British Journal of Pharmacology (1996) 117, 1565-1571

Haemodynamic and cardiac effects of kinin B_1 and B_2 receptor stimulation in conscious instrumented dogs

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1 Mongrel dogs were chronically instrumented with an intra-aortic catheter, a Königsberg intraventricular pressure transducer and a Döppler flow probe around the left coronary artery. After ganglionic blockade with hexamethonium, the cardiovascular effects of bradykinin B_1 and B_2 receptor agonists, des-Arg^9-bradykinin and bradykinin (BK), were investigated in the presence and absence of specific antagonists. The contribution of nitric oxide (NO) and prostanoids to the cardiovascular effects of kinins was also examined.

2 BK (1 μg kg^{-1} min^{-1}) and des-Arg^9-BK (1 μg kg^{-1} min^{-1}) both given as a 2 min i.v. infusion, produced a significant decrease in mean arterial pressure (MAP, $-34\pm4\%$ for BK and $-45\pm2\%$ for des-Arg^9-BK) and coronary vascular resistance (CVR, $-37\pm5\%$ for BK and $-50\pm2\%$ for des-Arg^9-BK), without affecting cardiac contractility, left ventricular end diastolic pressure, and coronary velocity. BK caused a significantly greater decrease in MAP and CVR than des-Arg^9-BK ($P<0.05$).

3 Pretreatment with the B_1 receptor antagonist, des-Arg^9-[Leu^8]-BK (25 μg kg^{-1}) significantly inhibited the decrease in MAP and CVR produced by des-Arg^9-BK but not by BK. Infusion of des-Arg^9-[Leu^8]-BK alone also induced a significant decrease in MAP and CVR ($P<0.05$). In the presence of the B_2 receptor antagonist, Hoe 140 (25 μg kg^{-1}), only the decreases in MAP and CVR caused by BK were significantly reduced ($P<0.05$).

4 Inhibition of NO synthase with N$^{\omega}$-nitro-L-arginine (L-NOARG, 45 mg kg^{-1}) significantly ($P<0.05$) prevented the decrease in CVR but not MAP induced by des-Arg^9-BK, whilst responses to BK were not affected by L-NOARG pretreatment. Inhibition of prostanoid synthesis with indomethacin (25 mg kg^{-1}) did not affect the reductions in MAP and CVR induced by des-Arg^9-BK or BK.

5 In conclusion, i.v. des-Arg^9-BK and BK administration induced reductions in MAP and CVR suggesting that in conscious instrumented dogs both B_1 and B_2 receptors are present and can affect systemic blood pressure and coronary resistance regulation. Our results also suggest that prostanoids are not involved in the vascular response to kinins and that coronary vascular B_1 receptors are at least in part coupled to the release of NO.

Keywords: des-Arg^9-bradykinin; bradykinin; kinin receptors; haemodynamics; coronary circulation; conscious dogs

Introduction

Kinins are important mediators of pain and inflammation as well as potent vasodilators (Hall, 1992). Bradykinin (BK) and its kininase I metabolite, des-Arg^9-BK, activate B_2 and B_1 receptor subtypes respectively (Regoli *et al.*, 1977), the existence of which has been recently confirmed by cloning and pharmacological characterization in man (Hess *et al.*, 1992; Menke *et al.*, 1994).

In the vasculature, B_2 receptors appear to be constitutive, are widely distributed on the arterial endothelium and mediate release of prostacyclin and of an endothelial-derived relaxing factor (Graier *et al.*, 1992). The reduction in arterial blood pressure in response to intravenous BK has been shown in various models to result from peripheral vasodilatation mediated by the activation of endothelial receptors (Berguer *et al.*, 1993; Santiago *et al.*, 1994). B_2 receptors are also present on venous smooth muscle cells mediating BK-induced contractions of the human isolated umbilical vein (Marceau *et al.*, 1994) and of the rabbit and guinea-pig jugular vein (Pruneau *et al.*, 1995b).

Kinin B_1 receptors, generally absent in normal vessels from most animal species, are instead inducible and functionally expressed in response to cytokines such as interleukin-1β (IL-1β) (deBlois *et al.*, 1991), bacterial lipopolysaccharides (Regoli *et al.*, 1981), and during *in vitro* tissue incubation (Regoli *et al.*, 1981; Bouthillier *et al.*, 1987; Pruneau & Bélichard, 1993) or *in vivo* arterial trauma (Pruneau *et al.*, 1994). In contrast, B_1 receptors appear to be constitutively expressed in the arterial system of the dog since both *in vitro* and *in vivo* experiments have shown a vascular relaxation and a reduction in blood pressure to des-Arg^9-BK in this species (Staszewska-Wooley *et al.*, 1991; Yen & Lai, 1992; Nakhostine *et al.*, 1993).

