Role of kinins in chronic heart failure and in the therapeutic effect of ACE inhibitors in kininogen-deficient rats

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In the present study, we used BNK rats, which are genetically deficient in kininogens, or B₂ kinin receptor knockout mice, blood pressure and severity of myocardial ischemia-reperfusion injury were no different from their wild-type controls, although they exhibited a diminished response to ischemic preconditioning (45). Liu et al. (20) also reported that in Lewis rats with chronic HF induced by coronary artery ligation, blockade of kinins with a B₂ kinin receptor antagonist did not aggravate cardiac remodeling or development of HF, but it did block part of the cardioprotective effect of angiotensin-converting enzyme inhibitors (ACEI). These results may suggest that under basal conditions, release or lack of kinins may not be important in either maintaining blood pressure and cardiac homeostasis or the pathophysiology of HF. However, in response to stimuli such as ischemic preconditioning or administration of ACEI, increased release or decreased degradation of kinins may have profound cardioprotective significance.

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IN RESPONSE TO MYOCARDIAL INFARCTION (MI), the heart undergoes a remodeling process characterized by early expansion of the infarcted area, progressive dilatation of the left ventricle (LV), hypertrophy of the viable myocardium, and hyperplasia of interstitial fibroblasts, ultimately leading to progressive left ventricular dysfunction and chronic heart failure (HF) (24, 32). This remodeling process appears to be critically dependent on the size of the infarct as well as pre- and afterloading of the heart (9, 32, 33). Activation of vasoactive hormones such as kinins, nitric oxide, and angiotensins may be an important adaptive and/or maladaptive mechanism in the pathophysiology of LV remodeling and development of HF (8, 13, 16, 22).

Kinins are released from high- and low-molecular-weight kininogens (HMWK and LMWK, respectively) by kininogenases such as plasma and tissue (glandular) kallikrein (6). Evidence suggests that a local kallikrein-kinin system exists in the heart, which enables it to synthesize and release kinins (25, 29, 30). Kinins released locally may act as autocrine/paracrine hormones, regulating cardiac function. Several studies have shown that during acute myocardial ischemia, release of kinins from the heart is rapidly increased. However, the influence or significance of increased kinin release on cardiac function or remodeling in response to ischemia or MI is not fully understood. We have previously shown that in animals that lack kinins, such as Brown Norway Katholiek (BNK) rats, which are genetically deficient in kininogens, or B₂ kinin receptor knockout mice, blood pressure and severity of myocardial ischemia-reperfusion injury were no different from their wild-type controls, although they exhibited a diminished response to ischemic preconditioning (45). Liu et al. (20) also reported that in Lewis rats with chronic HF induced by coronary artery ligation, blockade of kinins with a B₂ kinin receptor antagonist did not aggravate cardiac remodeling or development of HF, but it did block part of the cardioprotective effect of angiotensin-converting enzyme inhibitors (ACEI). These results may suggest that under basal conditions, release or lack of kinins may not be important in either maintaining blood pressure and cardiac homeostasis or the pathophysiology of HF. However, in response to stimuli such as ischemic preconditioning or administration of ACEI, increased release or decreased degradation of kinins may have profound cardioprotective significance.

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development of chronic HF, and the effect of ACEI on HF after coronary ligation. We compared BNK rats with Brown Norway rats (BN), which have normal circulating kininogen, testing whether: 1) kinins play a role in the regulation of cardiac function; 2) kinins influence MI size after coronary ligation; 3) kinins are involved in the development of HF after MI; and/or 4) kinins participate in the cardioprotective mechanism of ACEI.

MATERIAL AND METHODS

Male BNK (initially obtained from the Department of Pharmacology, Kitasato University School of Medicine, and bred in our laboratory) and BN rats (Charles River Laboratories, Wilmington, MA) weighing 255–275 g were housed in an air-conditioned room with a 12-h light/12-h dark cycle; they received standard laboratory rat chow (0.4% sodium) and drank tap water. Animals were given 5–7 days to adjust to their new environment and were anesthetized with pentobarbital sodium for all surgical procedures (50 mg/kg ip). The study was approved by the Henry Ford Hospital Care of Experimental Animals Committee.

