Stimulation of Bradykinin B₁ Receptors Induces Vasodilation in Conductance and Resistance Coronary Vessels in Conscious Dogs

Comparison With B₂ Receptor Stimulation

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Background—Constitutive bradykinin B₁ receptors have been identified in dogs; however, their physiological implications involving the coronary circulation remain to be determined. This study examined, in conscious dogs, the coronary response to des-Arg⁹-bradykinin (a B₁ receptor agonist) and the mechanisms involved.

Methods and Results—Eleven dogs were instrumented with a left ventricular micromanometer, a circumflex coronary catheter, a cuff occluder, a Doppler flow probe, and ultrasonic crystals to measure coronary blood flow velocity (CBFv) and coronary diameter (CD). Intracoronary des-Arg⁹-bradykinin (3 to 100 ng/kg) and bradykinin (0.1 to 10 ng/kg) did not modify systemic hemodynamics but dose-dependently increased CBFv and CD. Des-Arg⁹-bradykinin was less potent than bradykinin. Hoe 140 (a B₂ antagonist, 10 µg/kg) abolished the effects of bradykinin but did not influence the effects of des-Arg⁹-bradykinin. When CBFv increase was prevented by the cuff occluder, CD responses to bradykinin and des-Arg⁹-bradykinin were maintained. Intracoronary lisinopril (0.75 mg) increased the CD response to bradykinin, with only minimal effect on CBFv, and extended the duration of the effect. Lisinopril did not alter des-Arg⁹-bradykinin responses. Intracoronary N⁷-nitro-L-arginine (2 mg/kg) decreased the CD effect of bradykinin and prevented the CBFv and CD effects of des-Arg⁹-bradykinin. The relaxing effect of des-Arg⁹-bradykinin on isolated coronary rings was prevented by des-Arg⁹-[Leu⁸]-bradykinin.

Conclusions—In the conscious dog, B₁ receptors are present in coronary vessels, and their stimulation produces vasodilation in conductance and resistance vessels, which is mediated essentially by NO but not modulated by angiotensin-converting enzyme. However, the coronary vasodilation induced by B₁ receptor stimulation is not as great as that produced by B₂ receptor stimulation. (Circulation. 2000;101:1848-1853.)

Key Words: bradykinin ■ nitric oxide ■ receptors ■ vasodilation

Kinin receptors are classified as B₁ and B₂ receptors, according to their sensitivity to different agonists and antagonists.¹ The existence of B₁ and B₂ receptors has been confirmed by cloning and pharmacological characterizations in humans.²,³ Kinins exert their vascular effects through B₂ and B₁ receptors. B₂ receptors are constitutively present in most tissues of various species. B₂ receptors are sensitive to bradykinin and kallidin (Lys-bradykinin), whereas B₁ receptors are relatively insensitive to these compounds but sensitive to des-Arg⁹-bradykinin and Lys-des-Arg⁹-bradykinin, natural metabolites of bradykinin and Lys-bradykinin induced by kininase I (carboxypeptidase N).¹ The activation of B₂ receptors can elicit complex responses reflecting the multiple actions of agonists in endothelial cells, vascular smooth muscle, and nerve endings. B₁ receptors, absent in normal vessels from some animal species, are inducible and functionally expressed in response to cytokines, bacterial lipopolysaccharides, and in vitro tissue incubation.⁴–⁶ However, B₁ receptors are heterogeneously expressed depending on species and tissues.⁷ B₁ receptors are constitutively and functionally expressed in the arterial vessels and glomerulus of rats,⁸,⁹ in the pulmonary vascular bed of cats,¹⁰ and in the arterial vessels of pigs¹¹ and dogs.¹²–¹⁴ In humans, a recent in vitro study using immunolabeling techniques revealed the presence of B₁ receptors in vascular endothelial and smooth muscle cells of large elastic arteries, muscular arteries (such as coronary arteries), and muscular arterioles.¹⁵ The functional role of constitutive B₁ receptors in the arterial vessels of dogs is well documented.¹²–¹⁴ The stimulation of these receptors produces a vasorelaxation and a reduction in blood pressure.
pressure. However, their physiological implications in the coronary circulation remain to be determined. Therefore, the present study was designed to examine, in conscious dogs, the coronary effects of B1 receptor stimulation and the mechanisms involved. This was accomplished by injecting des-Arg9-bradykinin into the circumflex coronary artery through a chronically implanted catheter at doses that did not induce systemic changes. To determine the potency of B1 receptor stimulation, the effects of des-Arg9-bradykinin were compared with those of bradykinin. To distinguish the direct versus the flow-mediated effects of des-Arg9-bradykinin and bradykinin on large coronary vessels, the effects of des-Arg9-bradykinin and bradykinin on coronary diameter (CD) were examined when coronary blood flow (CBFv) increase was prevented by inflation of a cuff occluder. In addition, because angiotensin-converting enzyme (ACE) inhibition can potentiate the vasodilator effects of bradykinin, a series of experiments was performed to determine the influence of an ACE inhibitor, lisinopril, on the effects of intra coronary des-Arg9-bradykinin and bradykinin. Finally, to assess the role of NO in the effects of B1 receptor stimulation, a series of experiments was performed after the administration of N\textsuperscript{\textbullet}-nitro-L-arginine (LNA) to block NO synthase.