The present study was designed to characterize the physiological mechanisms underlying the direct haemodynamic and cardiac effects of des-Arg^9-BK and BK in the dog. To avoid possible interference due to anaesthetics, we used conscious dogs chronically instrumented with an arterial catheter, a pressure transducer implanted in the left ventricle and a Döppler flow probe around the circumflex branch of the left coronary artery. After ganglionic blockade, the effects of des-Arg^9-BK and BK were examined in the absence or presence of the specific B_1 and B_2 receptor antagonists, des-Arg^9-[Leu Arg^8]-BK and Hoe 140. In order to investigate the mechanism of action of des-Arg^9-BK and BK, a series of experiments was also conducted using indomethacin and N$^{\omega}$-nitro-L-arginine to block prostanoid synthesis and nitric oxide (NO) production, respectively.

Methods

Animal surgery

The experiments were conducted in seven adult mongrel dogs, of either sex, weighing 22 to 30 kg. Surgery was performed

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under halothane anaesthesia (0.5 to 1.5 vol 100 ml^{-1} in oxygen) after induction with i.v. sodium thiopentone (30 mg kg^{-1}). The animals were ventilated artificially with room air through an intratracheal tube connected to a Bird respirator (Mark VII, Bird Co, Palm Springs, U.S.A.) at a stroke volume and respiratory frequency of 18 cycles min^{-1} and 15 ml kg^{-1}, respectively. A left thoracotomy was performed through the sixth intercostal space. A miniature pressure gauge (Konigsberg P7A, Konigsberg Instruments, Pasadena, U.S.A.) was implanted into the left ventricular cavity *via* an apical stab wound. A polyvinylchloride catheter (internal diameter, 1 mm; external diameter, 2.5 mm; J17958, Gilson, Villiers, France) was placed in the descending aorta. A 10-MHz Döppler flow probe (diameter, 2.5 to 3 mm, Valpey-Fisher, Hopkinton, U.S.A.) was implanted around the left circumflex coronary artery, 2 to 4 cm from its origin.

All wires and catheters were passed subcutaneously to the back of the dog and brought through the skin between the scapulae. The thorax was then evacuated of air and closed.

Noramidopyrine (2 g per day), ciprofloxacine (250 mg per day) and amoxicilline (300 mg per day) were administered for five days postoperatively.

The arterial catheter was filled (2 ml) with sterile saline (0.9% NaCl) containing heparin (1000 iu ml^{-1}) and was flushed every week to prevent clot formation.

Animal handling and blood analysis

Dogs were kept in the animal house at a constant temperature of 20±2°C on a 12 h-light dark cycle (07 h 00 min–19 h 00 min light). Air volume was renewed 15 times per hour. Dogs were provided water *ad libitum* and food according to their body weight (30 g pet food kg^{-1} body weight day^{-1}). Haematological analysis (erythrocytes count, haemoglobin, haematocrit, mean corpuscular volume (MCV), leukocyte count, fibrinogen) was performed the week before experimentation and four weeks after surgery.

Haemodynamic measurements

Experiments were initiated at least two weeks after surgery in healthy, apyretic conscious dogs that were trained to lie quietly on their right side on a table. The aortic catheter was connected to a Statham P23ID blood pressure transducer (Gould Statham, U.S.A.). Döppler flow probes were connected to a Döppler flowmeter (VF1, Valpey Fischer, U.S.A.). Heart rate (HR, beats min^{-1}) was monitored by the use of a cardiotachometer (Gould Statham, U.S.A.) triggered by the blood pressure pulse. Phasic and mean arterial blood pressure (MAP, mmHg), left ventricular pressure (LVP, mmHg) and its first derivative (LV dP/dt, mmHg s^{-1}), left ventricular end diastolic pressure (LVEDP, mmHg) and left circumflex coronary velocity (CBFV, kHz) were simultaneously recorded on a multichannel polygraph system (ES1000, Gould Statham, U.S.A.). Coronary resistance (CVR, mmHg kHz^{-1}) which reflects responses of resistance coronary vessels was calculated as the ratio of mean arterial pressure to mean coronary blood flow. A multi speed transmission Harvard apparatus infusion pump (INF382, Datex, Switzerland) was used for drug administration.

Experimental protocol

The previously described haemodynamic parameters were continuously monitored in five dogs. Des-Arg^9-BK (1 μg kg^{-1} min^{-1}) and BK (1 μg kg^{-1} min^{-1}) were administered i.v. over a 2-min period after ganglionic blockade with 45±5 mg kg^{-1} hexamethonium bromide (30 mg kg^{-1} bolus, followed by intermittent injection to maintain effective ganglionic blockade). Adequacy of ganglionic blockade was confirmed by the absence of reflex changes in HR to boluses of 5 μg kg^{-1} i.v. nitroglycerin and 5 μg kg^{-1} i.v. phenylephrine (10 min after hexamethonium injection, every 30 min for N^{ω}-nitro-L-arginine and indomethacin experiments, and immediately after the end of agonist infusion in all cases).

