Chronic Infusion of Bradykinin Delays the Progression of Heart Failure and Preserves Vascular Endothelium-Mediated Vasodilation in Conscious Dogs

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Background—This study examined the effects of chronic bradykinin infusion on hemodynamics and myocardial and endothelial functions during the development of heart failure.

Methods and Results—Sixteen instrumented dogs were randomized to receive through the left atria either vehicle or bradykinin (1 μg/min) during ventricular pacing (250 bpm, 5 weeks). Hemodynamic and left ventricular (LV) parameters and the vasodilator responses to intravenous acetylcholine (0.3 to 3 μg/kg) and nitroglycerin (1 to 10 μg/kg) were examined in the control and after 3 and 5 weeks of pacing. The expression of endothelial NOS in femoral, carotid, and renal arteries was determined by Western blot analysis. After 3 weeks of pacing, changes in LV diastolic and systolic parameters were significantly lower in bradykinin-treated than vehicle-treated dogs (LV end-diastolic pressure, +10±3 versus +19±2 mm Hg; time constant of LV isovolumic relaxation, +11±2 versus +17±1 ms; LV wall thickening, −33±18% versus −75±9%; and cardiac output, −16±6% versus −32±6%; all P<0.05). Compared with vehicle-treated dogs, bradykinin-treated dogs had a reduced rightward shift of the diastolic LV pressure-diameter relation and a reduced diastolic LV wall stress. Similar trends were observed after 5 weeks. The vasodilator response to nitroglycerin was preserved in both groups. The response to acetylcholine was blunted in vehicle-treated but preserved in bradykinin-treated dogs. Vascular endothelial NOS expression decreased in vehicle-treated but was preserved in bradykinin-treated dogs.

Conclusions—In conscious dogs, chronic bradykinin infusion delays the heart failure progression by preserving LV diastolic and systolic functions and by preserving vascular endothelial function. (Circulation. 2003;109:⋯⋯⋯⋯⋯⋯)

Key Words: bradykinin ■ heart failure ■ endothelium

B radykinin (BK) is a potent endogenous vasodilator that can be released from vascular endothelial cells and metabolized by ACE and other peptidyl peptidases.1 BK exerts its multiple actions on myocytes, vascular endothelial and smooth cells, and cardiac nerve endings through 2 types of receptors, B1 and B2. There is growing evidence that BK may influence favorably heart failure (HF). In experimental HF, ACE inhibitors increase blood BK levels,2 and a BK B2 receptor antagonist reduces markedly the acute vasodilator effects of ACE inhibitors.3,4 In patients with HF chronically treated by ACE inhibitors, BK receptor blockade with a mixed B1 and B2 receptor antagonist produces a dose-dependent vasoconstriction that disappears after withdrawal of ACE inhibitor therapy, suggesting a role of BK at the vascular level in the chronic treatment with these drugs.5 In the coronary circulation, previous studies have shown that BK participates in the regulation of coronary vascular tone6 and improvement of coronary endothelial dysfunction and coronary flow reserve by ACE inhibitors.7,8 Recent studies have demonstrated that, in contrast with the impaired vasodilator response to acetylcholine usually observed in HF,9–11 the vasodilator effect of exogenous BK is preserved in pacing-induced HF in dogs4 and that endogenous BK plays a significant role in the vasomotor control in HF.12 Moreover, it has been demonstrated that BK B2 receptor knockout mice develop spontaneous congestive HF.13 These data strongly suggest that BK is involved in the pathogenesis of HF and in the beneficial effects of ACE inhibitor in HF. However, it remains unknown whether BK per se exerts protective effects during HF. Therefore, this study examined the effect of chronic BK infusion on myocardial function and hemodynamics during the development of HF induced by pacing in conscious dogs. Furthermore, because vascular endothelial dysfunction is usually associated with HF,4,7–11 the endothelial function was evaluated by examining the vascular responses to endothelium-dependent and -nondependent vaso-
dilators in the control and during pacing period. Because BK exerts its major effects on vascular endothelium through the NO pathway, the vascular endothelial NO synthase (eNOS) protein expression was determined in 3 major arterial beds by Western blot analysis.