Experimental Heart Failure Model

The left anterior descending coronary artery (LAD) was ligated as described previously (2, 3). Briefly, animals were intubated and ventilated with room air using a positive-pressure respirator (Harvard model 680, South Natick, MA). A left thoracotomy was performed via the fourth intercostal space, and the lungs were retracted to expose the heart. After the pericardium was opened, the LAD was ligated with a 7-0 silk suture near its origin (the edge of the left atrium); ligation was deemed successful when the anterior wall of the LV turned pale. The lungs were inflated by increasing positive end-expiratory pressure, and the thoracotomy site was closed in layers. The sham ligation group underwent a similar procedure except that the suture was not tightened around the coronary artery. Our previous study showed that 2 mo after a large MI (40% of the LV) was created by ligating the LAD, both ventricular volume and end-diastolic pressure were significantly increased, while the ejection fraction (EF) was decreased, clearly indicating the development of HF (19).

Experimental Protocols

Two months after sham surgery or coronary ligation, BN rats were divided into four groups: 1) sham ligation (n = 7); 2) HF treated with vehicle (tap water; n = 9); 3) HF treated with ramipril (1 mg·kg
1 ·day
21; n = 8); and 4) HF treated with ramipril + icatibant (HOE-140) via an osmotic pump (100 µg·kg
1 ·day
21; n = 6). BNK rats were randomly divided into three groups: 1) sham ligation (n = 9); 2) HF treated with vehicle (tap water; n = 9); and 3) HF treated with ramipril (n = 8). All treatments lasted 2 mo.

Measurement of Plasma Kininogen

Plasma levels of HMWK and LMWK were determined as described previously (23, 45) with some modification. Briefly, for HMWK, plasma was incubated with glass powder in the presence of o-phenanthroline. Kinins were released from HMWK by plasma kallikrein generated by plasma prekallikrein through activation of coagulation factor XII. Released bradykinin was measured by radiomunoassay. For LMWK, plasma was incubated with glass powder in the absence of o-phenanthroline. Thus kinins converted from HMWK by plasma kallikrein through activated factor XII were destroyed by kininases in plasma. The HMWK-depleted plasma was acidified to pH 2.0 to destroy kininase and then brought back to pH 7.8 and incubated with glandular kallikrein. Kinins released from LMWK by glandular kallikrein were measured by radiomunoassay.

Cardiac Hemodynamics and Ventriculography

At the end of 2 mo treatment, rats were anesthetized and a polyethylene catheter (PE-50) was inserted into the carotid artery of each rat. Mean blood pressure (MBP) and heart rate (HR) were measured with a P23 XL pressure transducer connected to a processor (Gould Brush 220, Cleveland, OH). A catheter was advanced into the LV via the right carotid artery, and left ventricular end-diastolic pressure (LVEDP) and systolic pressure were measured. To assess LV volume and performance, we used a direct angio graphic method developed by Yang et al. (46). With the catheter still in the LV, ventriculograms were recorded on 35-mm cine film at 60 frames/s during injection of 0.5 ml contrast material (Renomi-60, Squibb, New Brunswick, NJ). The films were projected, and the margins of the LV image traced during end diastole and end systole over three consecutive cardiac cycles. ESV and EDV were determined using the area-length method (44). LVEF was calculated as EF = (EDV − ESV)/EDV.

Histopathological Examination

After the hemodynamic and ventriculographic studies, the rats were killed, and the chest was opened. The heart was excised and weighed and the LV sectioned transversely into four slices from apex to base. Slices were rapidly frozen in isopentane solution precooled in liquid nitrogen and used for morphometric analysis of infarct size and histological examination. Only the noninfarcted myocardium was subjected to histological analysis.

Determination of myocardial infarct size. A 10-µm section was cut from each slice and stained with Masson’s trichrome. Sections from all four slices were projected on a screen. The circumference of the entire endocardium and epicardium as well as the infarcted portion was measured by computer-assisted planimetry (SigmaScan, Jandel). Circumferences from all four slices (entire endocardium and epicardium as well as the infarcted portion) were added together and averaged, respectively. The infarcted portion of the LV was calculated from these measurements and expressed as a percentage of the total circumference (34).

Cardiomyocyte size (cross-sectional area). Sections were pretreated with 3.3 U/ml neuraminidase type V (Sigma, St. Louis, MO) and then stained with fluorescein-labeled peanut agglutinin (Vector, Burlingame, CA) to delineate myocyte cross-sectional area. From each slice, three radially oriented microscopic fields were selected at random and photographed on 35-mm film at a magnification of ×100. Images were projected with a photomagnifier, and the cross-sectional area of each myocyte was measured with computer-based planimetry (SigmaScan, Jandel). An average cross-sectional area was calculated using data obtained from all four slices.

