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Bradykinin-induced contractions of canine saphenous veins: mediation by B_2 receptors and involvement of eicosanoids

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1 Experiments were designed to determine the subtype of kinin-receptors mediating the contraction of venous smooth muscle to bradykinin and to investigate the involvement of metabolites of arachidonic acid in this response.

2 Bradykinin (10^{-9} to 10^{-6} M) caused concentration-dependent contractions of the canine isolated saphenous vein without endothelium, which were potentiated by indomethacin (10^{-5} M, an inhibitor of cyclo-oxygenase). The concentration-response curve was biphasic, reaching an asymptote at 10^{-8} M and a secondary maximal response at 10^{-6} M.

3 Bradykinin (10^{-8} M to 3×10^{-6} M) caused a three fold stimulation in the release of the vasodilator prostaglandin E_2 (PGE_2) and a two fold stimulation of that of the vasodilator prostacyclin, measured by the production of 6-keto-$PGF_{1\alpha}$ (its stable breakdown product).

4 Under control conditions, nordihydroguaiaretic acid (NDGA, 10^{-5} M), an inhibitor of lipoxygenase, did not affect the response to bradykinin. In the presence of indomethacin (10^{-5} M), NDGA reduced contractions to bradykinin, suggesting the involvement of lipoxygenase metabolites in the potentiation evoked by the inhibitor of cyclo-oxygenase.

5 The selective B_1 receptor agonist [des-Arg^9]-bradykinin, in the concentration-range 10^{-6} to 10^{-5} M, induced contractions, which were abolished by the B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140, 10^{-6} M). The selective B_1 receptor antagonist [des-Arg^9,Leu^8]-bradykinin, (10^{-7} to 10^{-5} M) had no significant effect on bradykinin-induced contractions.

6 The B_2 receptor antagonists Hoe 140 (10^{-8} to 10^{-6} M) and D-Arg[Hyp^3,D-Phe^7]-bradykinin (10^{-7} to 10^{-5} M) shifted the concentration-response curve to bradykinin to the right in a concentration-dependent manner.

7 These results indicate that, in the canine saphenous vein, bradykinin causes contraction by activating B_2 receptors. This results in the production of metabolites of arachidonic acid, which play a key role in the contraction of canine saphenous venous smooth muscle.

Keywords: Bradykinin; canine saphenous vein; bradykinin receptor; cyclo-oxygenase; lipoxygenase

Introduction

Bradykinin is an autacoid released during trauma, pain and inflammatory reactions (Regoli & Barabé, 1980). In conscious dogs, bradykinin evokes a concentration-dependent reduction of the compliance of the saphenous vein (Müller-Schweinitzer, 1988). In most isolated veins, bradykinin causes contractions (Goldberg *et al*., 1976; Tsuru *et al*., 1976). The mechanisms underlying these contractions depend on the tissues and the species studied. In several preparations, the cyclo-oxygenase products prostaglandin H_2, thromboxane A_2 (Aksoy *et al*., 1990; Campos & Calixto, 1994) and prostaglandin $F_{2\alpha}$ (Limas, 1977; Wong *et al*., 1977) mediate the contraction evoked by the peptide. In the canine isolated saphenous vein, contractions induced by bradykinin are potentiated by an inhibitor of cyclo-oxygenase (Goldberg *et al*., 1976). In vascular smooth muscle, two subclasses of kinin-receptors, B_1 and B_2, are present (Regoli & Barabé, 1980; Burch & Kyle, 1992). The B_1 receptors are restricted to some vascular beds, and are particularly prominent in isolated vascular tissues of the rabbit. They exhibit a higher affinity for the kinase I metabolite [des-Arg^9]-bradykinin and are antagonized by the selective receptor antagonist [des-Arg^9,Leu^8]-bradykinin (Regoli *et al*., 1992). In contrast, B_2 receptors are widely distributed and possess a higher affinity for bradykinin than the B_1 subtype. The existence of isoforms of the B_2 subtype in different species is likely (Regoli *et al*., 1992).