**Methods**

**Instrumentation**

Eleven mongrel dogs (average weight 32.4±1.0 kg) were anesthetized with sodium thiopental, intubated, and ventilated with room air mixed with halothane (1.2±0.2 vol\%). Under sterile conditions, a left thoracotomy through the fifth intercostal space was performed. Tygon catheters (Norton Plastics) were implanted in the descending aorta and left atrium. Through an apical stab wound, a pressure transducer (model P7, Konigsberg Instruments) was implanted in the LV cavity. The circumflex coronary artery was isolated with minimal dissection, and a silastic catheter (0.30-mm ID, 0.63-mm OD; Dow Corning Co) was inserted into the proximal circumflex coronary artery. A 10-MHz Doppler flow probe was implanted around the circumflex artery distal to the tip of the intracoronary catheter. Two 5-MHz piezoelectric crystals attached on silk patches were sutured to the opposing surface of the circumflex artery near the Doppler flow probe to measure circumflex CD. A cuff occluder was placed around the circumflex artery upstream from the Doppler flow probe and CD crystals. The thoracic incision was closed in layers, and all wires and catheters were tunneled to the infrascapular area. Morphine (10 mg) was given subcutaneously when autonomic respiration had been restored in the dog. Postoperative care was made daily. Ampicillin (1 g/d) was given for 2 weeks. The intracoronary catheter was maintained patent with a continuous infusion of saline (Zyklomat BT1, Ferring SA) for 3 days. Thereafter, the catheter was flushed daily with heparinized saline. The animals used in the present study were maintained in accordance with the official regulations of the French Ministry of Agriculture.

**Experimental Protocols**

Experiments were initiated 3 to 6 weeks after surgery in conscious healthy dogs. In 6 dogs, before any drug administration, a blood sample was withdrawn by aortic catheter for hematological analysis. After baseline hemodynamic recordings, before drug injections, to verify the absence of changes related to the injection method, a bolus of warm saline (0.2 mL) was injected into the circumflex artery and flushed with 0.5 mL saline delivered with a pump-driven syringe over a 10-second period. Then, by use of the same method, the dose-response curves of des-Arg9-bradykinin (3, 10, 30, and 100 ng/kg) and bradykinin (0.1, 1, 3, and 10 ng/kg; both from Sigma Chemical Co) were established. In all experiments, saline solution (38°C) was infused continuously at 1 mL/min. All drugs used were dissolved in warm saline and freshly prepared before experiments. Between 2 injections, a 3-minute delay was allowed for 0.1 to 1 µg/kg bradykinin and 3 to 10 ng/kg des-Arg9-bradykinin, and a 15- to 30-minute delay was allowed for 1 to 10 µg/kg bradykinin and 30 to 100 ng/kg des-Arg9-bradykinin.

