Cardiac interstitial bradykinin release during ischemia is enhanced by ischemic preconditioning

HUI-LIN PAN,¹,² SHAO-RUI CHEN,¹ GLORIA M. SCICLI,³ AND OSCAR A. CARRETERO³

¹Department of Anesthesiology and ²Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157; and ³Division of Hypertension, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan 48202

Received 18 October 1999; accepted in final form 14 January 2000

Pan, Hui-Lin, Shao-Rui Chen, Gloria M. Scicli, and Oscar A. Carretero. Cardiac interstitial bradykinin release during ischemia is enhanced by ischemic preconditioning. Am J Physiol Heart Circ Physiol 279: H116–H121, 2000.—Ischemic preconditioning is known to protect the myocardium from ischemia-reperfusion injury. We examined the transmural release of bradykinin during myocardial ischemia and the influence of ischemic preconditioning on bradykinin release during subsequent myocardial ischemia. Myocardial ischemia was induced by occlusion of the left anterior descending coronary artery in anesthetized cats. Cardiac microdialysis was performed by implantation and perfusion of dialysis probes in the epicardium and endocardium. In eight animals, bradykinin release was greater in the endocardium than in the epicardium (14.4 ± 2.8 vs. 7.3 ± 1.7 ng/ml, P < 0.05) during 30 min of ischemia. In seven animals subjected to preconditioning, myocardial bradykinin release was potentiated significantly from 2.4 ± 0.6 ng/ml during the control period to 23.1 ± 2.5 ng/ml during 30 min of myocardial ischemia compared with the non-preconditioning group (from 2.7 ± 0.6 to 13.4 ± 1.9 ng/ml, P < 0.05, n = 6). Thus, this study provides further evidence that transmural gradients of bradykinin are produced during ischemia. The results also suggest that ischemic preconditioning enhances bradykinin release in the myocardial interstitial fluid during subsequent ischemia, which is likely one of the mechanisms of cardioprotection of ischemic preconditioning.

Keywords: cardiac microdialysis; angiotensin-converting enzyme inhibitors; captopril; kinin receptors; myocardium

The Kallikrein-Kinin System involves the sequential activation of several proteolytic enzymes. Activation of factor XII in blood at the site of tissue injury results in activation of kallikrein and subsequent generation of bradykinin cleavage of kininogen (9, 32). The generated bradykinin is degraded primarily by the action of kininase I and angiotensin-converting enzyme (ACE, also called kininase II). The kallikrein-kinin system has been located in the heart and endothelium (17, 31). Bradykinin is a potent vasoactive peptide derived from precursors during myocardial ischemia (10, 17, 20). Occlusion of a major coronary artery is known to lead to more severe ischemia in the endocardium than in the epicardium (21, 22). In this regard, during prolonged ischemia, tissue necrosis proceeds from the endocardium to the epicardium because of a greater energy imbalance in the endocardium (11, 21). Previous studies also demonstrated enhanced accumulation of many ischemic metabolites in the endocardium, including lactic acid and adenosine (1, 6). However, it is uncertain whether transmural gradients of bradykinin release exist during myocardial ischemia.

The sensory nerve endings of cardiac sympathetic afferent nerves are located in the myocardial interstitium near the epicardium (2). We have shown that endogenously produced bradykinin is involved in activation of cardiac sympathetic afferents during myocardial ischemia (25). Thus determination of myocardial interstitial bradykinin is important for our understanding of the mechanisms of chest pain in patients with myocardial ischemia. A cardiac microdialysis approach has been developed to assess the cardiac interstitial fluid levels of adenosine during zero-flow ischemia (6, 26). This technique is particularly useful, because cardiac tissues in humans and many experimental animals, such as cats, have little collateral blood flow, and it is impossible to obtain coronary sinus blood samples from the ischemic region during complete occlusion of the coronary arteries. Thus one specific aim of the present study was to use the cardiac microdialysis technique to determine whether there is transmural distribution of myocardial bradykinin release during myocardial ischemia.