The present study was designed to determine the receptor subtypes involved in the contraction evoked by bradykinin in the canine saphenous vein and to investigate the possible involvement of metabolites of arachidonic acid in this preparation.

Methods

Lateral saphenous veins were taken from male mongrel dogs (15–30 kg) anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, intravenously). Female dogs were not studied because of the poor responsiveness of their saphenous veins to bradykinin (unpublished observations). Immediately after excision, the tissues were placed into ice-cold modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, ethylenediamine tetraacetate 0.026 and glucose 11.1 (control solution). The veins were cleaned of adherent connective tissue and cut into rings (4–5 mm in length). Since bradykinin can stimulate the release of endothelium-derived relaxing factors both in arteries and veins (Cherry *et al*., 1982; Pawloski & Chapnick, 1991; Mombouli *et al*., 1992), experiments were performed after removal of the endothelium, acheived by gently rubbing the intimal surface with the tip of a small forceps.

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Isometric force

The rings were suspended between two stirrups in organ chambers (25 ml) filled with control solution (aerated with 95% O_2/5% CO_2, maintained at 37°C, pH 7.4). One of the stirrups was anchored to the organ chamber, and the other connected to a force transducer (Gould CT 2) to record changes in isometric force. The rings were stretched to the optimal point of their length-active tension relation, as determined by the contractile response to 60 mM KCl at progressive levels of stretch. The absence of endothelium was checked by the lack of relaxation to thrombin (up to 1 u/ml^{-1}) during contractions to prostaglandin $F_{2\alpha}$ (10^{-7} M). Preparations were used only when no relaxation to thrombin was observed.

Release of prostaglandin E_2 and prostacyclin

Saphenous vein rings, without endothelium, were placed in glass tubes containing 2 ml of control solution (oxygenated with 95% O_2/5% CO_2) and incubated for 30 min at 37°C. The incubation buffer was then replaced with 2 ml of fresh buffer containing perindoprilat (angiotensin-converting enzyme (E.C. 3.4.15.11) inhibitor, 5×10^{-6} M), Plummer's inhibitor (mergepta, MGTA, carboxypeptidase (including kinase I) inhibitor, 10^{-5} M) and, where specified, Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, 10^{-6} M). After an additional 45 min, the buffer was replaced once more with fresh solution containing the same drugs and increasing concentrations of bradykinin. After 30 min, the samples were freeze-dried and reconstituted in assay buffer for the measurement of prostaglandins (Gao & Vanhoutte, 1992). Prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ (the stable breakdown product of prostacyclin) were measured by use of radioimmunoassay kits (Amersham Corporation, Arlington Heights, IL, U.S.A.).

Experimental protocols

Only one concentration-contraction curve was obtained in each preparation. When receptor antagonists were used, the preparations were incubated for 45 min with the drugs, which remained in the bath solution during exposure of the tissue to the agonists. Experiments were performed in parallel, one ring serving as control, the others being exposed to increasing concentrations (one per ring) of antagonists. The incubation time for indomethacin (inhibitor of cyclo-oxygenase, 10^{-5} M), NDGA and BayG6575 (inhibitors of lipoxygenase, 10^{-5} M) was at least 45 min; these drugs remained in the solution throughout the experiments. To prevent the breakdown of bradykinin, the rings were treated with the angiotensin-converting enzyme (E.C 3.4.15.11) inhibitor perindoprilat (5×10^{-6} M), and with the carboxypeptidase (including kininase I) inhibitor MGTA (10^{-5} M) for 45 min before the experiment was started (Mombouli *et al.*, 1992).