To determine the specificity of the response to bradykinin and des-Arg9-bradykinin, additional experiments were performed in 6 dogs after intracoronary infusion of a bradykinin B1 receptor antagonist, Hoe 140 (10 µg/kg infused in 1 minute; icatibant, a kind gift of Dr B. Sholikens, Hoescht AG, Frankfurt, Germany). After baseline recordings, the responses to intracoronary bradykinin and des-Arg9-bradykinin were assessed.

To distinguish the flow-dependent phenomena and the direct effect of B1 or B2 receptor stimulation in the coronary diameter effect, the CD response to des-Arg9-bradykinin (100 ng/kg) and bradykinin (1 ng/kg) was examined before and after inflation of the coronary cuff occluder in 6 dogs.

To assess the influence of ACE inhibition on CBFv and CD responses to bradykinin and des-Arg9-bradykinin, intracoronary bradykinin and des-Arg9-bradykinin injections were repeated in 6 dogs after the administration of intracoronary lisinopril (0.75 mg, 1 mL/min for 5 minutes; ZENECA Pharma).

To determine the role of NO in the effects of B1 or B2 receptor stimulation, intracoronary des-Arg9-bradykinin and bradykinin injections were repeated in 6 dogs after pretreatment of LNA (2 mg/kg, 1 mL/min for 8 minutes).

To verify the constitutive existence of B1 receptors, 5 uninstrumented dogs were anesthetized with sodium pentobarbital, and their hearts were removed. The circumflex artery was isolated, cleaned of adherent connective tissue, and cut into rings ~3 mm in length. In some rings, endothelium removal was performed by rubbing the intimal layer with forceps. All rings were suspended in organ chambers filled with 20 mL control solution (modified Krebs-Ringer bicarbonate solution at 37°C) that was gassed with 95% O2/5% CO2 for isometric tension recording. After pretreatment with bestatin (10 µM) and mergetpa (10 µM/L) and 45 minutes of equilibration, rings were contracted with thromboxane A\textsubscript{2}, analogue U46619 (1 µM/L). Each ring was randomly assigned to either the control solution or the B1 receptor antagonist des-Arg9-[Leu\textsuperscript{8}]-bradykinin (10 µM/L). Cumulative responses to increasing concentrations of des-Arg9-bradykinin were obtained. The rings were then washed with the control solution and contracted with U46619 (1 µM/L), and relaxation to bradykinin (10 µM/L) was obtained in the presence of bestatin and mergetpa. Relaxations were measured at peak decrease in tension for each concentration of drugs by use of software from IOS-Laboratory (EMKA Technologies). Results are expressed as percentage of the maximal contraction to U46619.

**Data Collection and Analysis**

Statham P23ID pressure transducers were used to measure aortic and left atrial pressures. Absolute values of LV pressure were obtained by calibration of the micromanometer in 37°C water. CBFv was measured by a Doppler flowmeter (Triton Technology Inc). CD was measured by a sonomicrometer (Triton Instruments). The signals were monitored with an oscilloscope and calibrated by the sonomicrometer. All signals were input into a microcomputer with the use of Hem v1.5 software (Notocord Systems) and monitored on a multispectral graphic recorder.

Hemodynamic parameters were calculated at baseline and at peak increase in mean CBFv in response to various agents. The duration of CBFv and CD increases induced by bradykinin and des-Arg9-bradykinin was determined as the interval between the initial rise and the return to baseline CBFv and CD. At autopsy, the position, alignment, and orientation of the crystals were examined. The position of the intracoronary catheter was confirmed, and the coronary artery distal to the intracoronary catheter and the territory of left ventricle perfused by the circumflex artery were macroscopically examined.
Statistical Analysis

Values are expressed as mean±1 SEM. Experimental results were subjected to an ANOVA for repeated measures (superANOVA, Abacus Concepts, Inc). A 1-way ANOVA for repeated measurements was used for intragroup interactions. A 2-way ANOVA for repeated measurements with the same parameters was used for intergroup interactions. When a significant trend was observed by variance analysis, comparisons between means, such as the effects of the same dose of bradykinin or des-Arg⁹-bradykinin before and after lisinopril, LNA, or HOE 140 treatment, were performed by contrast analysis. When only 2 means were compared, a paired t test was used. A difference was considered statistically significant at P<0.05.