Short periods of ischemia, whether single or repeated, render the heart more resistant to subsequent longer ischemia, a process known as ischemic preconditioning (7, 15). Although the mechanisms of ischemic preconditioning remain to be established, several ischemic metabolites, including bradykinin, have been proposed to be responsible for this protective action afforded by ischemic preconditioning (7, 9, 10, 28). The role of bradykinin in cardioprotection has been determined by administration of exogenous bradykinin or kinin receptor antagonists and by sampling coronary sinus blood during ischemia (9, 10, 19, 20). Bradykinin

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
is an important mediator of cardiac protection during myocardial ischemia and infarction. Its cardioprotective profile resembles that of ACE inhibitors, which potentiate the beneficial effects of endogenous kinins on cardiovascular function, coronary blood flow, and myocardial energy metabolism (8–10, 12, 31). Furthermore, the contribution of bradykinin to the cardioprotective effect of ischemic preconditioning has been substantiated in kinin B2 receptor knockout mice and kininogen-deficient rats (32). However, to our knowledge, the impact of ischemic preconditioning on myocardial bradykinin release has not been determined directly. Therefore, in the present study, we tested the hypothesis that ischemic preconditioning promotes myocardial bradykinin release during subsequent prolonged ischemia.

METHODS

General Surgery and Procedures

The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Wake Forest University School of Medicine, and they adhere to the Guide for the Care and Use of Laboratory Animals (US Public Health Service). Adult cats of either gender were anesthetized initially with ketamine (30 mg/kg im), and anesthesia was maintained with α-chloralose (50–60 mg/kg iv). One femoral artery and vein were cannulated for measurement of blood pressure and administration of fluids and drugs, respectively. The animal was intubated, and respiration was maintained artificially (model CIV-101, Columbus Instruments, Columbus, OH). Arterial blood pressure was measured with a pressure transducer and monitored continuously on a recorder (model K2G, Astro-Med, West Warwick, RI). Arterial blood gases were analyzed with a Radiometer analytical Systems, West Lafayette, IN; 300 μm diameter, molecular weight cutoff 10,000 atomic mass units) and hollow silica tubes (Scientific Glass Engineering, Austin, TX; 120 μm OD, 170 μm OD). The known kinin-generating and degrading enzymes (mol wt >34,000) will not cross the dialysis membrane because of their size. For each probe, a silica tube was inserted into each end of the dialysis fiber and adjusted to keep the distance between the ends of the tubes at 4 mm (dialysis area). The silica tubes were then sealed in place within the dialysis fiber with cyanoacrylic glue. One end of the tube was inserted manually into the left ventricular wall and pulled through the myocardium until the portion of the dialysis fiber between the ends of the silica tubes was positioned within the region of myocardium perfused by the LAD. After the probe was implanted, the inflow silica tube was connected via a length of PE-10 tubing to a gastight glass syringe filled with Krebs-Henseleit buffer bubbled with 95% N2-5% CO2. The Po2, Pco2, and pH in the buffer were ~25 mmHg, 40 mmHg, and 7.35, respectively. The low Po2 was chosen to minimize the local supply of O2 by the microdialysis probe during myocardial ischemia (6). The buffer solution was infused through the inflow tubing of the probe at a constant rate of 1 μl/min with a four-channel syringe infusion pump (model MD-1020, Bioanalytical Systems). The effluent from each probe (dialysate) traveled through the outflow tubing and into capped microcentrifuge tubes containing 30 μl of a solution containing kininase and kininogenase inhibitor to prevent degradation of bradykinin in collected samples. The kininase and kininogenase inhibitor solution (1 ml) contained 20 mg of disodium EDTA, 10 mg of 1,10-phenanthroline, 800 mg of soybean trypsin inhibitor, and 10,000 kallikrein-inactivating units of aprotinin (18). The probes were perfused for 120 min before the beginning of the protocol to allow the tissue to recover from implantation of the microdialysis probe. In preliminary experiments, we determined the recovery efficiency of the dialysis probe in vitro by placing the membrane portion of the probe in a 35-mm culture dish containing a solution of 10 ng/ml of bradykinin at different perfusion rates. On the basis of five separate experiments, the recovery rate was 76 ± 13% at a perfusion rate of 1 μl/min. The recovery rate decreased to 34 ± 9% when the perfusion rate was increased to 2 μl/min; thus 1 μl/min was chosen for this study. Because of the limited sensitivity of the bradykinin assay, the dialysate was collected over a period of 30 min for bradykinin measurement.