Drugs and chemicals

The following drugs were used: bradykinin, [des-Arg^9]-bradykinin, [des-Arg^9,Leu^8]-bradykinin, D-Arg[Hyp^3,D-Phe^7]-bradykinin, nordihydroguaiaretic acid (NDGA), indomethacin (all from Sigma Chemical Co., St. Louis, MO, U.S.A.); perindoprilat (Servier, Neuilly sur Seine, France), Plummer's inhibitor (mergepta, MGTA, DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid, Calbiochem, La Jolla, CA, U.S.A.), Hoe 140 (Hoescht, Frankfurt, Germany), BayG6575 (nafazatrom, pyrazol-3-one,2,4-dihydro-5-methyl-2-[2-(2-naphthalenyloxy)ethyl]-(9Cl), Miles Pharmaceuticals, West Haven, CT, U.S.A.). Drugs were prepared in water except for indomethacin (dissolved in water and Na_2CO_3), NDGA and BayG6575 (dissolved in dimethylsulphoxide, Sigma).

Calculations and statistical analysis

Results are expressed either in absolute values (g) or as % of the maximal contraction induced by 60 mM KCl. The release of prostaglandins is expressed as picograms per milligram tissue. In each experimental group, n refers to the number of animals from which blood vessels were taken. Results are expressed as mean ± s.e.mean. The negative logarithm of the concentration of bradykinin causing half-maximal response is referred to as ED_{50}. Determination of the potency of kinin receptor antagonists (pA_2) was by the method of Arunlakshana and Schild (1959). Statistical comparisons were performed by means of a two-ways Analysis of Variance or Student's t test for paired and unpaired observations. Differences were considered to be statistically significant when $P<0.05$.

Results

Isometric force

Bradykinin (10^{-9} to 10^{-6} M) caused concentration-dependent contractions of the canine saphenous veins. The log concentration-response curve was biphasic, reaching a first asymptote at 10^{-8} M, and a secondary maximal response at 10^{-6} M (3.62 ± 0.71 g; Figure 1). The first phase of the log concentration-response curve was shifted significantly leftward and upward by indomethacin (10^{-5} M, Figure 1). In the presence of the inhibitor of cyclo-oxygenase, the maximal contraction evoked by bradykinin was significantly greater than in control solution (6.36 ± 1.01 *g*; Figure 1; $P<0.05$).

The inhibitor of lipoxygenase, NDGA (10^{-5} M) did not significantly affect the log concentration-contraction response curve to bradykinin (10^{-10} to 3×10^{-7} M) under control conditions (Figure 2a). In the presence of indomethacin (10^{-5} M), NDGA significantly reduced the response to the peptide (Figure 2b). Similar results were obtained with another inhibitor of lipoxygenase, BayG6575 (10^{-5} M, $n=4$; data not shown).

The selective B_1 receptor agonist, [des-Arg^9]-bradykinin (3×10^{-9} to 10^{-5} M) did not cause significant increases in tension under control conditions. In the presence of indomethacin (10^{-5} M), the peptide (10^{-6} to 10^{-5} M), caused concentration-dependent contractions (maximal contraction 2.1 ± 0.67 g; Figure 3, $n=5$) which were abolished by the B_2 receptor antagonist Hoe 140 (10^{-6} M; Figure 3).

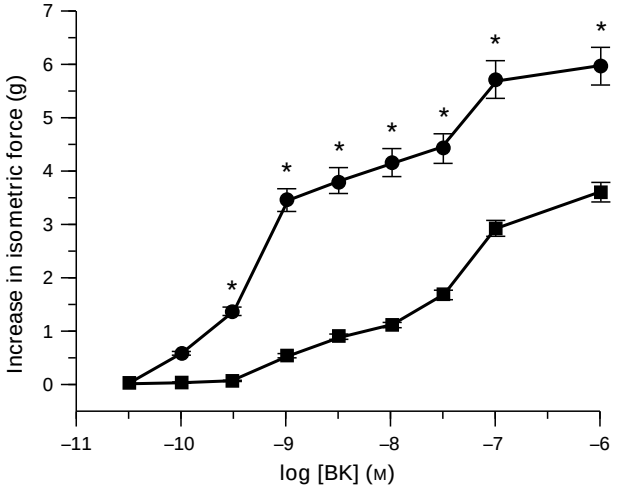

Figure 1 Log concentration-response curve to bradykinin (BK) under control condition (■) and in the presence of indomethacin (10^{-5} M, ●). Data are expressed in absolute values (g) and are shown as means with vertical lines indicating s.e.mean ($n=7$). The asterisks denote significant differences between control- and indomethacin-treated rings ($P<0.05$).