In the second part of this study, the cardiovascular effects of des-Arg^9-BK and BK (doses as above) were evaluated in five dogs, in the presence of the B_1 receptor antagonist, des-Arg^9-[Leu^8]-BK and of the selective B_2 receptor antagonist, Hoe 140.

After ganglionic blockade, des-Arg^9-BK or BK was injected i.v. over a 2-min period (again at a dose of 1 μg kg^{-1} min^{-1}), (a) before and immediately after a 5-min i.v. infusion of des-Arg^9-[Leu^8]-BK (25 μg kg^{-1}), (b) before and immediately after a 5-min i.v. infusion of Hoe 140 (25 μg kg^{-1}), (c) before and immediately after injection of their vehicle (saline).

In the third series of experiments the role of prostanoids and nitric oxide on the peripheral and coronary action of des-Arg^9-BK and BK was examined. After ganglionic blockade in 6 dogs, des-Arg^9-BK or BK was injected i.v. over a 2-min period at the dose of 1 μg kg^{-1} min^{-1}, (a) before and 60 min after a 10-min i.v. infusion of the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine (45 mg kg^{-1}), (b) before and 30 min after a 30-s i.v. bolus injection of the prostanoid synthesis inhibitor indomethacin (10 mg kg^{-1}), (c) before and immediately after injection of their vehicle (Na_2CO_3).

Statistical analysis

Experimental values are presented as mean±s.e.mean. The data were analyzed by a one-way ANOVA and Scheffé's F test. A value of $P<0.05$ was considered to be significant. Statistics were computed using Statview II (Abacus Concepts).

Drugs

Bradykinin acetate, des-Arg^9-BK acetate, des-Arg^9-[Leu^8]-BK acetate, hexamethonium bromide, nitroglycerin, phenylephrine, N^{ω}-nitro-L-arginine, and indomethacin were obtained from Sigma Chemical Co (St Louis, MO, U.S.A.). Hoe 140 ([D-Arg^0, Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK) was kindly provided by Prof J. Martinez (CNRS URA1845, Montpellier, France).

Noramidopyrine, amoxicillin, and ciprofloxacine were obtained from Hoechst laboratories (Puteau, France), Smith Kline Beecham (Nanterre, France), and Bayer Pharma (Sens, France), respectively. All drugs were freshly made up in saline or Na_2CO_3 and kept on ice and protected from light.

Results

Blood analysis

As shown in Table 1, erythrocytes, haematocrit, mean corpuscular volume (MCV) and platelets were not modified four weeks after instrumentation. Leukocyte number and fibrinogen levels were significantly increased four weeks after surgery.

Effects of ganglionic blockade on baseline haemodynamics

Hexamethonium did not significantly modify mean arterial pressure (MAP (mmHg): 107±7 baseline value vs 109±7 after ganglionic blockade), left ventricular pressure (LVP (mmHg): 142±5 vs 129±5) and its first derivative (dP/dt (mmHg s^{-1}): 2688±254 vs 2313±198), left ventricular end diastolic pressure (LVEDP (mmHg): 19.6±1.4 vs 15.8±1.9), coronary blood flow velocity (CBFV (kHz): 4.2±0.5 vs 4.6±0.3) and coronary vascular resistance (CVR (mmHg kHz): 25±4 vs 25±4). Heart rate increased significantly by 85% after hexamethonium administration (HR (beats min^{-1}): 72±10 vs 125±11, $n=5$, $P<0.05$).

Haemodynamic effect of des-Arg^9-BK and BK

Preliminary experiments demonstrated that 1 μg kg^{-1} min^{-1} of des-Arg^9-BK or BK produced a submaximal decrease in

MAP and CVR close to the plateau of the dose-response curves (0.3, 1, 3 $\mu g\ kg^{-1}\ min^{-1}$) (data not shown). This dose was thus selected for the rest of our study. Infusion lasted for 2 min and resulted in transient haemodynamic effects peaking 1 min after the beginning of the infusion (Figure 1). Table 2 illustrates the changes in the measured parameters induced by des-Arg^9-BK and BK, 1 min after the beginning of peptide infusion. Basal systemic, left ventricular and coronary haemodynamic values are also shown in Table 2. There was no difference between basal haemodynamic values of des-Arg^9-BK and BK-treated dogs (Table 2). Des-Arg^9-BK significantly decreased MAP from 95±5 mmHg (baseline value) to 63±5 mmHg (1 min after beginning of infusion) ($n=5$, $P<0.05$) but did not affect HR (125±11 vs 125±10 beats min^{-1}), LVP (125±4 vs 108±3 mmHg), dP/dt (2344±175 vs 2100±100 mmHg s^{-1}), LVEDP (15±2 vs 10±2 mmHg), and CVBF (4.02±0.42 vs 4.32±0.51 kHz). Coronary vascular resistance was significantly decreased by des-Arg^9-BK infusion (from 25.5±4.2 to 15.3±1.4 mmHg kHz^{-1}, $n=5$, $P<0.05$).