**Methods**

**Instrumentation**

As previously described, in 16 beagle dogs weighing 13 to 21 kg anesthetized with 1 vol% halothane, a left thoracotomy was performed under sterile conditions for implantation of Tygon catheters in the descending aorta and left and right atria, a micromanometer in the left ventricular (LV) cavity, 2 pacing leads on the right ventricle, an ultrasonic flow probe around the root of aorta, and piezoelectric crystals in the LV to measure the internal short-axis and wall thickness in the same equatorial plane. The maintenance of animals, including postoperative care, analgesia, and antiinfectious prophylaxis, were kept in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1986). The animals used in this study were supplied by CEDS (Mézilles, France).

**Experimental Protocol**

Experiments in the control were performed in conscious healthy dogs. After recording of systemic and LV parameters, the vasodilator responses to acetylcholine (0.3 to 3 µg/kg) and nitroglycerin (1 to 10 µg/kg) were assessed. Each dose of these drugs was prepared in 3 mL saline and infused intravenously in 30 seconds.

After completing these experiments, dogs were randomized to receive, through the left atrial catheter, a continuous infusion of either vehicle (veh) group, 90 µL/min BK (BK group, 1 µg/min) or BK (BK group, 90 µL/min, a dose producing acutely minimal pressure-lowering changes and a blood concentration of BK within the range observed during acute ACE inhibition in dogs). Vehicle and BK infusions (using a portable pump [Microject, Sorenson Medical]) and right ventricular pacing (250 bpm) were simultaneously initiated and continued for 5 weeks. Because our previous studies showed that dogs developed HF after 3 weeks of pacing, the experiments were repeated after 3 and 5 weeks of pacing. To measure hemodynamics during BK infusion after stopping the pacemaker, a 15-minute period after interrupting the pacemaker was respected. To get a steady state for measuring the baseline value after stopping BK infusion (to avoid any potential interaction between drugs), an additional 15-minute period was allowed. Previous studies and preliminary data have shown that these time periods of stabilization are enough to get stable baseline hemodynamics in the absence or presence of BK infusion (1 µg/min). After euthanasia or in cases of premature death, animals were autopsied.

**Determination of Vascular eNOS Protein Expression by Western Blot Analysis**

Under anesthesia (thiopental), femoral, carotid, and renal arterial segments were harvested from 4 vehicle-treated and 4 BK-treated dogs, frozen with liquid nitrogen, and stored at −80°C. In addition, samples from 6 instrumented nonpacing control dogs were also investigated. The frozen segments were pulverized, homogenized, and centrifuged (1000g, 5 minutes). The supernatant was used for Western blot analysis. Sample proteins (25 µg/lane) were size fractionated by SDS-polyacrylamide gels (7.5%, 3 hours) and transferred to PVDF membranes by overnight electroblotting. A prestained protein-weight marker (Amersham International) and a control sample from an animal (used for the normalization) were added on each gel. The membranes were blocked at room temperature in 3% BSA prepared with 0.1% Tween 20 Tris-buffer saline (TTBS) for 3 hours, incubated with rabbit polyclonal antibody of eNOS (Santa Cruz Biotechnology, 1:1000 diluted in 1% BSA TTBS, 2 hours), and then washed 5 times with TTBS (5 minutes each).

Subsequently, the membranes were incubated with horseradish peroxidase–conjugated anti-rabbit IgG (Santa Cruz Biotechnology, 1:5000, 1 hour) and washed 5 times. Specific immunoreactive proteins were detected using enhanced chemiluminescence (Amersham International). The bands on the X-ray film were quantified by scanning densitometry and expressed as percentage of the control.