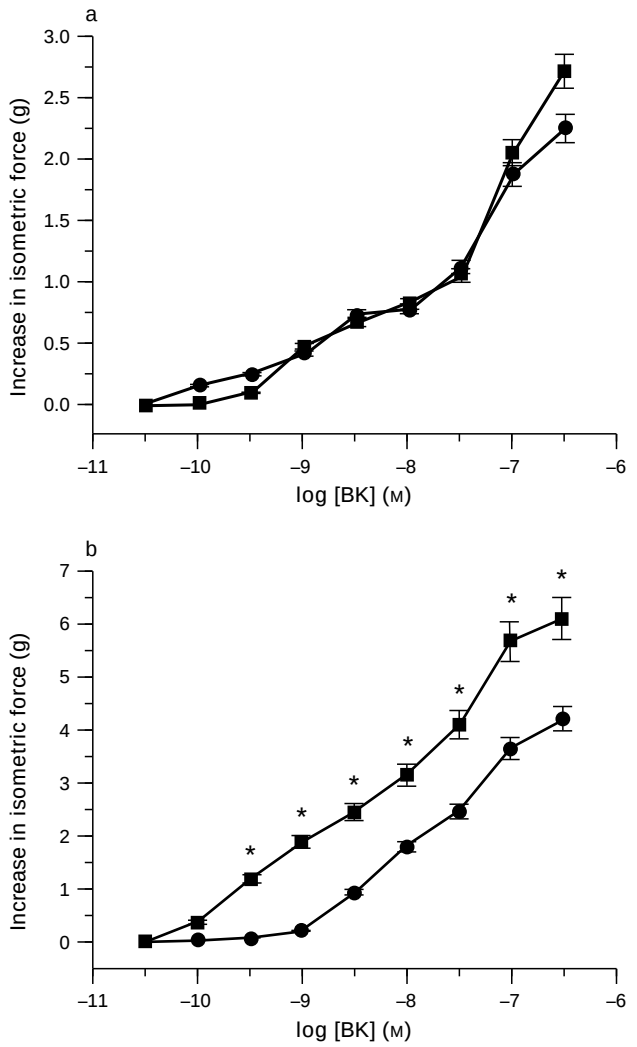

Figure 2 (a) Log concentration-response curve to bradykinin (BK) under control conditions (■) and in the presence of NDGA (10^{-5} M, ●). (b) Log concentration-response curve to bradykinin in the presence of indomethacin (10^{-5} M, ■) and in the presence of indomethacin plus NDGA (10^{-5} M, ●). Data are expressed in absolute values (g) and are shown as means with vertical lines indicating s.e.mean ($n=4$). The asterisks denote significant differences between indomethacin- and indomethacin plus NDGA-treated rings ($P<0.05$).

In rings incubated with indomethacin (10^{-5} M), the concentration-dependent contractions evoked by bradykinin (3×10^{-10} to 10^{-6} M) were not affected significantly by the selective B_1 receptor antagonist [des-Arg^9,Leu^8]-bradykinin (Figure 4a); the latter also did not significantly affect contractions to [des-Arg^9]-bradykinin (data not shown). The selective B_1 receptor antagonists D-Arg[Hyp^3,D-Phe^7]-bradykinin (Figure 4b) and Hoe 140 (Figure 4c) caused a shift to the right of the concentration-response curve to bradykinin. In the presence of the selective B_2 receptor antagonists, the concentration-response curve to bradykinin became apparently monophasic (Figure 4). The Schild plots derived from the data shown in Figure 4 yielded slopes not different from unity [correlation coefficients of 0.94 (s.d. = 0.29) and 0.99 (s.d. = 0.03) and pA_2 values of 7.0 and 8.25 for D-Arg[Hyp^3,D-Phe^7]-bradykinin and Hoe 140, respectively].

