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Mediation by B_1 and B_2 receptors of vasodepressor responses to intravenously administered kinins in anaesthetized dogs

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1 Vasodepressor responses to intravenous (i.v.) injection of bradykinin (BK) and des-Arg^9-BK, a selective B_1 kinin receptor agonist, were characterized following i.v. pretreatment with selective B_1 ([Leu^8]-des-Arg^9-BK) and B_2 (Hoe 140) kinin receptor antagonists in anaesthetized dogs.

2 Des-Arg^9-BK (0.05–3.3 nmol kg^{-1}) produced dose-dependent decreases in mean arterial blood pressure with a ED_{50} 0.4 nmol kg^{-1}. The vasodepressor effects evoked by des-Arg^9-BK (0.6 nmol kg^{-1}) and BK (0.2 nmol kg^{-1}) were greater after i.v. and i.a. injections, respectively.

3 The vasodepressor response to BK (0.6 nmol kg^{-1}) but not to des-Arg^9-BK (0.6 nmol kg^{-1}) was significantly ($P<0.001$) blocked by pretreatment with the B_2 receptor antagonist, Hoe 140.

4 The vasodepressor response to des-Arg^9-BK (0.6 nmol kg^{-1}) but not to BK (0.6 nmol kg^{-1}) was significantly ($P<0.001$) reduced by pretreatment with the selective B_1 receptor antagonist, [Leu^8]-des-Arg^9-BK. Although both B_1 and B_2 receptor antagonists caused a transient fall in blood pressure, their inhibitory action was unlikely to be related to a desensitization mechanism.

5 Inhibition of prostaglandin synthesis with indomethacin prevented the vasodepressor response induced by arachidonic acid (1 mg kg^{-1}, i.v.) but not that to BK or des-Arg^9-BK (0.6 nmol kg^{-1}).

6 These results suggest, firstly, that the vasodepressor responses to i.v. BK and des-Arg^9-BK are mediated by the activation of B_2 and B_1 receptors, respectively; secondly, that prostaglandins are not involved in the vasodepressor responses to kinins. These findings provide pharmacological evidence for the existence of functionally active B_1 receptors in canine cardiovascular homeostasis.

Keywords: Bradykinin; des-Arg^9-BK; kinin receptors; kinin antagonists; blood pressure

Introduction

Bradykinin (BK) is a potent vasodilator the action of which appears to be mediated by the release of an endothelium-derived relaxing factor and/or prostacyclin, resulting from the activation of B_2 receptors on the vascular endothelium (Taylor *et al.*, 1989). Despite the very well known B_2 receptor-mediated vasodepressor effect of kinins (Regoli & Barabé, 1980; Bhoola *et al.*, 1992), the cardiovascular activity of the natural kininase I metabolite, des-Arg^9-BK, which is a selective B_1 receptor agonist, has been less studied. This fact is due in part to the belief that B_1 receptors are not expressed in normal tissues but are synthesized *de novo* during tissue incubation *in vitro* and as a result of inflammation or exposure of tissue to chemical noxious stimuli *in vivo* (Marceau *et al.*, 1983; Bouthillier *et al.*, 1987). For instance, des-Arg^9-BK had no significant effect on the cardiovascular system of rats and rabbits whereas exogenous des-Arg^9-BK lowered mean arterial blood pressure through the activation of peripheral vascular B_1 receptors in rabbits pre-injected intravenously 5 h earlier with a bacterial lipopolysaccharide (Regoli *et al.*, 1981; Marceau *et al.*, 1983; Bouthillier *et al.*, 1987). Nevertheless, under normal conditions, both B_1 and B_2 receptors appear to mediate the vasodilator action of kinins in the canine renal artery *in vitro* (Rhaleb *et al.*, 1989) as well as the vasorelaxation responsible for an increased renal blood flow in the dog *in vivo* (Lortie *et al.*, 1992). In the latter study, mediation of the natriuretic response to intrarenal infusion of BK was ascribed to a tubular B_1 receptor.

Selective antagonists for the B_1 and B_2 receptors are now available. The compound [Leu^8]-des-Arg^9-BK is considered as the prototype antagonist for the B_1 receptor (Regoli & Barabé, 1980) while Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7, Oic^8]BK) is the most potent, long-acting and selective B_2 receptor antagonist so far described *in vitro* and *in vivo* (Wirth *et al.*, 1991; Hock *et al.*, 1991; Bao *et al.*, 1991; Lembeck *et al.*, 1991; Rhaleb *et al.*, 1992).

