, et al. Captopril potentiates the myocardial infarct size-limiting effect of ischemic preconditioning through bradykinin B<sub>2</sub> receptor activation. *J Am Coll Cardiol* 1996;28:1616-22.
- Bugge E, Ytrehus K. Bradykinin protects against infarction but does not mediate ischemic preconditioning in the isolated rat heart. *J Mol Cell Cardiol* 1996;28:2333-41.
- Matsuki T, Shoji T, Yoshida S, et al. Sympathetically induced myocardial ischaemia causes the heart to release plasma kinin. *Cardiovasc Res* 1987;21:428-32.
- Baumgarten CR, Linz W, Kunkel G, Schölkens BA, Wiemer G. Ramiprilat increases bradykinin outflow from isolated hearts of rat. *Br J Pharmacol* 1993;108:293-5.
- Martorana PA, Kettenbach B, Briepohl G, Linz W, Schölkens BA. Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. *Eur J Pharmacol* 1990;182:395-6.
- Hartman JC, Wall TM, Hullinger TG, Shebuski RJ. Reduction of myocardial infarct size by ramiprilat: reversal by the bradykinin antagonist HOE 140. *J Cardiovasc Pharmacol* 1993;21:996-1003.
- Liu GS, Thornton J, Van Winkle DM, Stanley AWH, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A<sub>1</sub> adenosine receptors in rabbit heart. *Circulation* 1991;84:350-6.
- Thornton JD, Liu GS, Olsson RA, Downey JM. Intravenous pretreatment with A<sub>1</sub>-selective adenosine analogues protects the heart against infarction. *Circulation* 1992;85:659-5.
- Morris SD, Yellon DM. Angiotensin-converting enzyme inhibitors potentiate preconditioning through bradykinin B<sub>2</sub> receptor activation in human heart. *J Am Coll Cardiol* 1997;29:1599-1606.
- ACE Inhibitor Myocardial Infarction Collaborative Group. Indication for ACE inhibitors in the early treatment of acute myocardial infarction: systematic overview of individual data from 100,000 patients in randomized trials. *Circulation* 1998;97:2202-12.
- Brown NJ, Vaughan DE. Angiotensin-converting enzyme inhibitors. *Circulation* 1998;97:1411-20.
- SOLVD. Effect of enalapril on mortality and development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med* 1992;327:685-91.
- SAVE. Effects of captopril on ischemic events after myocardial infarction. *Circulation* 1994;90:1731-8.
- Kober L, Torp-Pederson C, Carlson JE, et al. for the Trandolapril Cardiac Evaluation (TRACE) Study Group. A clinical trial of the angiotensin-converting enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med* 1995;333:1670-6.
- Ehring T, Baumgart D, Krajcar M, Hummelgen M, Kompa S, Heusch G. Attenuation of myocardial stunning by the ACE inhibitor ramiprilat through a signal cascade of bradykinin and prostaglandins but not nitric oxide. *Circulation* 1994;90:1368-85.
- Linz W, Wiemer G, Schölkens BA. Role of kinins in the pathophysiology of myocardial ischemia: in vitro and in vivo studies. *Diabetes* 1996;45 Suppl 1:S51-S8.
- Sayer JW, Timmis AD. Angiotensin-converting enzyme inhibitors and coronary artery disease. *Cardiovasc Drugs Ther* 1996;10:631-7.
- Deutsch E, Berger M, Kussmaul WG, Hirschfeld JW, Herrmann HC, Laskey WK. Adaptation to ischemia during percutaneous transluminal coronary angioplasty: clinical, hemodynamic, and metabolic features. *Circulation* 1990;82:2044-51.
- Cribier A, Korsatz L, Koning R, et al. Improved myocardial ischemic response and enhanced collateral circulation with long repetitive coronary occlusion during angioplasty: a prospective study. *J Am Coll Cardiol* 1992;20:578-86.
- Tomai F, Crea F, Gaspardone A, et al. Mechanisms of cardiac pain during coronary angioplasty. *J Am Coll Cardiol* 1993;22:1892-6.
- Tomai F, Crea F, Gaspardone A, et al. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K<sup>+</sup> channel blocker. *Circulation* 1994;90:700-5.
- Tomai F, Crea F, Gaspardone A, et al. Effects of A<sub>1</sub> adenosine receptor blockade by bamifylline on ischaemic preconditioning during coronary angioplasty. *Eur Heart J* 1996;17:846-53.