Experimental Protocols

Transmural myocardial bradykinin release during ischemia. Twenty-two animals were used for this protocol (4 animals died of ventricular fibrillation during ischemia and thus were not included in the following protocol). In eight animals, four microdialysis probes were implanted in the heart. Two were implanted in the myocardial region perfused by the distal LAD, such that one dialysis probe was located in the endocardium and the other in the epicardium (within 1–1.5 mm from the epicardial surface). Correct position of the probes in the ischemic region was verified visually by cyanosis on occlusion of the LAD. Another two probes were implanted in the endocardium and epicardium, respectively, but located in the areas not perfused by the distal LAD (as evidenced by the lack of change in tissue color after LAD occlusion). In all animals studied, precise position of the probe in the myocardium was verified under a microscope (model M-900, D. F. Vasconcellos, São Paulo, Brazil) at the end of the experiment. After collection of two 30-min preischemic control samples, myocardial ischemia was induced for 30 min by snaring the suture around the LAD. After ischemia, reperfusion was allowed for 30 min by releasing the occluder. Dialysate samples were collected during 30 min of ischemia and 30 min of reperfusion.

Two dialysis probes were positioned in the endocardium in six animals. To evaluate the ability of cardiac microdialysis to reflect alterations in kinin metabolism during ischemia, dialysates were collected before and after treatment with captopril, an inhibitor of ACE (kininase II). Captopril
H118  ISCHEMIC PRECONDITIONING INCREASES MYOCARDIAL BRADYKININ

(4 mg/kg iv; Sigma Chemical, St. Louis, MO) was injected 2 h after an initial cycle of 30 min of myocardial ischemia-30 min of reperfusion. At 15 min after captopril injection, the dialysates were collected again during a repeated 30 min of control period, 30 min of ischemia, and 30 min of reperfusion for measurement of bradykinin. Four animals served as the control group for repeat ischemia, in which the above procedures were performed, except captopril treatment was omitted.

Effect of ischemic preconditioning on myocardial bradykinin release during ischemia. Thirteen animals were used for this protocol. In seven animals, two microdialysis probes were implanted in the portion of the endocardium perfused by the LAD. After collection of two control samples, ischemic preconditioning was produced by 5 min of LAD occlusion followed by 5 min reperfusion. The ischemia-reperfusion cycle was repeated immediately after initial reperfusion. Subsequently, the LAD was occluded for 30 min. Reperfusion was allowed for 30 min by releasing the snare around the LAD. Six additional animals not subjected to ischemic preconditioning served as controls (non-preconditioning group).

The heart was removed at the end of the experiment, and the myocardial tissues were stained with triphenyltetrazolium chloride (Sigma Chemical) to assess possible tissue infarction. Bradykinin levels in the dialysate samples were measured by RIA, as described previously (18). Values are means ± SE. Differences between means were determined by ANOVA followed by Tukey’s post hoc test. Statistical significance was considered when P < 0.05.

RESULTS

Hemodynamic Data Profile

The hemodynamic parameters were stable for all animals throughout the experiments. The mean arterial pressure and heart rate at the beginning of the protocol were 94 ± 23 mmHg and 124 ± 17 beats/min, respectively. At the end of experiments, the mean arterial pressure and heart rate were 76 ± 15 mmHg and 119 ± 12 beats/min, respectively. Absence of myocardial infarction was confirmed in all animals studied by staining the myocardium with triphenyltetrazolium chloride. Four animals were eliminated because of ventricular fibrillation during ischemia and reperfusion. Data from two animals were excluded from analysis because of misplacement of the cardiac microdialysis probes.