The present study was designed to test the hypothesis that vascular B_1 and B_2 receptors can mediate the vasodepressor response to kinins in the dog under normal conditions. Thus, the selective antagonists of the B_1 ([Leu^8]-des-Arg^9-BK) and B_2 (Hoe 140) receptors were used to characterize kinin receptors that mediate the blood pressure effects of BK and des-Arg^9-BK in anaesthetized dogs. BK-induced vasorelaxation appears to be mediated by prostaglandins in arteries of some species (Barabé *et al.*, 1979; Taylor *et al.*, 1989). Moreover, unlike the endothelial B_2 receptor, canine muscular B_1 and B_2 receptors mediate relaxation of the arterial smooth muscle by promoting the release of prostaglandins (Rhaleb *et al.*, 1989). The second objective of the present study was therefore to examine the contribution of prostaglandins in the vasodepressor response to kinins. A preliminary account of this work has been published in an abstract form (Nakhostine *et al.*, 1992).

Methods

Surgical preparation of the animal

Adult mongrel dogs of either sex ($n=20$) weighing 17.5 ± 0.7 kg were anaesthetized with sodium thiopenone (25 mg kg^{-1}, i.v.) and alpha-chloralose (80 mg kg^{-1} followed by 15–20 mg kg^{-1} h^{-1}, i.v.). The animals were heparinized (200

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i.u. kg^{-1}, i.v.) and ventilated artificially with room air through an endotracheal tube by means of a Harvard pump (model 607). The right femoral artery was cannulated with a polyethylene catheter (PE-90) filled with physiological saline to measure the arterial blood pressure. The right and left femoral veins were also cannulated to enable the intravenous infusion or bolus injections of drugs. The data were recorded on a polygraph system (Nihon Kohden, model RM-6000) and the multi speed transmission Harvard apparatus infusion pump (model 940) was used for drug administration.

Care of the animals and haematological analysis

The research protocol and the care of the animals conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on animal care and approved by the ethical committee of University of Montreal for animal research.

All dogs were housed and maintained at a constant temperature of 20–22°C on a 12 h light/dark cycle (lights on 06 h 00 min–18 h 00 min) and provided with food and water *ad libitum*. Each animal underwent a veterinary medical examination before experimentation. Furthermore, a complete blood analysis was performed on three canine specimens to confirm that these animals were pathogen-free. Haematological analysis was made the day of their arrival at the animal house (initial values) and two weeks later just prior to experimentation. As shown in Table 1, the haematological values of white and red blood cells, haemoglobin, haematrocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets, sodium, potassium, calcium, glucose, urea, creatinine, alkaline phosphatase, arpartate aminotransferase (GOT) and total protein were all within normal limits.

Experimental protocol

The first series of experiments was designed to measure the i.v. effects of several increasing doses of des-Arg^9-BK (0.05 to 3.3 nmol kg^{-1}) and of three doses of BK (0.2, 0.6 and 2.2 nmol kg^{-1}) on mean arterial blood pressure (MAP). The two kinins were injected to the same animals in a volume of 3 ml followed by a 2 ml saline to wash out the cannula. The interval between injection was 10 min. In addition, the vasodepressor effects induced by BK (0.2 nmol kg^{-1}) and des-Arg^9-BK (0.6 nmol kg^{-1}) were compared after i.v. and i.a. injections.

The second series of experiments aimed to characterize the vasodepressor responses to BK and des-Arg^9-BK with the use of [Leu^8]-des-Arg^9-BK, a B_1 receptor selective antagonist and of Hoe 140, a B_2 receptor selective antagonist. BK or des-Arg^9-BK was injected at the same dose of 0.6 nmol kg^{-1}, (a) before and 3–5 min after the i.v. injection of [Leu^8]-des-Arg^9-BK (6.0 nmol kg^{-1} followed by an infusion of 0.6 nmol kg^{-1} min^{-1} for 5 min); (b) before and 10 min after the i.v. injection of Hoe 140 (4.5 nmol kg^{-1}) or (c) 5 min after the i.v. injection of 5 ml saline followed by an infusion of saline at 7.6 ml min^{-1} for 5 min as control group.