After BK administration (1 $\mu g\ kg^{-1}\ min^{-1}$), MAP significantly decreased from 98±3 mmHg (baseline value) to 54±2 mmHg (1 min after beginning of infusion) ($n=5$, $P<0.05$). HR (122±8 vs 127±10 beats min^{-1}), LVP (135±5 vs 105±3 mmHg), dP/dt (2305±150 vs 2050±166 mmHg s^{-1}), LVEDP (15±2 vs 11±2 mmHg), and CBFV (4.02±0.43 vs 5.15±0.87 MHz) were not significantly modified by BK. Coronary vascular resistance was significantly decreased by BK (from 26.5±4.1 to 13.2±1.9 mmHg kHz^{-1}, $P<0.05$). The effects of 1 $\mu g\ kg^{-1}\ min^{-1}$ BK was 1.3 fold greater on MAP and CVR than that measured with the same dose of des-Arg^9-BK ($P<0.05$).

Effect of des-Arg^9-[Leu^8]-BK and Hoe 140 on the haemodynamic response to des-Arg^9-BK and BK

Figure 2 shows that injection of the B_1 receptor antagonist, des-Arg^9-[Leu^8]-BK (25 $\mu g\ kg^{-1}$) induced a significant decrease ($n=5$, $P<0.05$) in MAP (−36±3%) and in CVR

Table 1 Blood analysis

	Values 1 week before surgery	*Values 4 weeks after surgery*	*Normal values*
Erythrocytes	6.5±0.3	6.0±0.1	$5.5-8.5\times10^{12}\ l^{-1}$
Haematocrit	45±2	42±1	37–55%
MCV	68.9±1.0	69.4±0.7	66–77 μm^3
Platelets	271±32	275±24	$200-900\times10^9\ l^{-1}$
Leukocytes	10.6±0.1	16.8±0.9*	$6-18\times10^9\ l^{-1}$
Fibrinogen	2.19±0.12	3.36±0.19*	2–4 g l^{-1}

Abbreviations: MCV, mean corpuscular volume.
Values 1 week before surgery and 4 weeks after are means±s.e.mean, *$P<0.05$.
Normal values are taken from Kirk & Bistner (1975).