**Data Collection and Statistical Analysis**

All signals were recorded on a microcomputer and analyzed with HEM (v1.5, Notocord Systems). Aortic, left, and right atrial pressures were measured by Statham P23ID transducers. LV pressure (LVP) and LVEDP/dt were measured by the micromanometer. Cardiac output (CO) was measured using a flowmeter (T108, Transonic system). LV wall thickness and diameter were measured using a sonomicrometer (TRITON Technology). The time constant of isovolumic LV pressure–time integral was calculated using LVP = P/payment rate, where LVP is LVP at the start of decay and t is time. LV chamber stiffness coefficient (K) was calculated by fitting simultaneous paired LV pressure–volume data derived from the diastolic portion of 8 successive beats to the monoexponential curve with a zero asymptote (LVP = A.exp(−St)) and with a variable asymptote (LVP = A.exp(−St) + B), where A is a curve-fitting constant, D is LV diameter, and B is the pressure intercept). LV end-diastolic (ED) wall stress (g/cm²) was calculated according to a simplified cylindrical model (LV long axis being not measured) as 1.36 LVEDP × LVEDD² / LVED wall thickness, where LVEDP is LVED pressure and LVEDD is LVED diameter.

Results are expressed as mean±SEM. One-way and 2-way ANOVA for repeated measures was performed with SuperANOVA software (v1.11, Abacus concept), and comparisons between means were performed by contrast analysis when appropriate. When only 2 means were compared, an appropriate Student’s t test was performed, χ² test was used to compare the difference in the mortality between vehicle-treated and BK-treated animals. A value of P<0.05 was considered significant.

**Results**

In the vehicle-treated group, 4 of 9 dogs died during the fourth week of pacing. At autopsy, patent signs of acute pulmonary edema were observed in the dogs that died prematurely. All 7 dogs randomized to the BK-treated group were alive at the end of the protocol. The difference in mortality between the 2 groups was significant (P<0.05).

Throughout the experiments, no side effects, such as cough or angio-edema, were observed in BK-treated dogs.

**Changes in Hemodynamic and LV Parameters After 3 and 5 Weeks of Pacing**

In the control, hemodynamic and LV dimensional values were similar in both vehicle-treated and BK-treated dogs (Table 1). During pacing with continuous vehicle or BK infusion, mean aortic pressure (MAP) was similar in both vehicle-treated and BK-treated dogs after 3 and 5 weeks of pacing (80±3 and 77±4 mm Hg versus 85±2 and 80±2 mm Hg, respectively), indicating the absence of any pressure-lowering effect of BK at the dose used. Under this condition, compared with the control (69±4 mm Hg/L per min), total peripheral resistance (TPR) increased in vehicle-treated dogs after 3 weeks of pacing (89±9 mm Hg/L per min, P<0.05) and remained elevated at 5 weeks (83±11 mm Hg/L per min). In contrast, in BK-treated dogs, compared with the control (77±4 mm Hg/L per min), TPR remained unchanged after 3 weeks of pacing (76±9 mm Hg/L per min) and decreased after 5 weeks (64±5 mm Hg/L per min, P<0.05). This difference was attributable to a lower CO in vehicle-treated animals.
whereas n = 9 in the control state and after 3 weeks of pacing, whereas n = 7 for vehicle-treated animals and BK-treated dogs, respectively. *P < 0.05 vs vehicle-treated dogs.

After 3 weeks of pacing and after interruption of pacing and infusion, heart rate and LVEDP increased significantly, whereas MAP, LVdP/dt max, LV wall thickening, and CO decreased significantly compared with the control in both groups (Table 1). TPR increased only in vehicle-treated dogs. However, despite similar changes in MAP, the alterations in LVEDP, LV wall thickening, CO, and τ were greater in vehicle-treated than in BK-treated dogs (Table 1 and Figure 1). LVEDD increased in both groups, but shortening fraction decreased more in vehicle-treated than in BK-treated dogs. LVED stress tended to be greater in vehicle-treated than in BK-treated animals (0.88±0.10 and 0.84±0.14 L/min after 3 and 5 weeks of pacing) than in BK-treated animals (1.10±0.11 and 1.15±0.08 L/min, P < 0.05).

Figure 1. Changes in hemodynamic and LV parameters (compared with the control) in vehicle-treated and BK-treated dogs after 3 weeks of pacing. WTh indicates LV wall systolic thickening. Compared with vehicle-treated animals, BK-treated animals had a lesser degree of heart failure, n = 9 and n = 7 for vehicle-treated and BK-treated dogs, respectively. *P < 0.05 vs vehicle-treated dogs.