The third series of experiments was to examine the participation of prostaglandins in the BK and des-Arg^9-BK induced vasodepressor responses. Each agonist was tested at a dose of 0.6 nmol kg^{-1}, 1 h after the i.v. injection of either indomethacin (10 mg kg^{-1}) or the vehicle (Trizma base 0.2 M). To ascertain the effectiveness of this treatment, the vasodepressor effect of arachidonic acid (1 mg kg^{-1}) (precursor of prostaglandins) was measured in 2 dogs before and after indomethacin treatment.

Peptides and drugs

Des-Arg^9-BK was purchased from Hukabel Scientific Ltd. Montréal, Québec, Canada. BK, [Leu^8]-des-Arg^9-BK, indomethacin, Trizma base, arachidonic acid and heparin sodium salt were all purchased from Sigma Chemical Co., St-Louis, MO, U.S.A. Hoe 140 was made available from Hoechst AG (Frankfurt, Germany).

Stock solutions of peptides (1–10 mg ml^{-1}) were made in saline, divided into 100 µl aliquots and stored at −20°C until used. Indomethacin was prepared in Trizma base (0.2 M) just before use while arachidonic acid was prepared in 25% ethanol.

Statistical analysis

Values represent the mean ± s.e.mean of (n) animals. Statistical significance of differences between means were calculated with Student's t test for paired samples. Only probability values (P) smaller than 0.05 were considered to be statistically significant.

Table 1 Haematological values for 3 dogs

	Normal values[1]	*Initial values*	*Values before experimentation*
Leukocytes	6.0–18.5 × 10^9 l^{-1}	11.6 ± 0.7	12.7 ± 1.1
Erythrocytes	5.5–8.5 × 10^{12} l^{-1}	7.5 ± 0.9	7.2 ± 0.8
Haemoglobin	133–192 g l^{-1}	172.0 ± 15.6	164.0 ± 14.0
Haematocrit	36.8–54.4%	48.7 ± 4.3	47.2 ± 3.7
MCV	59.9–75.2 fl	65.8 ± 3.4	66.4 ± 3.4
MCH	21.5–27.2 pg	23.2 ± 1.1	23.0 ± 1.0
MCHC	336–383 g l^{-1}	353.0 ± 1.5	347.0 ± 3.2
Platelets	200–900 × 10^9 l^{-1}	375 ± 79	381 ± 59
Sodium	140–170 mmol l^{-1}	151.0 ± 2.5	150.1 ± 1.9
Potassium	3.5–6.7 mmol l^{-1}	4.3 ± 0.2	4.8 ± 0.3
Calcium	2.1–3.1 mmol l^{-1}	2.7 ± 0.04	3.7 ± 0.1
Glucose	3.0–6.5 mmol l^{-1}	5.2 ± 0.4	5.4 ± 0.5
Urea	3.6–9.0 mmol l^{-1}	8.6 ± 2.6	2.7 ± 0.1
Creatinine	85–175 µmol l^{-1}	110.1 ± 8.5	85.0 ± 9.6
Alkaline phosphatase	5–135 Ul^{-1}	33.0 ± 6.1	55.0 ± 18.0
GOT	17–58 Ul^{-1}	37.7 ± 1.8	41.7 ± 7.9
Total proteins	50–80 g l^{-1}	65.3 ± 2.2	59.3 ± 5.8

[1]Normal values are taken from Tvedten (1989).
Abbreviations: MCV, mean corpuscular volume, MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration, GOT, aspartate aminotransferase.

Results

Vasodepressor effects of des-Arg⁹-BK and BK

Baseline mean arterial pressure (MAP) prior to des-Arg⁹-BK or BK injections was 126 ± 5 mmHg ($n = 8$). Des-Arg⁹-BK produced dose-dependent decreases in MAP between 0.05 and 3.3 nmol kg^{-1} ($ED_{50} = 0.4$ nmol kg^{-1}); the maximal fall in MAP (− 41 ± 3 mmHg) was elicited at 2.2 nmol kg^{-1} (Figure 1). The depressor response to 0.6 nmol kg^{-1} des-Arg⁹-BK peaked at 27 ± 2 s post-injection and lasted for 3 to 4 min (Figure 2). The effect of 0.6 nmol kg^{-1} BK on MAP was 1.5 fold geater than that observed with the same dose of des-Arg⁹-BK (− 48 ± 2 mmHg vs − 30 ± 3 mmHg; $P <$ 0.001), while the time course effect was similar for both peptides (Figure 2). The dose of 2.2 nmol kg^{-1} BK produced a vasodepressor response (− 51 ± 5 mmHg, $n = 3$) which was similar to that induced by the dose of 0.6 nmol kg^{-1}. The vasodepressor response to 0.6 nmol kg^{-1} des-Arg⁹-BK was significantly ($P < 0.05$, $n = 6$) greater after i.v. injection than after i.a. administration. In contrast, the changes in MAP elicited by 0.2 nmol kg^{-1} BK was significantly ($P < 0.05$, $n = 6$) higher after i.a. administration (Figure 3). Thus, BK appeared more potent than des-Arg⁹-BK in reducing MAP whatever the route of administration.