Interstitial fibrosis (interstitial collagen fraction). Sections were stained with fluorescein-labeled peanut agglutinin. The interstitial space (including the capillaries) was visualized and counterstained with rhodamine-labeled Griffonia simplicifolia lectin I. The procedure for sample selection and imaging was the same as that for the myocytes. Measurements were made with computer-assisted videodensitometry (SigmaScan, Jandel). The volume fraction of interstitial fibrosis was calculated as the percent total surface area (microscopic field)
occupied by the interstitial space (stained with peanut agglutinin) minus the percent total surface area occupied by the capillaries. The average interstitial collagen fraction was calculated using data obtained from all four slices (3 samples from each slice).

Oxygen diffusion distance. To measure the intercapillary distance, only the capillaries around the myocytes were included, and the oxygen diffusion distance was expressed as half the intercapillary distance (14, 26). Average values were calculated using data obtained from all four slices.

Data Analysis

All data are expressed as means ± SE. Student's t-test was used to compare mean values of sham, HF vehicle, HF-ACEI, and HF-ACEI + kinin antagonist treatment groups in BN or BNK rats. Holm's procedure was used to adjust the multiple testing. The P value indicated significance if it was < 0.015 in BN and < 0.02 in BNK rats. The effects of drug treatment in the two types of rats (BN and BNK) were compared using two-way analysis of variance (ANOVA); changes from sham ligation to HF-vehicle or from HF-vehicle to HF-ACEI between BN and BNK rats were also compared. P < 0.05 was considered significant.

RESULTS

Early and Late Mortality

Two months after sham or coronary ligation, the animals received either vehicle or drug treatment for another 2 mo, and all parameters were measured at the end of the experiment. Of the 25 BN rats that underwent ligation, 1 died after the operation, for a surgical mortality rate of 4%. In addition, 1 animal in the HF vehicle group (n = 10) died 3 mo after MI, for a mortality rate of 10% due to heart failure. No rats in the drug treatment or sham-ligated group died. Of the 19 BNK rats that underwent coronary artery ligation, 2 of those in the HF-vehicle group (n = 11) died within 3 mo after MI, for a heart failure mortality rate of 18%. No rats in the drug treatment or sham-ligated group died.

Plasma Kininogen

Plasma HMWK was 38-fold higher in BN rats than that in BNK rats (1.11 ± 0.16 vs. 0.029 ± 0.01 µg/ml; P < 0.001), whereas LMWK was 16.5-fold higher in BN (0.50 ± 0.01 vs. 0.03 ± 0.001 µg/ml; P < 0.001).

Hemodynamic and Cardiac Function in BN and BNK Rats

There were no statistically significant differences between sham-ligated BN and BNK rats with regard to MBP (113 ± 4 vs. 116 ± 5 mmHg), LVEDV (0.37 ± 0.03 vs. 0.4 ± 0.02 ml), LVESV (0.14 ± 0.01 vs. 0.12 ± 0.01 ml), LVEF (0.63 ± 0.01 vs. 0.69 ± 0.01%), or LVEDP (0.2 ± 0.13 vs. 0.8 ± 1 mmHg) (Figs. 1–3; Table 1). Histological findings were also similar in both groups, including myocyte cross-sectional area (352 ± 9 vs. 398 ± 17 µm²), interstitial collagen fraction (4.91 ± 0.3 vs. 5.1 ± 0.3%), oxygen diffusion distance (11 ± 0.3 vs. 11.2 ± 0.4 µm), and heart weight (251 ± 7 vs. 267 ± 7 mg/100 g body wt) (Fig. 4; Table 1).

In comparison with sham-ligated rats, there was a significant increase in LVEDV, LVESV, and LVEDP associated with a significant decrease in LVEF in the HF-vehicle group in both BN and BNK rats. The increase in LV volume and LVEDP and decrease in LVEF were similar in both strains (Figs. 1–3).

Myocardial Infarct Size, Heart Weight, and Histological Findings in BN and BNK Rats

Myocardial infarct size and heart weight for BN and BNK rats is shown in Table 1. All rats with coronary artery ligation developed a large MI, ranging from 41 to 48% of the LV circumference. There were no significant differences in infarct size among groups or between BN
and BNK rats 4 mo after MI. Compared with sham ligation, heart weight was increased in the HF vehicle group in both strains (BN: 327 ± 19 vs. 251 ± 7 mg/100 g; P < 0.01; BNK: 323 ± 18 vs. 267 ± 7 mg/100 g; P < 0.01). Myocyte cross-sectional area, interstitial collagen fraction, and oxygen diffusion distance were significantly larger in the HF-vehicle group compared with sham ligation in both BN and BNK rats. This remodeling process was similar in both strains (Table 1 and Fig. 4).