Results

At autopsy, macroscopic examination did not reveal any lesion in the catheterized circumflex artery or in the region of myocardium supplied by this vessel. Blood analysis at the beginning of the experimental protocol showed that erythrocytes (5.5±0.3×10¹²/L), hematocrit (38.7±2.2%), leukocytes (13.6±0.5×10⁹/L), and fibrinogen (3.2±1 g/L) were within the normal range of values for dogs.

Coronary Effects of Bradykinin and des-Arg⁹-Bradykinin

Baseline systemic and coronary parameters were similar before the administration of bradykinin and des-Arg⁹-bradykinin (Table). Intracircumflex injection of bradykinin and des-Arg⁹-bradykinin did not modify heart rate, mean arterial pressure, left ventricular (LV) systolic and end-diastolic pressures, or LV dP/dt max. Bradykinin and des-Arg⁹-bradykinin increased dose-dependent CBF velocity (CBFv) and CD (Figure 1). However, the CBFv and CD increases induced by des-Arg⁹-bradykinin were smaller than those produced by bradykinin (P<0.05). The dose of 100 ng/kg des-Arg⁹-bradykinin produced changes in CBFv and in CD similar to those produced by 1 ng/kg bradykinin (Figure 1).

Coronary Responses to Bradykinin and des-Arg⁹-Bradykinin in the Presence of Hoe 140

Lisinopril did not produce significant changes in systemic hemodynamics, CBFv, or CD. After the administration of
lisinopril, intracoronary bradykinin or des-Arg<sup>9</sup>-bradykinin did not change systemic hemodynamics at any dose. Lisinopril did not modify the peak response of CBFv to bradykinin but significantly increased the peak response of CD (Figures 4 and 5, top left panels) and prolonged the duration of CBFv and CD responses (Figures 4 and 5, bottom left panels), indicating that ACE inhibitors could affect the coronary response to bradykinin. In contrast, lisinopril did not affect the CBFv and CD effects of des-Arg<sup>9</sup>-bradykinin (Figures 4 and 5, right panels).

**Coronary Responses to Bradykinin and des-Arg<sup>9</sup>-Bradykinin After Pretreatment With LNA**

LNA (2 mg/kg) did not produce any significant changes in systemic hemodynamics other than a slight but significant decrease in heart rate (from 81±5 to 72±2 bpm). LNA did not significantly modify mean CBFv but decreased CD (from 3375±359 to 3279±352 μm, \(P<0.05\)). The peak effect of bradykinin on mean CBFv tended to decrease and the peak effect on CD decreased after LNA pretreatment \(P<0.05\); Figure 6, left panels), confirming the role of NO in the coronary vasodilator effect of bradykinin. After LNA pretreatment, des-Arg<sup>9</sup>-bradykinin did not modify systemic hemodynamics. The effects of des-Arg<sup>9</sup>-bradykinin on CBFv and CD were prevented by LNA (Figure 6, right panels), indicating the implication of NO in the coronary effects of des-Arg<sup>9</sup>-bradykinin.

**In Vitro Coronary Responses to des-Arg<sup>9</sup>-Bradykinin**

The successive contractions of rings to U46619 were of similar magnitude (16.2±1.6 g). des-Arg<sup>9</sup>-bradykinin induced a concentration-dependent relaxation that was endo-
thelium dependent (Figure 7). des-Arg<sup>9</sup>-[Leu<sup>8</sup>]-bradykinin markedly reduced the des-Arg<sup>9</sup>-bradykinin–induced relaxation. The bradykinin-induced relaxation was 5-fold larger than that induced by des-Arg<sup>9</sup>-bradykinin and was not altered by des-Arg<sup>9</sup>-[Leu<sup>8</sup>]-bradykinin (Figure 7).