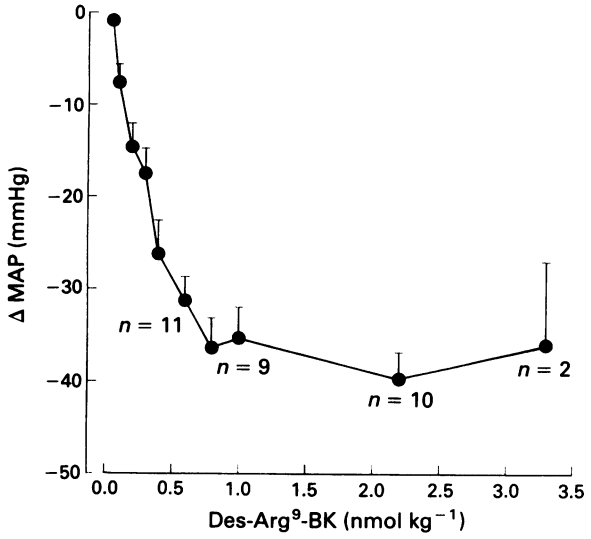

Figure 1 Dose-response curve showing the changes in mean arterial blood pressure (MAP) induced by the i.v. injection of des-Arg⁹-BK in anaesthetized dogs. Values are the mean ± s.e.mean of 8 dogs unless otherwise indicated by n.

Effect of [Leu⁸]-des-Arg⁹-BK, a selective B_1 receptor antagonist

Baseline MAP before [Leu⁸]-des-Arg⁹-BK infusion was 115 ± 4 mmHg ($n = 10$). [Leu⁸]-des-Arg⁹-BK injection (6.0 nmol kg^{-1}) induced a significant ($P < 0.001$, $n = 10$) decrease in MAP (− 38 ± 3 mmHg) which peaked at 27 ± 1 s and returned rapidly to pretreatment value (Figure 4). No tachyphylaxis was observed when des-Arg⁹-BK (0.6 nmol kg^{-1}) or [Leu⁸]-des-Arg⁹-BK (6.0 nmol kg^{-1}) were injected three times at intervals of 10 min (Figure 4). However, the vasodepressor effect of 0.6 nmol kg^{-1} des-Arg⁹-BK was significantly ($P <$ 0.001, $n = 10$) blocked 3–5 min after the i.v. injection of [Leu⁸]-des-Arg⁹-BK (6.0 nmol kg^{-1} plus 0.6 nmol kg^{-1} min^{-1} × 5 min) (Figures 4 and 5). In contrast, [Leu⁸]-des-Arg⁹-BK had no significant effect on the vasodepressor effect elicited by 0.6 nmol kg^{-1} BK (Figure 5).

Effect of Hoe 140, a selective B_2 receptor antagonist

Baseline MAP before Hoe 140 injection was 118 ± 5 mmHg ($n = 10$). The i.v. injection of 4.5 nmol kg^{-1} Hoe 140 significantly ($P < 0.001$, $n = 10$) decreased MAP (− 17 ± 3 mmHg), an effect which peaked at 34 ± 2 s and returned rapidly to pretreatment value (Figure 4). Although no tachyphylaxis occurred when 0.6 nmol kg^{-1} BK was injected three times at intervals of 10 min, the depressor response to 4.5 nmol kg^{-1} Hoe 140 was no longer present after the second and third injections. The decrease in MAP induced by 0.6 nmol kg^{-1} BK was significantly blocked ($P < 0.001$, $n = 10$) 10 min after the prior i.v. injection of 4.5 nmol kg^{-1} Hoe 140 (Figures 4, 6). In contrast, the vasodepressor response to 0.6 nmol kg^{-1} des-Arg⁹-BK remained unaffected by the pre-injection of Hoe 140 (Figure 6). The vasodepressor re-