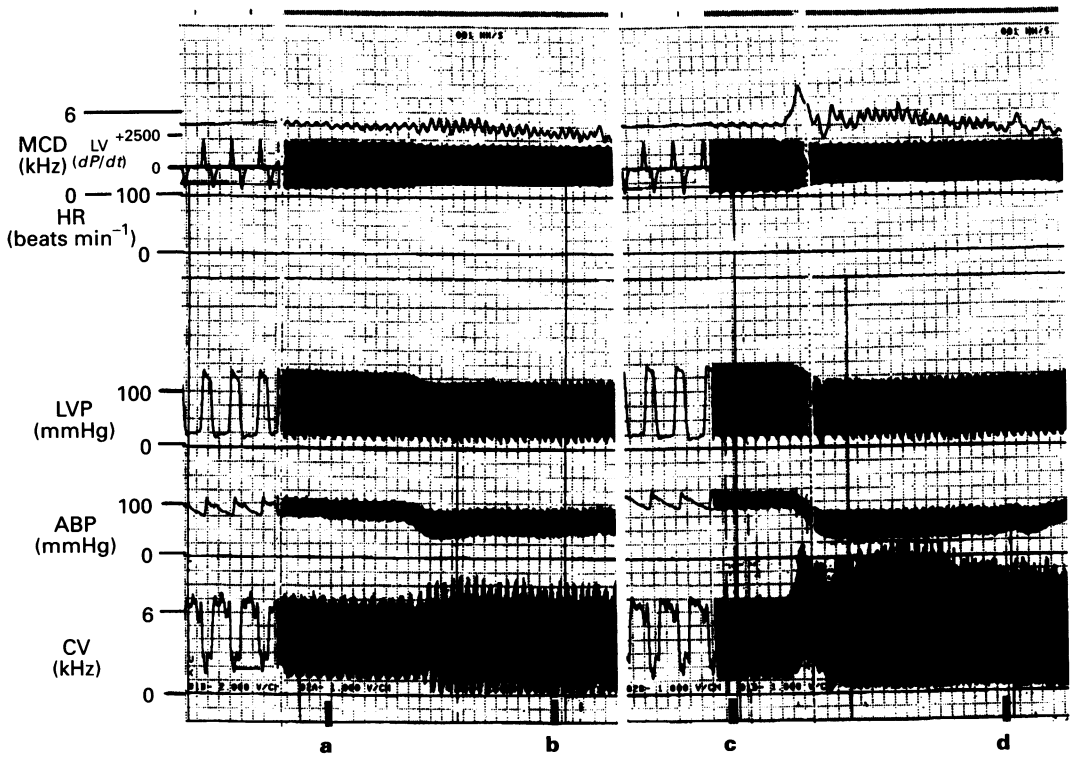

Figure 1 Recording of mean coronary velocity (MCV), the first derivative of left ventricular pressure over time (LV dP/dt), heart rate (HR), left ventricular pressure (LVP), arterial blood pressure (ABP) and coronary velocity (CV), before and during administration of 1 $\mu g\ kg^{-1}\ min^{-1}$ i.v. des-Arg^9-bradykinin (beginning in a and ending in b) and of 1 $\mu g\ kg^{-1}\ min^{-1}$ i.v. bradykinin (beginning in c and ending in d).

Table 2 Effects of des-Arg^9-bradykinin (DBK) and bradykinin (BK) (1 μg kg^{-1} min^{-1}) on haemodynamics

	MAP (mmHg)	*HR* (beats min^{-1})	*LVEDP* (mmHg)	*dP/dt* (mmHg s^{-1})	*CBFV* (kHz)	*CVR* (mmHg kHz^{-1})
Baseline						
DBK	95±5	125±11	15±2	2344±400	4.0±0.4	25.5±4.2
BK	98±3	122±8	15±2	2305±150	4.0±0.4	26.5±4.1
% change from baseline						
DBK	*[-34±4¶	0	-33±7	-16±8	*[7±5	*[-37±5¶
BK	[-45±2¶	4±1	-28±6	-13±6	[26±12	[-50±2¶

Values are means±s.e.mean. *$P<0.05$ DBK vs BK; ¶ $P<0.05$ vs baseline values.

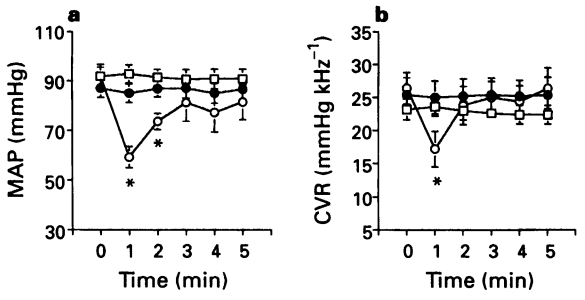

Figure 2 Time-course effect of a 5-min i.v. infusion of des-Arg^9-[Leu^8]-BK (○), Hoe 140 (●), or vehicle (□) on mean arterial pressure (MAP) and coronary vascular resistance (CVR). Values are means±s.e.mean. *$P<0.05$, $n=5$.

(−35±2%), peaking 1 min after the beginning of the 5 min i.v. infusion and returning promptly to basal values. Other parameters (HR, LVP, *dP/dt*, LVEDP, and CBFV) were not significantly affected by des-Arg^9-[Leu^8]-BK infusion. The B_2 receptor antagonist, Hoe 140 (25 μg kg^{-1}) was without significant effect either on MAP and CVR (Figure 2), or on other measured parameters (HR, LVP, *dP/dt*, LVEDP, and CBFV).

Des-Arg^9-[Leu^8]-BK inhibited the effects of des-Arg^9-BK on MAP and CVR by 72±3% and 92±3% respectively ($n=5$, $P<0.05$, in comparison to vehicle values) (Figure 3). In contrast, Hoe 140 had no effect on MAP and CVR reduction induced by des-Arg^9-BK (Figure 3).

In the presence of Hoe 140, the magnitude of the MAP and CVR decrease caused by BK were reduced by 91±2% and 94±4% respectively ($n=5$, $P<0.05$) (Figure 3). In contrast des-Arg^9-[Leu^8]-BK did not affect the response to BK.

Effects of the NO synthase inhibitor, N$^\omega$-nitro-L-arginine (L-NOARG) and the cyclo-oxygenase inhibitor on the haemodynamic response to des-Arg^9-BK and BK

MAP, LVP, and LVEDP all significantly increased 1 h after the 10-min i.v. injection of L-NOARG (45 mg kg^{-1}) (MAP: 97±4 vs 177±3 mmHg; LVP: 119±4 vs 216±5 mmHg; LVEDP: 17±0.9 vs 28±2.6 mmHg, $n=6$, $P<0.05$). HR (123±6 vs 109±4 beats min^{-1}), *dP/dt* (2075±166 vs 2160±114 mmHg min^{-1}), CBFV (3.8±0.8 vs 5.2±0.9 kHz), CVR (29±5 vs 34±7 kHz mmHg^{-1}) were unaffected by L-NOARG. L-NOARG caused a non-significant 22% inhibition of the decrease in MAP induced by des-Arg^9-BK and a significant ($P<0.05$, $n=6$), 50% inhibition in the CVR reduction induced by the B_1 receptor agonist (Figure 3). L-NOARG failed to inhibit both MAP and CVR decreases induced by i.v. injection of BK (Figure 3).