Release of prostanoids

Bradykinin (10^{-8} to 3×10^{-6} M) caused a concentration-dependent increase in the release of both prostaglandin E_2 (Figure 5a) and 6-keto-prostaglandin $F_{1\alpha}$ (Figure 5b). The increase in the release of these prostanoids was abolished by Hoe 140 (10^{-6} M).

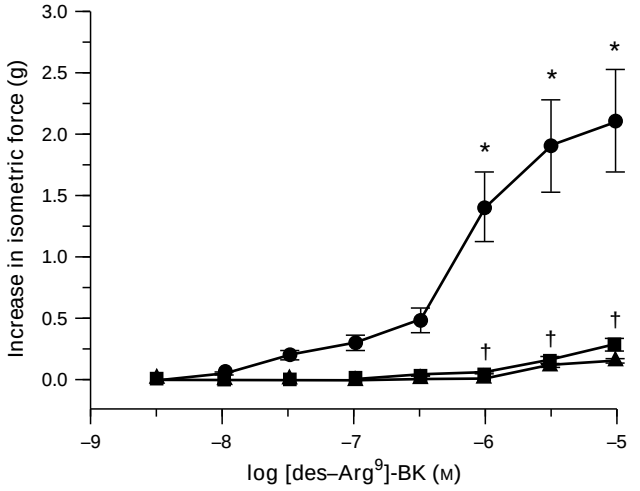

Figure 3 Log concentration response curve to [des-Arg^9]-bradykinin under control conditions (■), in the presence of indomethacin (10^{-5} M, ●) and in the presence of indomethacin plus Hoe 140 (10^{-6} M, ▲). Data are expressed in absolute values (g) and are shown as means with vertical lines indicating s.e.means ($n=5$). The asterisks denote significant differences between control- and indomethacin-treated rings ($P<0.05$). The daggers denote significant differences between indomethacin- and indomethacin plus Hoe 140-treated rings ($P<0.05$).

Discussion

The present study demonstrates that bradykinin causes a concentration-dependent contraction of the canine isolated saphenous vein. In several venous preparations, bradykinin-induced contractions are associated with the production of metabolites of arachidonic acid generated through the cyclo-oxygenase pathway. Thus, inhibitors of cyclo-oxygenase reduce the contraction caused by bradykinin in human foetal placental veins (Tulenko, 1981) and in bovine mesenteric veins (Wong *et al*., 1977). Prostaglandin H_2 and thromboxane A_2 mediate the contraction evoked by the peptide in several preparations (Aksoy *et al*., 1990; Campos & Calixto, 1994). In mesenteric veins from certain species, the contraction evoked by bradykinin depends on the production of prostaglandin $F_{2\alpha}$ (Wong *et al*., 1977; Limas, 1977). The present findings demonstrate that in canine saphenous veins, inhibitors of cyclo-oxygenase potentiate the contraction induced by the peptide, indicating that endogenous prostanoids do not mediate but rather antagonize the contraction evoked by bradykinin. These could include prostaglandin E_2 and prostacyclin which are the major products of cyclo-oxygenase that induce relaxation of vascular smooth muscle. The present study focused on the effects of bradykinin on smooth muscle, hence the endothelium was removed. Indeed, the endothelium is a source of vasodilator mediators, including prostacyclin (Moncada *et al*., 1976), endothelium-derived relaxing factor (which is either nitric oxide or a nitroso compound; Palmer *et al*., 1987; Myers *et al*., 1989) and an endothelium-derived hyperpolarizing factor (Nagao & Vanhoutte, 1992). This study demonstrates that, in the canine isolated saphenous vein, bradykinin induces the release of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin. During contractions of the same preparations, bradykinin evoked an endothelium-independent, indomethacin-sensitive relaxation; the peptide also induces the production of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ by cultured smooth muscle cells of the canine