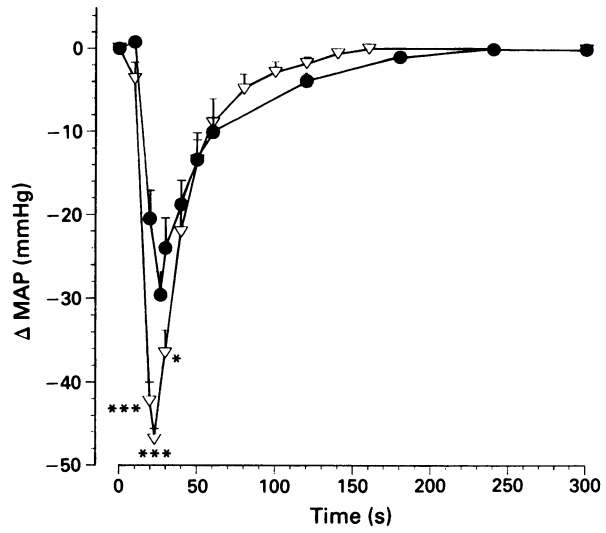

Figure 2 Time course of the changes in mean arterial blood pressure (MAP) induced by the i.v. injection of 0.6 nmol kg^{-1} of either bradykinin (BK) (▽) or des-Arg⁹-BK (●) in anaesthetized dogs. Values are the mean ± s.e.mean of 11 dogs. The statistical significance between bradykinin (BK) and des-Arg⁹-BK is indicated by $*P < 0.05$ and $***P < 0.001$.

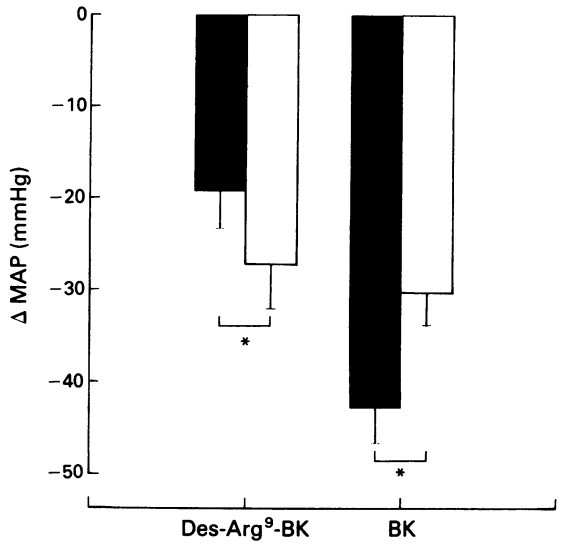

Figure 3 Maximal changes in mean arterial blood pressure (MAP) induced by the i.v. (open columns) or i.a. (solid columns) injection of 0.6 nmol kg^{-1} des-Arg⁹-BK or 0.2 nmol kg^{-1} bradykinin (BK) in anaesthetized dogs. Values are the mean ± s.e.mean of 6 dogs. The statistical significance between i.v. and i.a. values is indicated by $*P < 0.05$.