Effect of ACEI in HF After MI in BN and BNK Rats

Heart weight. The ACEI ramipril decreased heart weight in BN rats (286 ± 17 vs. 321 ± 12 mg/100 g body wt; P < 0.01) but not in BNK rats (323 ± 18 vs. 322 ± 14 mg/100 g body wt) (Table 1).

Hemodynamics and cardiac function. In BN rats, ramipril significantly decreased LV volumes and increased LVEF compared with the vehicle group (LVEDV: 0.63 ± 0.03 vs. 0.94 ± 0.04 ml, P < 0.01; LVEF: 0.36 ± 0.04 vs. 0.43 ± 0.01, P < 0.01). These effects were blunted in BNK rats (LVEDV: 0.75 ± 0.11 vs. 0.84 ± 0.09, P = 0.48; LVEF: 0.54 ± 0.09 vs. 0.60 ± 0.06 ml, P = 0.52; LVEF: 0.31 ± 0.04 vs. 0.27 ± 0.02, P = 0.28) (Figs. 5 and 6).

LVEDP was markedly reduced in the HF-ramipril group compared with HF-vehicle in BN rats. This effect was absent in BNK rats with HF. The decrease in LVEDP caused by ACEI was significantly larger in BN rats than that in BNK rats (10.8 vs. 1.8 mmHg; P < 0.05) (Fig. 7). However, the decrease in MBP induced by ramipril was significantly greater in BNK rats than in BN (Table 1).

Histopathological changes. In BN rats, ramipril significantly reduced myocyte cross-sectional area from 0.22 ± 0.02% to 0.01% (LVEDP: 0.75 ± 0.11 vs. 0.84 ± 0.09, P = 0.48; LVEF: 0.54 ± 0.09 vs. 0.60 ± 0.06 ml, P = 0.52; LVEF: 0.31 ± 0.04 vs. 0.27 ± 0.02, P = 0.28) (Figs. 5 and 6).

Table 1. Body weight, mean blood pressure, heart rate, and histopathological changes in BN and BNK rats

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<td>ACEI</td>
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<td>BW, g</td>
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<td>MBP, mmHg</td>
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<td>HR, beats/min</td>
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<td>ODD, µm</td>
<td>11 ± 3</td>
<td>15.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>14.8 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>MI-IS, % of LV</td>
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<td>LVW, mg/100 g BW</td>
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<td>RVW, mg/100 g BW</td>
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<td>HW, mg/100 g BW</td>
<td>251 ± 7</td>
<td>321 ± 11&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>HR, beats/min</td>
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<td>MI-IS, % of LV</td>
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Values are means ± SE; n, number of rats; BN, Brown Norway; BNK, BN Katholiek rats; BW, body weight; MBP, mean blood pressure; HR, heart rate; ODD, oxygen diffusion distance; MI, myocardial infarction; IS, infarct size; LVW, left ventricular weight; RVW, right ventricular weight; HW, heart weight; K-ant, kinin antagonist. <sup>a</sup>P < 0.05, vehicle vs. sham; <sup>b</sup>P < 0.01, vehicle vs. sham; <sup>c</sup>P < 0.05, ACEI vs. vehicle; <sup>d</sup>P < 0.01 and angiotensin-converting enzyme inhibitors (ACEI). ACEI vs. vehicle; <sup>e</sup>P < 0.01, ACEI + K-ant vs. ACEI.
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658 ± 19 to 493 ± 18 μm² (P < 0.001), interstitial collagen fraction from 10.4 ± 0.5 to 6.9 ± 0.5% (P < 0.05), and oxygen diffusion distance from 15.2 ± 0.4 to 13 ± 0.1 μm (P < 0.01) compared with vehicle-treated rats; in BNK rats, these effects were absent (Fig. 8; Table 1). In addition, in BN rats the kinin receptor antagonist icatibant partially blocked the effect of ACEI on myocyte cross-sectional area (607 ± 6 vs. 493 ± 18 μm² with ramipril alone; P < 0.001), interstitial collagen fraction (9.24 ± 0.27 vs. 6.9 ± 0.5% with ramipril alone; P < 0.001), and oxygen diffusion distance (14.8 ± 0.4 vs. 13 ± 0.1 m with ramipril alone; P < 0.001). These results confirmed our previous finding that in Lewis inbred rats, the cardioprotective effect is partially mediated by kinins (20).