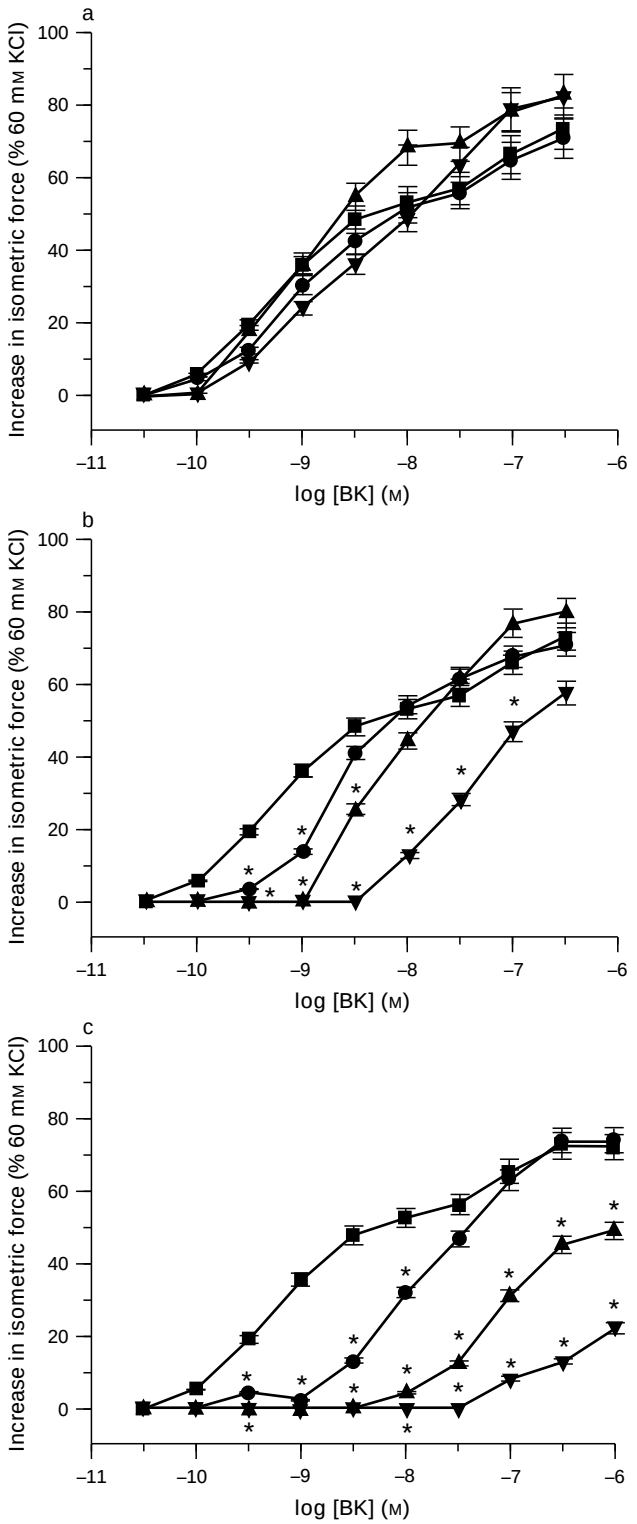

Figure 4 (a) Log concentration-response curve to bradykinin in the presence of increasing concentrations of the B_1 receptor antagonist [des-Arg^9,Leu^8]-bradykinin. Antagonist concentrations: (●) 10^{-7} M; (▲) 10^{-6} M; (▼) 10^{-5} M. (b) Concentration-response curve to bradykinin in the presence of increasing concentrations of the B_2 receptor antagonist D-Arg[Hyp^3,D-Phe^7]-bradykinin. Antagonist concentrations: (●) 10^{-7} M; (▲) 10^{-6} M; (▼) 10^{-5} M. (c) Log concentration-response curve to bradykinin in the presence of increasing concentrations of the B_2 receptor antagonist Hoe 140. Antagonist concentrations: (●) 10^{-8} M; (▲) 10^{-7} M; (▼) 10^{-6} M. All experiments were performed in the presence of indomethacin 10^{-5} M. Data are expressed as percentage of the maximal response to KCl (60 mM) and are shown as means with vertical lines indicating s.e.mean ($n=5$). The asterisks denote significant differences from control ($P<0.05$). (■) Control curve to BK in the absence of antagonists.