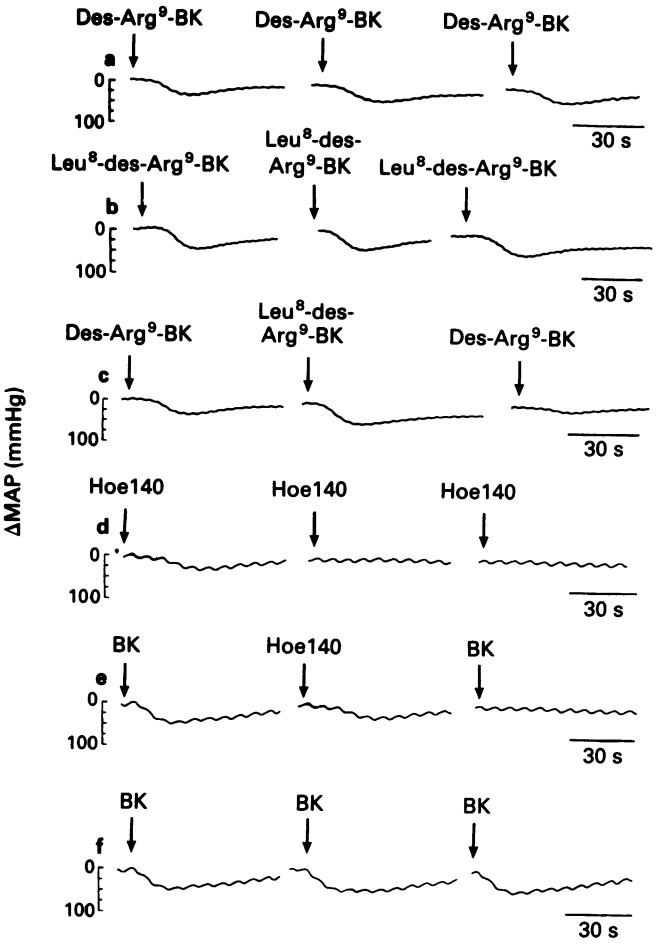

Figure 4 Example of traces showing the vasodepressor effects induced by three i.v. injections of 0.6 nmol kg^{-1} des-Arg^9-BK (a), 6.0 nmol kg^{-1} [Leu^8]-des-Arg^9-BK (b), 4.5 nmol kg^{-1} Hoe 140 (d) and 0.6 nmol kg^{-1} bradykinin (BK) (f). The vasodepressor effect of 0.6 nmol kg^{-1} des-Arg^9-BK is blocked 5 min after the prior i.v. infusion of [Leu^8]-des-Arg^9-BK (6.0 nmol kg^{-1} plus 0.6 nmol kg^{-1} min^{-1} × 5 min) (c). The vasodepressor effect of 0.6 nmol kg^{-1} BK is blocked 10 min after the prior i.v. injection of Hoe 140 (4.5 nmol kg^{-1}) (e). Each injection is separated by a period of 10 min. These experiments were conducted in dogs; haematological values are provided in Table 1.

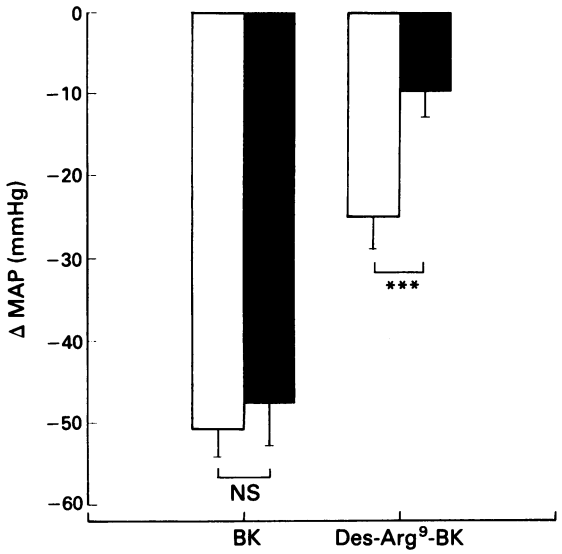

Figure 5 Effects of [Leu^8]-des-Arg^9-BK on the changes in mean arterial blood pressure (MAP) induced by the i.v. injection of 0.6 nmol kg^{-1} bradykinin (BK) or des-Arg^9-BK in anaesthetized dogs. Values are the mean ± s.e.mean of 5 (BK) and 10 (des-Arg^9-BK) dogs in the absence (open columns) and presence (solid columns) of the B_1 receptor antagonist (6.0 nmol kg^{-1} plus 0.6 nmol kg^{-1} min^{-1} for 5 min). Statistical significance is indicated by $***P<0.001$ and NS = not significant.

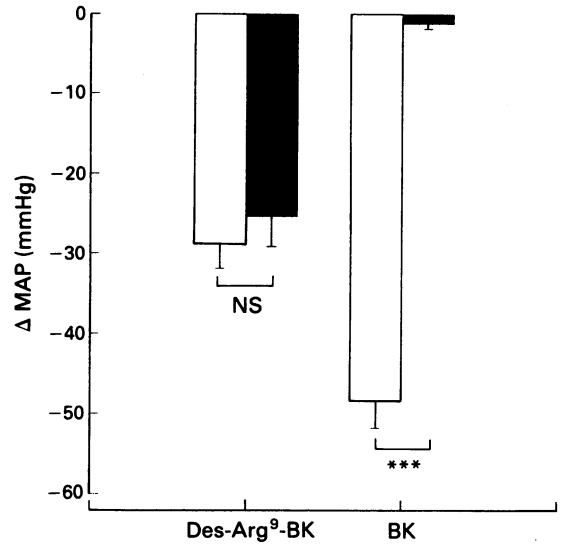

Figure 6 Effects of Hoe 140 on the changes in mean arterial blood pressure (MAP) induced by the i.v. injection of 0.6 nmol kg^{-1} bradykinin (BK) or des-Arg^9-BK in anaesthetized dogs. Values are the mean ± s.e.mean of 10 dogs in the absence (open columns) and presence (solid columns) of the B_2 receptor antagonist (4.5 nmol kg^{-1}). Statistical significance is indicated by $***P<0.001$ and NS = not significant.

sponse to BK was still impaired by 92%, 30 min after the injection of the antagonist (data not shown).