Transmural Myocardial Bradykinin Release During Ischemia-Reperfusion

In the nonischemic region, myocardial bradykinin levels in the dialysate were stable for 120 min. Bradykinin levels in the dialysate from probes in the ischemic zone during the control period were also similar (Fig. 1). During the preischemic control period, the bradykinin level was similar in the epicardium and the endocardium (Fig. 1). Occlusion of the LAD increased the bradykinin concentration in the dialysate significantly in the epicardium and endocardium in eight animals. There was a greater increase in dialysate bradykinin in the endocardium than in the epicardium during ischemia (P < 0.05; Fig. 1). During reperfusion, dialysate bradykinin in the epicardium and endocar-
The transmural distribution of cardiac bradykinin during ischemia has not been determined previously. Our study demonstrated that bradykinin was significantly higher in the endocardium than in the epicardium during 30 min of ischemia, which is likely due to a higher metabolic imbalance induced by ischemia in the endocardium (11, 21, 22). This observation is consistent with previous studies showing a time-dependent release of cardiac bradykinin during ischemia (19, 20). Although bradykinin is increased during myocardial ischemia in humans and experimental animals (3, 10, 19, 20), the cellular source of bradykinin production during ischemia has not been established. Recently, Matoba et al. (12) compared bradykinin release from cultured rat myocytes and endothelial cells in vitro during hypoxia-reoxygenation. Although both cell types are capable of producing bradykinin during hypoxia-reoxygenation, myocytes are an important source of bradykinin generation through a local kinin-kallikrein system (12). Because the vascular endothelium is a highly active metabolic tissue, it may be difficult to interpret concentrations of metabolites in the venous effluent. Estimates of ischemic metabolites in the coronary sinus blood may be complicated further by the fact that the venous blood drains the normal and the ischemic myocardium. Thus the cardiac microdialysis technique has an advantage, in that the metabolites released into the myocardial interstitium are measured directly. The increased accumulation of bradykinin in the myocardial interstitium may play a role in stimulation of the nerve endings of cardiac afferents during ischemia. For example, we have shown that endogenously produced bradykinin contributes to activation of cardiac sympathetic afferents during ischemia, since pretreatment with kinin B2-receptor antagonists attenuates the response of these afferent fibers to myocardial ischemia (25).

A variety of vasoactive substances are released from the coronary vascular endothelial cells and myocytes during myocardial ischemia. Some of these metabolites, such as thromboxane and endothelin, are detrimental to cardiac function and survival (5, 7), whereas others appear to be protective and are part of the response of the heart to acute injury. Endogenously produced adenosine, prostacyclin, nitric oxide (NO), and bradykinin have been shown to protect the myocardium against ischemia-reperfusion injury (14, 20). Release of such protective mediators also contributes to the protective effects of ischemic preconditioning (14, 20, 27). Increased cardiac bradykinin release during subsequent ischemia may be one of the important mechanisms of cardioprotection afforded by ischemic preconditioning. Although bradykinin release from the myocardium is increased during 3–5 min of ischemia (16, 23), the influence of ischemic preconditioning on myocardial bradykinin release during subsequent prolonged ischemia has not been determined directly. Our results indicate that ischemic preconditioning is capable of activating the cardiac kallikrein-kinin system in the feline model of myocardial ischemia. Our data suggest that increased bradykinin release during 30 min of ischemia is unlikely due to tissue infarction, as shown by staining the cardiac tissue with triphenyltetrazolium chloride. However, a technical limitation should be acknowledged, since infarct size is typically measured after 2 h of reperfusion. Using the cardiac microdialysis technique, Schulz et al. (23) demonstrated that myocardial bradykinin is increased with 3 or 10 min, but not 2 min, of ischemic preconditioning in a pig model of myocardial ischemia, which is consistent with the beneficial effect of bradykinin on the myocardium. The cardioprotective effect of bradykinin has been demonstrated in several animal models of myo-

![Graph A](image1.png)

**Fig. 2.** A: bradykinin levels in the endocardium during initial and repeated 30-min periods of ischemia, separated by 2 h, in the control group (n = 4). B: bradykinin levels in the endocardium during the control period, 30 min of ischemia, and 30 min of reperfusion before and after treatment with captopril (n = 6). Values are means ± SE. *P < 0.05 compared with control. **P < 0.05 compared with bradykinin level during ischemia before treatment with captopril.