**Discussion**

The principal results of the present study show that in conscious dogs, intracoronary injection of des-Arg<sup>9</sup>-bradykinin, a bradykinin B<sub>1</sub> receptor agonist, produces a dose-dependent coronary vasodilation in both conductance and resistance vessels. However, des-Arg<sup>9</sup>-bradykinin is less potent than bradykinin in coronary vessels, and in contrast to bradykinin, these coronary effects are not affected by bradykinin B<sub>2</sub> receptor blockade or by ACE inhibition. In addition, NO synthase blockade prevents the coronary vasodilator effect of des-Arg<sup>9</sup>-bradykinin.

The present study provides the first evidence that under physiological conditions (distant from anesthesia and acute surgical trauma), coronary vasodilation in both conductance and resistance vessels produced by intracoronary des-Arg<sup>9</sup>-bradykinin is mediated by bradykinin B<sub>1</sub> receptors. This is supported by the fact that Hoe 140, a selective B<sub>2</sub> receptor antagonist, did not block the effects of des-Arg<sup>9</sup>-bradykinin and that this effect was blocked in vitro by a B<sub>1</sub> receptor antagonist, des-Arg<sup>9</sup>-[Leu<sup>8</sup>]-bradykinin. In anesthetized greyhounds, intracoronary injection of a small dose of des-Arg<sup>9</sup>-bradykinin increased CBFv but did not modify CD, whereas a larger dose of des-Arg<sup>9</sup>-bradykinin increased both CBFv and CD in association with systemic effects. In this latter study, the question of whether the CD response was receptor- or flow-mediated was unresolved. The present study performed in conscious dogs, in the absence of systemic effects, shows that the CD increase in response to des-Arg<sup>9</sup>-bradykinin was essentially due to B<sub>1</sub> receptor stimulation rather than a flow-dependent phenomenon because CBF limitation by inflating a coronary cuff occluder did not affect the CD response to des-Arg<sup>9</sup>-bradykinin.

Normal vessels from most animal species are generally considered to be constitutively lacking bradykinin B<sub>1</sub> receptors. However, B<sub>1</sub> receptors are heterogeneously expressed depending on species and tissues. In normal rats, intra-arterial injections of des-Arg<sup>9</sup>-bradykinin produce weak but measurable hemodynamic responsiveness, and the B<sub>1</sub> receptor antagonist des-Arg<sup>9</sup>-[Leu<sup>8</sup>]-bradykinin reduces the glomerular filtration rate and urine concentration, suggesting the presence of constitutive B<sub>1</sub> receptors in the arterial trees and in the kidneys of rats. In pigs, des-Arg<sup>9</sup>-bradykinin also produces measurable hypotensive effects. In cats, the pulmonary vascular bed contains functional B<sub>1</sub> receptors; the stimulation of these receptors produces tone-dependent changes in pulmonary artery pressure. In dogs, B<sub>1</sub> receptors are also present in the isolated renal artery and mediate vasorelaxation, and in intact preparations, B<sub>1</sub> receptor stimulation by des-Arg<sup>9</sup>-bradykinin produces a hypotensive effect. By use of a specific antibody directed to the peptide sequences of B<sub>1</sub> receptors, it has recently been shown in humans that B<sub>1</sub> receptors are present not only in vascular endothelial and smooth muscle cells of large elastic arteries but also in muscular arteries, such as coronary arteries and muscular arteries. The notion that B<sub>1</sub> receptors are constitutively expressed in coronary vessels is supported by the present study, which shows the functional coronary effects of des-Arg<sup>9</sup>-bradykinin in the absence of evidence of inflammation by blood analysis. Although bradykinin can be metabolized into des-Arg<sup>9</sup>-bradykinin in dogs, the lack of effects of bradykinin through B<sub>1</sub> receptor stimulation after Hoe 140 pretreatment that we observed may be related to the fact that injected doses of bradykinin were lower than the dose of des-Arg<sup>9</sup>-bradykinin producing any significant effect. Finally, in coronary rings obtained from uninstrumented dogs, des-Arg<sup>9</sup>-bradykinin produced a concentration-dependent relaxation.