Pretreatment with indomethacin (10 mg kg^{-1}) resulted in a significant ($P<0.05$) $n=5$, increase in resting levels of MAP and LVP 30 min after i.v. bolus injection (MAP: 90±4 (baseline) vs 115±6 mmHg (indomethacin); LVP: 87±5 vs 105±5 mmHg). Indomethacin did not affect *dP/dt* (2200±232 vs 2220±204 mmHg min^{-1}), LVEDP (14.5±1.7 vs 15.0±1.6 mmHg), HR (124±2 vs 118±3 beats min^{-1}), CBFV (4.0±0.4 vs 4.9±0.5 kHz), or CVR (24±2 vs 25±2 kHz mmHg^{-1}).

In the presence of indomethacin both MAP and CVR were not significantly modified by B_1 and B_2 receptor agonists (Figure 3).

Discussion

Bradykinin and related kinins are potent vasodilator peptides (Regoli, 1984). Most evidence points to an involvement of the B_2 receptor in mediating the effects of kinins on circulatory control, although B_1 receptors, which are absent in normal conditions in most animal species, could also be involved in cardiovascular regulation in the dog (Staszewska-Wooley *et al.*, 1991; Nakhostine *et al.*, 1993).

While previous studies examining the effects of B_1 and B_2 receptor activation on haemodynamic parameters in the dog have been confined to anaesthetized animals (Staszewska-Wooley *et al.*, 1991; Nakhostine *et al.*, 1993) or isolated tissues (Rhaleb *et al.*, 1989; Yen & Lai, 1992), we chose instead to use chronically instrumented conscious dogs where the potential confounding influence of acute surgical trauma and of anaesthesia was absent. In addition, autonomic reflexes are known to modulate the effects of systemically administered vasodilators. Thus we examined the effects of des-Arg^9-BK and BK in the presence of ganglionic blockade to avoid the influence of cardiovascular reflexes on the measured circulatory and cardiac responses (Kiuchi *et al.*, 1993). Our results show that systemic arterial blood pressure, coronary vascular resistance and blood flow velocity along with cardiac contractility were not affected by ganglionic blockade. However, the heart rate was significantly increased by blockade of the autonomic nervous system as previously demonstrated in the conscious instrumented dog (Wynsen *et al.*, 1987).

In the present study we have shown that i.v. infusion of des-Arg^9-BK induced a significant reduction in mean arterial blood pressure (MAP) and coronary vascular resistance (CVR). Cardiac contractility, left ventricular and diastolic pressure and coronary blood flow velocity were not affected by des-Arg^9-BK infusion.

The effects of des-Arg^9-BK on MAP and CVR were antagonized by the kinin-B_1 receptor antagonist, des-Arg^9-[Leu^8]-BK, but not by the kinin-B_2 receptor antagonist, Hoe 140, suggesting that the vasodilator response to des-Arg^9-BK was mediated by the activation of kinin B_1 receptors. These data confirm previous results (Staszewska-Wooley *et al.*, 1991; Nakhostine *et al.*, 1993) showing the existence of a constitutive kinin B_1 receptor in the arterial vasculature of the dog.

Originally, B_1 receptor activation has been defined as producing a contractile response in the rabbit isolated aorta (Regoli *et al.*, 1977; Bouthillier *et al.*, 1987). On another hand, des-Arg^{9}BK when injected i.v. in rabbits pretreated with bacterial lipopolysaccharides, was shown to reduce arterial blood pressure (Regoli *et al.*, 1981; Bouthillier *et al.*, 1987). In isolated vessels, vasorelaxation mediated by B_1 receptor activa-