Figure 2. LV diastolic pressure-diameter curves obtained in vehicle-treated and BK-treated dogs in the control (C) and after 3 weeks of pacing. Pressure-diameter curves were shifted rightward in vehicle-treated dogs. BK treatment limited this shift (Figure 2). The chamber stiffness
The effect of nitroglycerin on heart rate was similarly altered in both vehicle-treated and BK-treated animals (Table 1). In vehicle-treated dogs, LV chamber stiffness decreased despite the loss of animals in the vehicle-treated group (Table 1). In BK-treated dogs, the decrease in CO was not significant because of an additional LV dilation. In BK-treated dogs, the vasodilator response to acetylcholine was impaired in vehicle-treated dogs but preserved in BK-treated dogs. n=9 and n=7 for vehicle-treated and BK-treated dogs, respectively.

Vasodilator Responses to Nitroglycerin and Acetylcholine in the Control and After 3 and 5 Weeks of Pacing

The effect of nitroglycerin on heart rate was similarly altered in both vehicle-treated and BK-treated animals (10 μg/kg; +56±8 in control versus +15±3 bpm after 3 weeks of pacing for vehicle-treated dogs and +50±11 versus +15±5 bpm for BK-treated dogs, all P<0.02). The effect of nitroglycerin on MAP was similar in both the control and after 3 weeks of pacing for both groups (10 μg/kg; −15±3 and −16±1 mm Hg for vehicle-treated dogs versus −12±4 and −17±1 mm Hg for BK-treated dogs). Similar results were also obtained for CO (+0.4±0.1 L/min in both control and after 3 weeks of pacing for vehicle-treated dogs and +0.3±0.1 L/min in both states for BK-treated animals) and for TPR (−31±5% and −38±5% in the control and after 3 weeks of pacing, respectively, in vehicle-treated dogs versus −27±3% and −34±4% in BK-treated dogs). After 3 weeks of pacing, heart rate response to acetylcholine was similarly altered in both vehicle-treated and BK-treated dogs (Table 2). In contrast with vehicle-treated dogs showing a reduced pressure-lowering effect of acetylcholine, BK-treated animals showed a preserved pressure-lowering effect of acetylcholine (Table 2). The TPR response to acetylcholine was impaired in vehicle-treated dogs but preserved in BK-treated dogs (Figure 3). Similar results were observed after 5 weeks of pacing in the surviving animals (data not shown).

Vascular eNOS in BK-Treated and Vehicle-Treated Dogs

Figure 4A illustrates representative blots of immunoreactive eNOS proteins of renal arteries from 1 nonpacing control dog, 1 vehicle-treated dog, and 1 BK-treated dog. In the presence of a blocking peptide, the eNOS band was absent, indicating the specificity of this antibody to detect eNOS (data not shown). Compared with nonpacing control animals, eNOS protein expression in femoral, carotid, and renal arteries of vehicle-treated animals decreased significantly. In contrast, in BK-treated animals, vascular eNOS protein expression in the 3 examined territories was preserved (Figure 4B).

Discussion

Pacing-induced HF is a well-established HF model.3,4,11,12,15–17 In this model, the development and severity of HF is associated with a decrease in cardiac output and an increase in peripheral resistance. In our study, we observed similar trends in both vehicle-treated and BK-treated dogs. However, the vasodilator response to acetylcholine was impaired in vehicle-treated dogs but preserved in BK-treated dogs. These findings suggest that BK infusion may have a protective effect on the vasodilator response to acetylcholine, which may contribute to the preserved cardiac output in BK-treated dogs.

Figure 4. Vascular expression of eNOS proteins after 5 weeks of pacing. A. Representative blots obtained from renal arteries from 1 nonpacing control, 1 vehicle-treated, and 1 BK-treated dog. B. Mean values of eNOS proteins in 3 arterial beds obtained in 6 nonpacing control, 4 vehicle-treated, and 4 BK-treated dogs after 5 weeks of pacing. †P<0.05 vs vehicle-treated dogs.
HF depend on the rate and duration of pacing. In the present study, a pacing rate of 250 bpm was used. It resulted in a 32% reduction in CO after 3 weeks of pacing, and 4 of 9 vehicle-treated dogs died during the pacing period.