Several studies have shown that NO is involved in the relaxing effect of kinins. In the present study, pretreatment with LNA significantly decreased the peak response of CD to bradykinin but only slightly decreased the CBFv response. The minimal influence of LNA on the CBFv response is consistent with that reported in porcine coronary resistance arteries. This CBFv response to bradykinin, which is resistant to inhibitors of NO synthase, may be mediated by endothelium-derived hyperpolarizing factor (EDHF), because it has been shown that the relaxing response of coronary vessels to bradykinin is also mediated by EDHF, which probably acts through calcium-activated potassium channels. However, the in vivo role of EDHF remains unknown because EDHF has not been identified. The coronary effects of des-Arg<sup>9</sup>-bradykinin on conductance and resistance vessels were prevented by LNA, indicating that the effects of des-Arg<sup>9</sup>-bradykinin are, in a large part, mediated by NO, which is consistent with a previous study in which NO synthase inhibitors and des-Arg<sup>9</sup>-bradykinin were administrated intravenously.

In vitro, the bradykinin-induced vasorelaxation is potentiated by ACE inhibitors. In humans, bradykinin is involved in the vascular effect of ACE inhibitors. A recent study from our laboratory has shown that enalaprilat extends the duration of the effect of bradykinin with no significant changes in the peak CBFv response to bradykinin. The present study, using lisinopril, demonstrates a similar result in association with an increased CD response to bradykinin. However, lisinopril did not modify the coronary responses to des-Arg<sup>9</sup>-bradykinin, which is in accordance with observa-
tions in the pulmonary circulation of cats, indicating that in the coronary circulation in vivo, ACE plays only a small role in des-Arg²-[Leu⁸]-bradykinin metabolism. Although it has been shown in vitro that ACE inhibitors may prevent the degradation of des-Arg⁹-bradykinin, in vivo experiment did not find an increased blood concentration of des-Arg⁹-bradykinin after enalapril administration. This may explain why ACE inhibitors do not influence the effects of des-Arg⁹-bradykinin.

Previous in vitro and in vivo studies have shown that B₂ receptors mediate the major physiological effects of kinins. Our data indicating that bradykinin is more potent than des-Arg⁹-bradykinin in conductance and resistance coronary vessels support this notion. However, the possible role of B₁ receptors under pathological circumstances remains to be investigated because the plasma concentration of des-Arg⁹-bradykinin is higher than that of bradykinin in patients with hypertension and because B₁ receptors can be upregulated and exhibit less desensitization than do B₂ receptors, which may lead to a predominant effect of B₁ receptors. In human coronary vessels, as previously mentioned, B₁ receptors are present and can be upregulated, with their in vitro stimulation producing a vasorelaxing effect. In addition, a recent study reports that B₁ receptors are more abundant than B₂ receptors in atheromatous plaques of human coronary vessels, suggesting that B₁ receptors may be involved in atheromatous disease.

In conclusion, the present study shows for the first time that under physiological conditions, bradykinin B₁ receptors are functionally present in canine coronary vessels and that their stimulation produces vasodilation in conductance and resistance vessels that is mediated in large part by NO and is not modulated by ACE inhibition. Although the coronary vasodilation induced by B₁ receptor stimulation is less potent than that produced by B₂ receptor stimulation under physiological conditions, the stimulation of B₁ receptors may exert potent vasoactive coronary effects in pathological conditions (such as local or systemic inflammation and sepsis) in which B₁ receptors can be upregulated.

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References

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