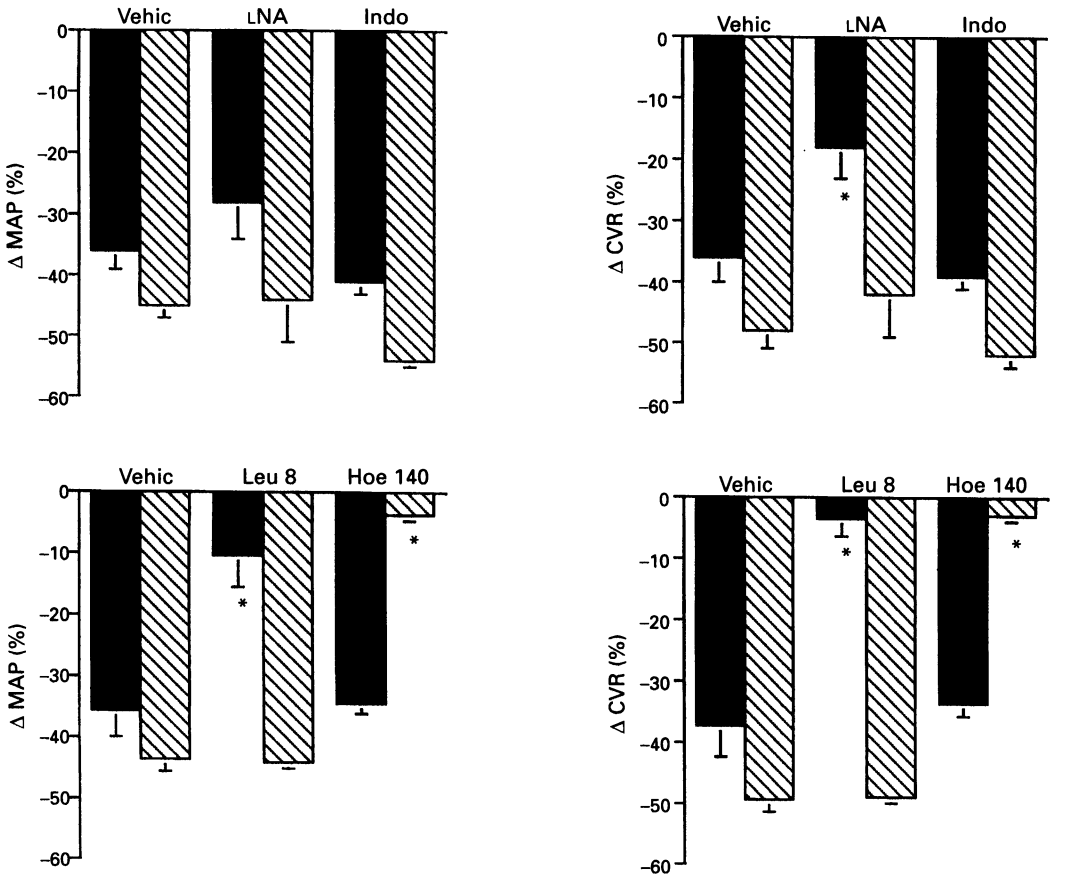

Figure 3 Effects of pretreatments with N$^{\omega}$-nitro-L-arginine (LNA: 45 mg kg^{-1}), indomethacin (Indo: 10 mg kg^{-1}), des-Arg^9-[Leu Arg^8]-BK (Leu 8: 25 μg kg^{-1}) and Hoe 140 (25 μg kg^{-1}), on the effects of i.v. infusion of des-Arg^9-BK (1 μg kg^{-1} min^{-1}, solid columns) or BK (1 μg kg^{-1} min^{-1}, hatched columns) on mean arterial pressure (MAP) and coronary vascular resistance (CVR). Values are means ± s.e.mean. *$P<0.05$, $n=6$.

tion has been described in the dog renal artery (Rhaleb *et al.*, 1989), rabbit mesenteric artery (deBlois & Marceau, 1987), rabbit carotid artery (Pruneau & Bélichard, 1993), pig coronary artery (Pruneau *et al.*, 1995c), bovine coronary artery (Drummond & Cocks, 1995a), and human coronary artery (Drummond & Cocks, 1995b). The mechanism underlying this vasorelaxation is different according to the species and to the vascular territory. Prostaglandins appear involved in the B_1 receptor-dependent vasorelaxation of the rabbit mesenteric artery (deBlois & Marceau, 1987) and of the dog mesenteric vein (Toda *et al.*, 1987), whereas NO seems to mediate the des-Arg^9-BK-induced vasorelaxation of the rabbit carotid artery (Pruneau & Bélichard, 1993), the porcine coronary artery (Pruneau *et al.*, 1995c) and the cat pulmonary vasculature *in vivo* (DeWitt *et al.*, 1994). NO and two further non-prostanoid factors contribute to endothelium-dependent relaxation to kinins in the bovine coronary artery (Drummond & Cocks, 1995a), whilst the mechanisms underlying B_1 receptor-induced relaxation in human coronary arteries are unknown (Drummond & Cocks, 1995b).

In our experimental conditions, the cyclo-oxygenase inhibitor, indomethacin, did not block the haemodynamic effects of des-Arg^9-BK whilst N$^{\omega}$-nitro-L-arginine inhibited only partially but significantly the reduction of coronary vascular resistance produced by des-Arg^9-BK. These data show that dilatation of coronary resistance arteries mediated by the B_1 receptor agonist is at least partly coupled to the release of nitric oxide.