This study demonstrated that LV myocardial contractile function is less altered in BK-treated than in vehicle-treated dogs. Several mechanisms may be evoked. First, because the present study used a dose of BK that did not modify arterial pressure and TPR, it is unlikely that the preserved myocardial contractile function was related to a reduction in afterload. Second, a recent study showed that an acute infusion of a BK analogue improved transmural myocardial blood flow in this model. It is also the case during chronic BK infusion, it could have in turn contributed to the improvement of myocardial function. Third, it has been shown that ACE inhibitors exert a BK-mediated favorable effect on LV diastolic function. Thus, one mechanism by which BK exerts its protective effect on myocardial contractile function may be related to its effects on LV diastolic function. In agreement with previous reports, this study showed a rightward shift of LV diastolic pressure-diameter curve, with an unchanged LV chamber stiffness after 3 weeks of pacing in vehicle-treated dogs. Chronic BK treatment reduced the rightward shift of the LV diastolic pressure-diameter relation but did not change LV chamber stiffness, which is in agreement with a recently developed concept. This resulted in a lesser LVEDP and LVED wall stress with a preserved LV distensibility in BK-treated dogs. In addition, a lesser alteration in \( \tau \) was observed in BK-treated dogs. All these effects of BK on the LV diastolic function may lead to a lower degree of stress-triggered myocardial maladaptive response. Finally, and more importantly, the beneficial effect of chronic BK infusion on myocardial function and hemodynamics may be related to a preserved NO-mediated endothelial function. It is known that endothelial dysfunction occurs in HF and ACE inhibitors improve endothelial dysfunction. Some studies showed that this effect of ACE inhibitors is BK-mediated. In accordance with previous reports, this study showed a reduced vasodilator response to acetylcholine after 3 weeks of pacing in vehicle-treated dogs. In contrast, this response was preserved in BK-treated dogs. Concomitantly, in vitro experiments performed in 3 major arterial beds showed that the eNOS expression was reduced in vehicle-treated dogs but preserved in BK-treated dogs. The present study is the first to demonstrate such effect during chronic BK infusion. This result seems to be in the same line as recent reports showing that chronic treatment with quinapril enhanced eNOS protein expression through a BK-dependent mechanism in normal rats and that chronic treatment with enalapril preserved eNOS mRNA level, which was attenuated by concomitant BK B2 receptor antagonist in the pacing-induced HF. The preserved eNOS expression could maintain NO production. By limiting afterload increase and by inhibiting myocardial mitochondrial respiration, NO may have attenuated myocardial \( \text{O}_2 \) consumption during rapid pacing. By its effect on cardiac remodeling, NO may have mediated the beneficial effect of BK on LVED functions and thereby preserved myocardial contraction.

It is known that BK is a full agonist at the level of B2 receptors and B2 receptors are predominant in various tissues. Although BK can be metabolized into des-arg9-BK (a B1 receptor agonist) by kininase I, BK is essentially degraded into other inactive metabolites by kininase II (ACE and neutral endopeptidase), and because B1 receptors are expressed constitutively in only a few blood vessels and in low quantity, their activation produces weak effects. Furthermore, a recent study demonstrated a preserved B2 receptor-mediated vasodilatation in response to BK in pacing-induced HF. All of these results suggest that BK may exert its effects through B2 receptors. In endothelial cells, the B2 receptor activation increases intracellular \( \text{Ca}^{2+} \) that activate eNOS to release NO and activate phospholipase A2 (which can alternatively be activated by B2 receptors) to produce arachidonic acid that is metabolized into prostacyclin or endothelium-derived hyperpolarizing factor. The preserved vascular eNOS observed in BK-treated dogs may allow BK to exert its effects through NO pathway.

In summary, this study shows that chronic BK infusion exerts a beneficial effect against the progression of HF by limiting the degradation of LV diastolic and systolic function. This preserved myocardial function may be the consequence of a preservation of the endothelial function, as indicated by a preserved vasodilator response to acetylcholine and, more importantly, a preserved vascular eNOS. However, because this study only examined the effects of chronic BK infusion during the development of HF rather than in animals with an established HF, where endothelial function is already impaired, the real consequence of chronic BK treatment in the latter situation needs to be additionally investigated. Nevertheless, the present results indicate a potential clinical implication of BK in HF.

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**References**


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