Effect of indomethacin on the vasodepressor responses to BK and des-Arg^9-BK

Baseline MAP before indomethacin treatment was 114 ± 4 mmHg (n = 10). The decrease in MAP to indomethacin injection (− 21 ± 4 mmHg) peaked at 21 ± 2 s and was stable at 109 ± 5 mmHg 1 h later, when BK and des-Arg^9-BK were injected. The indomethacin's vehicle (Trizma base) caused a similar fall in MAP (− 25 ± 3 mmHg) that peaked at 21 ± 1 s and the blood pressure returned to baseline within 5 min.

Indomethacin had no effect on vasodepressor responses to BK or des-Arg^9-BK. In contrast, the vasodepressor effect induced by the i.v. injection of arachidonic acid (1 mg kg^{-1}) was abolished in two animals pretreated with indomethacin (Figure 7).

Discussion

This study has shown that des-Arg^9-BK reduces systemic blood pressure in a dose-dependent manner in the dog. To our knowledge, this is the first species in which des-Arg^9-BK can lower blood pressure under non-pathological conditions. This effect has been well studied in pathological conditions such as following the injection of a sublethal dose of bacterial lipopolysaccharide (endotoxin) in the rabbit (Regoli *et al.*, 1981; Bouthillier *et al.*, 1987; Drapeau *et al.*, 1991b). The vasodepressor response to i.v. injection of BK was smaller than that observed after i.a. administration which is consistent with previous reports showing that 80–95% of the biological activity of BK is inactivated by kininase II during the first passage through the pulmonary circulation (Regoli & Barabé,

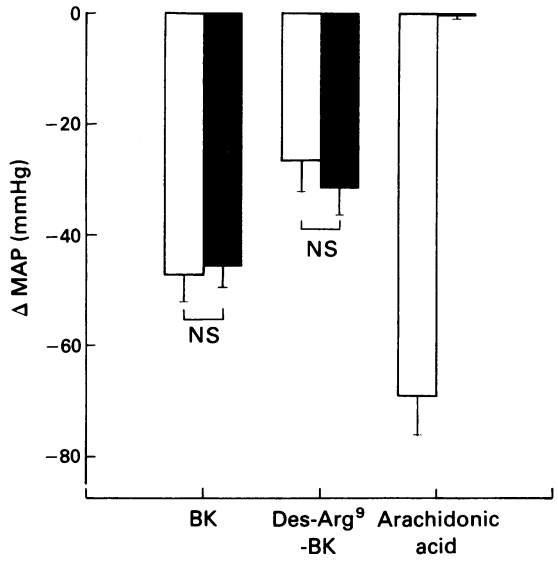

Figure 7 Effects of indomethacin on the changes in mean arterial blood pressure (MAP) induced by the i.v. injection of 0.6 nmol kg^{-1} bradykinin (BK) or des-Arg^9-BK and 1 mg kg^{-1} arachidonic acid in anaesthetized dogs. Values are the mean ± s.e.mean of 10 (kinins) or 2 (arachidonic acid) dogs in the absence (open columns) and presence (solid columns) of indomethacin (10 mg kg^{-1}, i.v. 1 h earlier). NS = not significant.

1980). However, the vasodepressor effect of des-Arg^9-BK described here was significantly greater after i.v. administration, suggesting a slower catabolism of this naturally occurring peptide through the pulmonary circulation compared to BK. This is in agreement with the recent observation that kininase II acts on des-Arg^9-BK at a slow rate and with a low affinity as compared to its action on BK (Drapeau *et al.*, 1991a,b). The higher potency of des-Arg^9-BK after i.v. administration has never been observed previously because in most species (e.g. rabbit, rat and guinea-pig), the selective B_1 receptor agonist has less than 1% of the activity of BK on systemic blood pressure (Regoli & Barabé, 1980; Bhoola *et al.*, 1992). The mechanism of the higher potency of des-Arg^9-BK after i.v. injection awaits elucidation but might be due to changes in the pulmonary circulatory resistance or to the release of an endogenous mediator from the pulmonary circulation which is probably not a prostaglandin.