DISCUSSION

In the present study, we found that 1) in rats genetically deficient in kinins, basal cardiac hemodynamics and function as well as morphology and histology were no different from rats with an intact kallikrein-kinin system; 2) kinin deficiency did not aggravate cardiac remodeling after MI, because infarct size and progression or development of heart failure were similar in normal and kininogen-deficient rats; and 3) the ACEI ramipril had a far less beneficial effect on LV remodeling and cardiac function in kininogen-deficient rats than in controls, indicating that potentiation of endogenous kinins or inhibition of kinin degradation may be largely responsible for the therapeutic effect of ACEI in rats with heart failure. Taken together, our
data may suggest that endogenous kinins play a minor role in maintaining basal blood pressure and cardiac hemodynamics under normal conditions or after MI in rats; however, kinins appear to be important mediators in the cardioprotective mechanism of ACEI. Some of these findings agree with our previous study in Lewis rats with chronic HF (20). However, animals such as BNK rats, which genetically lack kininogen, may be a more useful model to study the role of kinins. Because kininogen is almost absent before surgical manipulation and throughout the experimental period, this allows us to study the role of kinins in normal cardiac function as well as MI size, myocardial remodeling in the development of chronic HF, and the effect of ACEI on HF after coronary ligation.

Role of Kinins in Regulation of Blood Pressure and Cardiac Function

Kinins are generated from the precursors HMWK and LMWK by plasma and tissue kallikrein, destroyed rapidly by kininases (mainly ACE), and circulate at low concentrations in plasma (10–50 pg/ml). To test the hypothesis that kinins contribute to blood pressure regulation, many investigators have used either a kinin receptor antagonist or antibodies against kinins to see whether blockade of endogenous kinins increases blood pressure or alters cardiac hemodynamics. Pharmacological blockade of kinins was found to have no effect on blood pressure or cardiac function, although it did block the vasodepressor effect of exogenous bradykinin (5, 21, 39). We recently reported that basal blood pressure in mice genetically lacking B2 kinin receptors is no different from their wild-type controls, although they had a greater hypertensive response to high salt intake (1). In the present study, we further showed that deficiency of kinins did not alter either cardiac physiology or histology. Thus we may say that an intact kallikrein-kinin system is not essential for maintaining systemic blood pressure and cardiac hemodynamic hemostasis; however, kinins may be important paracrine/autocrine hormones involved in regulation of local blood flow, vascular resistance, and organ function, a possibility that calls for further investigation.

Role of Kinins in Myocardial Ischemia and Infarct Size

It is evident that a kallikrein-kinin system exists in the heart, which enables it to synthesize and release kinins. After stimuli such as myocardial ischemia or ischemic preconditioning, release of kinins from the heart is rapidly increased (27, 38). In addition, intracoronary infusion of kinins reduces infarct size (4, 12), indicating that kinins released from the heart may be an important cardioprotective factor in myocardial ischemia. We have previously tested the hypothesis that in B2 kinin receptor knockout mice or kinin-deficient BNK rats, myocardial ischemia-reperfusion injury would be more severe. However, we found that infarct size in B2 kinin receptor knockout mice or BNK rats was similar to controls, though they had a lesser response to preconditioning (28). In agreement with our previous finding, we now observed that BNK rats subjected to MI had similar infarct size and cardiac function in comparison to controls. These data clearly show that lack of kinins affects neither infarct size nor cardiac function, indicating that kinins may not be an important cardioprotective factor in myocardial ischemia and scar formation. However, in response to stimuli such as ischemic preconditioning or administration of ACEI, increased release of kinins may be an important component in cardioprotection.