- Clayns MJ, Vrints CJ, Bosmans JM, Conraads VM, Snoeck JP. Aminophylline inhibits adaptation to ischaemia during angioplasty: role of adenosine in ischaemic preconditioning. *Eur Heart J* 1996;17:539-44.
- Lim R, Laskey WK. Ischemic preconditioning in unstable coronary syndromes: evidence for time dependence. *J Am Coll Cardiol* 1997;30:1461-5.
- Sakata Y, Kodama K, Kitakaze M, et al. Different mechanisms of ischemic adaptation to repeated coronary occlusion in patients with and without recruitable collateral circulation. *J Am Coll Cardiol* 1997;30:1679-86.
- Tomai F, Crea F, Gaspardone A, et al. Phentolamine prevents adaptation to ischemia during coronary angioplasty: role of  $\alpha$ -adrenergic receptors in ischemic preconditioning. *Circulation* 1997;96:2171-7.
- Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R. Preconditioning of human myocardium with adenosine during coronary angioplasty. *Circulation* 1997;95:2500-7.
- Shattock MJ, Lawson CS, Hearse DJ, Downey JM. Electrophysiological characteristics of repetitive ischemic preconditioning in the pig heart. *J Mol Cell Cardiol* 1996;28:1339-47.
- Cohen MV, Yang XM, Downey JM. Attenuation of ST-segment elevation during repetitive coronary occlusions truly reflects the protection of ischemic preconditioning and is not an epiphenomenon. *Basic Res Cardiol* 1997;92:426-34.
- Leesar M, Bhatti Z, Masden R, Xenopoulos N, Bolli R. Adenosine preconditions human myocardium against the metabolic effects of ischemia during PTCA (abstr). *J Am Coll Cardiol* 1997;29:316A.

43. Stoddard MF, Hasnie AMA, Leesar M, Bolli R. Adenosine preconditions against ischemia-induced systolic and diastolic dysfunction during percutaneous coronary angioplasty (abstr). *J Am Coll Cardiol* 1998;31:458A.
44. Huskisson EC. Measurement of pain. *Lancet* 1974;2:1127-31.
45. Doucette LW, Corl D, Payne HM, et al. Validation of a Doppler guide wire for intravascular measurement of coronary artery blood flow velocity. *Circulation* 1992;85:1899-1911.
46. Vanyi J, Bowres TR, Jarvis G, White CW. Can an intracoronary Doppler wire accurately measure changes in coronary blood flow velocity? *Cathet Cardiovasc Diagn* 1993;29:240-6.
47. Bartunek J, Sys SU, Heyndrickx GR, Pijls NH, De Bruyne B. Quantitative coronary angiography in predicting functional significance of stenoses in an unselected patient cohort. *J Am Coll Cardiol* 1995;26:328-34.
48. Kern MJ, Donohue TJ, Bach RG, Aguirre FV, Caracciolo EA, Ofili E. Quantitating coronary flow velocity in patients during coronary angioplasty using a Doppler guidewire. *Am J Cardiol* 1993;71:34D-40D.
49. Assmann PE, Slager CJ, van der Borden SG, et al. Comparison of models for quantitative left ventricular wall-motion analysis from two-dimensional echocardiograms during acute myocardial infarction. *Am J Cardiol* 1993;71:1262-9.
50. Schiller NB, Shah PM, Crawford M, et al. Recommendation for quantitation of the left ventricle by two-dimensional echocardiography. *J Am Soc Echocardiogr* 1989;2:358-67.
51. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ Res* 1980;47:1-9.
52. Bolli R, Leesar M, Ahmed M, Stoddard M, Broadbent J. Letter to the Editor. *Circulation* 1998;98:824-5.
53. Billinger M, Fleisch M, Eberli FR, Garachemani A, Meier B, Seiler C. Is the development of myocardial tolerance to repeated ischemia in humans due to preconditioning or to collateral recruitment? *J Am Coll Cardiol* 1999;33:1027-35.
54. Kyriakidis MK, Petropoulakis PN, Tentolouris CA, et al. Relation between changes in blood flow of the contralateral coronary artery and the angiographic extent and function of recruitable collateral vessels arising from this artery during balloon coronary occlusion. *J Am Coll Cardiol* 1994;23:869-78.
55. Parratt JR, Vegh A, Zeitlin J, et al. Bradykinin and endothelial-cardiac myocyte interactions in ischemic preconditioning. *Am J Cardiol* 1997;80:124A-31A.