As previously described in the anaesthetized dog (Nakhostine *et al.*, 1993), we observed that an i.v. infusion of des-Arg^9-[Leu^8]-BK induced a significant reduction of both MAP and CVR. This may suggest that the B_1 receptor antagonist possesses a partial agonist activity in the dog vasculature which however is absent in most of the B_1 receptor systems so far described (Marceau, 1995). Another explanation could be related to the recently described property of des-Arg^9-[Leu^8]-BK of antagonizing the effect of angiotensin II on AT_1 receptors (Pruneau *et al.*, 1995a). Such a property could explain the hypotensive response obtained with des-Arg^9-[Leu^8]-BK infusion, since the non-peptide angiotensin II antagonist, losartan, has been shown to induce a dose-dependent decrease in blood pressure when administered i.v. in conscious normotensive dogs (Wong *et al.*, 1991) and this would perhaps be augmented in the presence of ganglion-blockade (a condition where angiotensin II may support the circulation).

Our data also indicates that i.v. infusion of BK induced a significant decrease in systemic blood pressure and coronary vascular resistance which appeared to be specifically mediated by B_2 receptor activation since in contrast to Hoe 140, des-Arg^9-[Leu^8]-BK did not affect this response. In addition, BK had no inotropic effect since it did not influence the index of cardiac contractility and left ventricular end diastolic pressure. There was no change in heart rate in response to BK infusion. This is in contrast with results of Ribuot *et al.* (1993) who observed a direct negative chronotropic effect of BK in the dog. The difference between these results and ours could be explained by the fact that BK was infused directly into the sinus node artery in their experiment.

The mechanism by which BK induces vasodilatation depends on the tissue and the species (Cherry *et al.*, 1982). BK can induce the release of NO from the vascular endothelium of the rat (Berguer *et al.*, 1993) or human mesenteric artery (Cherry *et al.*, 1982) whilst it relaxes coronary arteries in the dog (Pelc *et al.*, 1991) or in the rabbit (Lamontagne *et al.*, 1992) through a PGI_2-dependent mechanism. In the present study, neither N$^{\omega}$-nitro-L-arginine nor indomethacin pretreat-

ment affected the BK-induced reduction of MAP. Moreover, in resistance coronary arteries, the dilator response to BK was not blocked by either N^{ω}-nitro-L-arginine or indomethacin as previously demonstrated in porcine small coronary arteries (Tschudi *et al.*, 1990). Thus, the vasodilatation mediated by BK was not dependent on NO or prostacyclin release in the dog. The recently described endothelium-derived hyperpolarizing factor (EDHF) (Beny & Brunet, 1988) which was shown to participate in the endothelium-dependent vasodilator response to BK in the human coronary artery (Nakashima *et al.*, 1993) could be responsible for the effects of BK. A vagally mediated component has also been shown to contribute to the dilator action of BK in coronary resistance vessels in the dog (Clozel *et al.*, 1985). However, this cannot explain the decrease in CVR induced by BK infusion as observed in our study since autonomic reflexes were blocked.

Limitation of the study

Although it is important to observe that our experiments were conducted in the absence of anaesthesia and of acute surgical trauma that could potentially trigger the induction of B_1 receptors, we are aware that in our experimental set up the presence of sterile foreign bodies (intraventricular pressure recorder, intraaortic catheter and Döppler flow probe) in the heart and vessels of the instrumented dogs could be the origin of the significant increase in leukocyte number and fibrinogen levels that, even if they are within normal limits (Kirk & Bistner, 1975), could reflect the presence of a moderate but chronic inflammatory state favouring the induction of B_1 receptors. On the other hand, we must point out results (not shown) of an acute experimental study done in our laboratory on one anaesthetized dog during which we measured the effects of a 2 min des-Arg^9-BK infusion (1 $\mu g\ kg^{-1}\ min^{-1}$) on MAP. This experiment was done within the limits of 1 h between induction of anaesthesia and infusion of des-Arg^9-BK, and showed that the systemic vasodepressor response to 1 $\mu g\ kg^{-1}\ min^{-1}$ des-Arg^9-BK was already present in these conditions (36% decrease in MAP), strongly suggesting the presence of constitutive B_1 receptors in the dog systemic vasculature.

In conclusion, the results of the present study suggest that activation of B_1 receptors could participate in the vasodilator action of kinins on the resistance vessels in the canine coronary and systemic circulation. This depressor response was reduced by N^{ω}-nitro-L-arginine in the coronary vasculature suggesting that it was mediated in part by the release of nitric oxide.

Stimulation of bradykinin-B_2 receptors resulted in more pronounced effects on MAP and CVR than those obtained with the activation of B_1 receptors. These systemic and coronary vascular responses were resistant to inhibitors of nitric oxide synthase and of cyclo-oxygenase, which implies that an endothelial mediator other than nitric oxide or prostacyclin might be involved.

The skilful technical assistance of Yves Jamait is gratefully acknowledged. We also wish to thank Dr Tom Cocks for helpful suggestions regarding the manuscript.

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(Received October 23, 1995
Revised November 28, 1995
Accepted December 1, 1995)