Role of Kinins in Development of HF

Wollert et al. (42, 43) recently reported that chronic administration of a kinin B2 receptor antagonist 1 wk after MI in rats significantly increased LV interstitial collagen deposition but not myocyte cross-sectional area. However, in the present study we found that the severity of interstitial fibrosis and myocardial hypertrophy after MI, as well as LV chamber dilatation, was similar between kinin-deficient BNK rats and rats with normal kinins, which agrees with our previous finding that in Lewis inbred rats with chronic HF induced by MI, a kinin receptor antagonist did not worsen any of these parameters compared with the vehicle group (20). Although we do not have a good explanation for this discrepancy between our study and Wollert's, we believe it may be related to the experimental setting, because they began treatment 1 wk after MI and observed the animals for 3 wk, whereas we started treatment 2 mo after MI and observed them for 2 more mo, by which time rats were already in the chronic phase of HF (19). We know that LV remodeling following MI is a time-dependent process; there may be a temporal dissociation between collagen deposition and myocyte hypertrophy, and different processes are involved (13, 31). Nevertheless, the fact that kinin deficiency did not aggravate LV remodeling or development of cardiac dysfunction suggests that kinins may not be an important component in the pathophysiology of HF. It is possible that the trace amount of kininogen in blood of genetically deficient rats may partially account for the lack of differences in cardiac physiological and histological parameters between BNK and BN rats either under basal conditions or in the development of heart failure. Although we have unpublished data in kinin B2 receptor knockout mice with MI, we did not observe differences in the development of heart failure compared with their wild-type controls.

Role of Kinins in the Cardioprotective Effect of ACEI

Data obtained from BN rats in the present study agree with our previous finding that ACEI improved cardiac function and attenuated LV remodeling by decreasing LV volume, LVEDP, interstitial fibrosis, and cardiac hypertrophy and increasing EF in Lewis inbred rats with HF, and that a kinin receptor antagonist partially blocked these beneficial effects (20). We have now shown that the cardioprotective effect of ACEI was almost abolished in kininogen-deficient rats, which further supports the hypothesis that kinins are impor-
tiant mediators in the cardioprotective mechanism of ACEI.

ACE (kininase II) regulates the balance between the vasodilator and natriuretic properties of bradykinin and the vasoconstrictor and salt-retentive properties of angiotensin II. ACEI alter this balance by reducing the formation of angiotensin II and breakdown of kinins, leading to natriuresis and vasodilatation; and this in turn reduces preload, afterload, and wall stress (7, 15). However, it is not clear whether ACEI have a direct action on the heart besides their hemodynamic effect. We found that in BN rats ACEI decreased LV volume and LVEDP, accompanied by a decrease in MBP; a kinin antagonist blocked the cardiac effect but not their action on MBP. In BNK rats, ACEI decreased MBP as well but had no effect on LV function and remodeling. These data may suggest that the cardioprotective effect of ACEI relies more on local autocrine and paracrine actions involving the kallikrein-kinin system rather than blood pressure reduction (17, 41).

The cardioprotective effect of kinins may be partially mediated by release of prostacyclin and nitric oxide. Kinins are known to be potent stimulators of the release of prostaglandin and nitric oxide from the endothelium (10, 21, 47). It has been shown that myocardial ischemia increases kinin release, accompanied by an increase in cGMP (an indicator of nitric oxide production) and 6-keto-PGF1α, [a metabolite of prostacyclin (18, 35)] and that inhibiting prostaglandin synthesis diminishes or blocks the protective effect of kinins (11, 21, 40). Furthermore, a recent study showed that incubation of coronary microvessels or myocardial slices with ACEI or kininogen caused a significant increase in nitric oxide production and a decrease in myocardial oxygen consumption (48), both of which were blocked by a B2 kinin receptor antagonist. These data may indicate that NO-induced reduction of oxygen consumption contributes significantly to the cardioprotective action of kinins. Kinins may also be involved in myocardial energy metabolism. It was found that perfusing the ischemic heart with bradykinin increases production of myocardial high-energy phosphates as well as glycogen content, along with a reduction in lactate dehydrogenase and creatinine kinase activity (36, 37).

In summary, we have demonstrated that 1) kinins do not participate in maintaining normal cardiac hemodynamics and function; 2) kinin deficiency does not increase myocardial infarct size, aggravate LV remodeling, or worsen LV dysfunction; and 3) ACEI regress LV remodeling and improve cardiac function, and these effects are diminished in kininogen-deficient rats. We postulate that kinins may not be essential for maintaining blood pressure and cardiac hemodynamic homeostasis under either normal physiological conditions or the development of chronic HF. However, with pharmacological interventions such as ACE inhibition, activation of kinins appears to be an important mediator in the cardioprotective effect of ACEI.

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REFERENCES

17. Linz, W., and B. A. Schölkens. Influence of local converting enzyme inhibition on angiotensin and bradykinin effects in