![Graph B](image2.png)

**Fig. 3.** Myocardial interstitial bradykinin levels during the control period, 30 min of ischemia, and 30 min of reperfusion in animals with (n = 7) and without (n = 6) ischemic preconditioning. Values are means ± SE. *P < 0.05 compared with baseline. **P < 0.05 compared with bradykinin level during ischemia in animals without ischemic preconditioning (non-preconditioning).
cardiac ischemia. In this regard, pretreatment with Hoe-140 (icatibant, a bradykinin B₂-receptor antagonist) attenuates or abolishes the reduction in infarct size and ventricular arrhythmias induced by ischemic preconditioning (23, 28, 30).

Release of kinins has been shown in patients during coronary angioplasty and coronary artery bypass surgery (10, 20). However, it remains uncertain how increased release of bradykinin protects myocardial tissue during ischemia. We observed that ischemic preconditioning increased the myocardial bradykinin during ischemia but not during reperfusion. Thus it is possible that increased bradykinin release after preconditioning protects against tissue injury caused by myocardial ischemia. However, it remains uncertain whether the preconditioning-elicited increase in myocardial bradykinin also protects against reperfusion injury. Bradykinin generated during ischemia leads to the generation of prostacyclin and NO through kinin B₂ receptors (8–10, 12). Some studies have shown that NO diffuses into cardiac myocytes and stimulates soluble guanylyl cyclase, resulting in increased levels of cGMP to reduce myocardial O₂ demand (12, 24, 29). We observed in this study that an initial and a repeated period of ischemia, if separated by 2 h, had no potentiating effect on myocardial bradykinin release during subsequent prolonged ischemia. Thus the action of preconditioning on bradykinin metabolism appears to be transient in this feline model of myocardial ischemia. Others reported that cardiac protection is lost if the interval between the preconditioning stimulus and prolonged coronary artery occlusion is increased to 1 h (27, 28), which supports a role of labile mediators in this protection. A recent in vitro study has provided substantial evidence that endogenous bradykinin protects myocytes against hypoxia-reoxygenation injury by inducing NO production and increasing cGMP synthesis, which contributes to cardioprotection through energy preservation (12, 29) and modulation of myocyte contractility (24). The protective action of bradykinin may also be mediated by protein kinase C (4, 8) and generation of NO from endothelial cells (7, 20). Additionally, activation of protein kinase C may protect the myocardium from ischemic injury by opening ATP-sensitive K⁺ channels (13).

In summary, a cardiac microdialysis technique was used in the present study to determine bradykinin levels in the myocardial interstitial fluid and to examine the influence of myocardial ischemic preconditioning on ischemia-induced cardiac bradykinin release. We demonstrated that the bradykinin level was greater in the endocardium than in the epicardium during ischemia. We also found that ischemic preconditioning augmented the cardiac release of bradykinin during subsequent ischemia. Thus this study provides new information about transmural gradients of cardiac bradykinin and the potential role of myocardial bradykinin in cardioprotection induced by ischemic preconditioning.

This study was supported by the American Heart Association, Mid-Atlantic Affiliate, Grant GS-30 (to H.-L. Pan) and by National Heart, Lung, and Blood Institute Grants HL-60026 and HL-04199 (to H.-L. Pan) and HL-28982 to O. A. Carretero. H.-L. Pan was supported by a National Heart, Lung, and Blood Institute Independent Scientist Career Award during this study.

REFERENCES

20. Parratt JR, Vegh A, Zeitlin LJ, Ahmad M, Oldroyd K, Kaszala K, and Papp JG. Bradykinin and endothelial-cardiac...

21. Reimer KA and Jennings RB. The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 40: 633–644, 1979.