Infusion of [Leu^8]-des-Arg^9-BK, a selective and competitive antagonist of the B_1 receptor (Regoli & Barabé, 1980), reduced significantly the vasodepressor response to des-Arg^9-BK but not to BK. In contrast, Hoe 140, a selective and potent antagonist of B_2 receptors that was found to inhibit BK-induced hypotensive responses in rats (Wirth *et al.*, 1991; Bao *et al.*, 1991), antagonized only the vasodepressor response to BK. These results suggest that the vasodepressor responses to BK and to des-Arg^9-BK in dogs are mediated by vascular B_2 and B_1 receptors, respectively. Until recently, it was believed that B_1 receptors are not present on vessels isolated from healthy animals. However, it has been demonstrated that the dog isolated renal artery contains a population of functional B_1 receptors involved in the vasodilator action of kinins (Rhaleb *et al.*, 1989). Furthermore, renal B_1 receptors were implicated in the action of BK on increased renal blood flow and sodium excretion in dogs (Lortie *et al.*, 1992).

The fact that [Leu^8]-des-Arg^9-BK and Hoe 140 caused a transient vasodepressor response would suggest that these antagonists maintain partial agonist activities on their respective receptors. In conscious dogs, i.v. administration of Hoe 140 (0.01 and 0.1 mg kg^{-1}) did not affect MAP or HR during a 15 min observation period when recorded indirectly from the tail artery (Wirth *et al.*, 1991). In our study, Hoe 140 ($\sim$6 μg kg^{-1}) caused only a transient decrease of MAP which may have escaped detection in an indirect recording system. It is of particular interest to note that [Leu^8]-des-Arg^9-BK and related selective antagonists at the B_1 receptor are unequivocally devoid of intrinsic activity in most of the reported *in vitro* and *in vivo* systems containing the B_1 receptor (Barabé *et al.*, 1979; Couture *et al.*, 1981; Marceau *et al.*, 1983; Drapeau *et al.*, 1991b). This finding suggests that the functional B_1 receptor described in the present *in vivo* model under non-pathological conditions is somewhat different from the inducible B_1 receptor which has been described under pathological conditions. Further studies are under way to define further the pharmacological profile of this so-called atypical B_1 receptor.

Under the experimental conditions, no tachyphylaxis occurred after repeated injections of the B_1 receptor agonist or antagonist, suggesting that the inhibitory effect of [Leu^8]-des-Arg^9-BK is not due to a desensitization mechanism but to pharmacological antagonism. Similarly, no tachyphylaxis occurred with BK while Hoe 140 produced a small vasodepressor response which could not be reproduced at the second and third injection. Since the direct effect of Hoe 140 on blood pressure is much smaller than that evoked by BK, the blockade of the BK response by Hoe 140 is probably related to a pharmacological antagonism as reported earlier (Wirth *et al.*, 1991; Hock *et al.*, 1991; Lembeck *et al.*, 1991). Hoe 140 did not alter the B_1 receptor response evoked by des-Arg^9-BK in our paradigm and thus appears specific for B_2 receptors.

The finding that [Leu^8]-des-Arg^9-BK and Hoe 140, at doses sufficient to block exogenous des-Arg^9-BK and BK, did not cause increases in baseline arterial blood pressure casts some doubt concerning the contribution of endogenous kinins to blood pressure maintenance in normotensive dogs. One cannot exclude, however, the possibility that kinins might control vascular resistance and blood flow in specific vascular territories or interact with other vasoregulatory mechanisms such as the renin-angiotensin system (Van Den Buuse & Kerkhoff, 1991).

In order to evaluate the role of prostanoids in the peripheral action of BK and des-Arg^9-BK, we investigated the effect of indomethacin, a cyclo-oxygenase inhibitor. The vasodepressor effect of arachidonate, the natural precursor of prostaglandin synthesis, was totally inhibited in the presence of indomethacin, thus confirming the effectiveness of indomethacin in preventing formation of prostaglandins. The action of both BK and des-Arg^9-BK on the systemic circulation was not altered by indomethacin which rules out the participation of prostglandins in this response. This supports the belief that the involvement of prostacyclin/prostaglandins in the vasodilator action of BK is tissue- and species-dependent (Taylor *et al.*, 1989).

In conclusion, des-Arg^9-BK, the naturally occurring bradykinin metabolite, and BK administered intravenously are potent hypotensive peptides in dogs; their action appears to be mediated by peripheral B_1 and B_2 receptors, respectively.

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