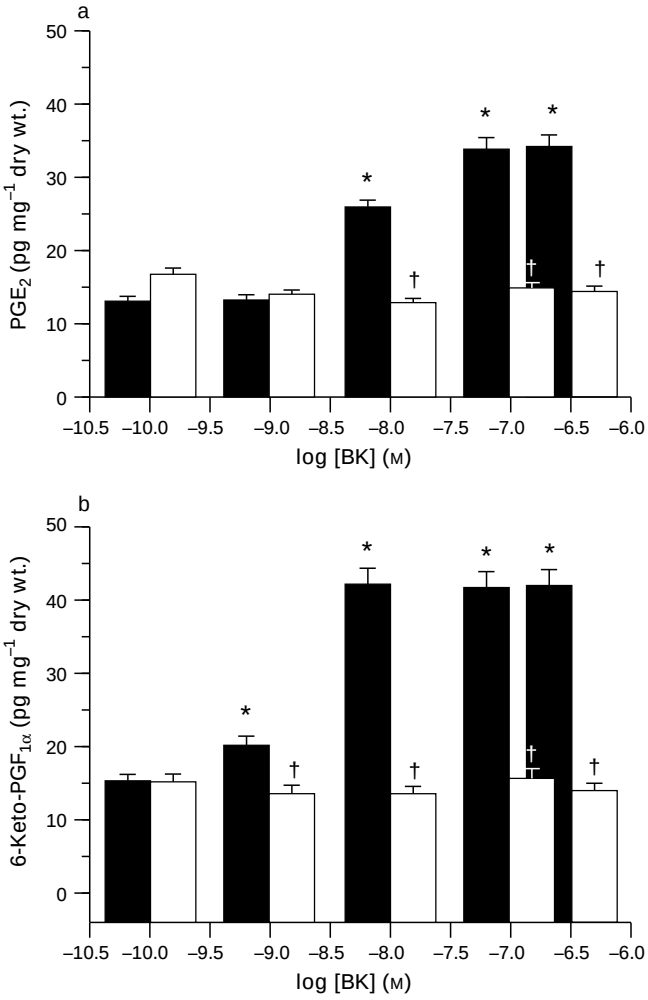

Figure 5 (a) Prostaglandin E_2 (PGE_2) levels in canine saphenous veins without endothelium after stimulation by increasing concentrations of bradykinin. The experiments were performed either in control conditions (solid columns) or in the presence of Hoe 140 (10^{-6} M, stippled columns). (b) 6-Keto-prostaglandin $F_{1\alpha}$ levels in canine saphenous veins without endothelium after stimulation by increasing concentrations of bradykinin. The experiments were performed either in control conditions (solid columns) or in the presence of Hoe 140 (10^{-6} M, stippled columns). Data are expressed relative to the dry weight of the tissues and represent means ± s.e.mean of 3 independent experiments. The asterisks denote significant differences from basal level ($P<0.05$). The daggers denote significant differences between control and Hoe 140-treated preparations ($P<0.05$).

saphenous vein (unpublished observations). Taken together, these data suggest that the cyclo-oxygenase products released by bradykinin originate in the venous smooth muscle and antagonize the contraction evoked by the peptide.

Under control conditions, the inhibitor of lipoxygenase NDGA does not affect the bradykinin-evoked contraction. However, potentiation of responses to bradykinin by indomethacin are not observed in the presence of NDGA. Similar findings have been obtained in the canine saphenous vein for acetylcholine and noradrenaline (Rimele & Vanhoutte, 1983). These results could be explained if inhibition of cyclo-oxygenase results in the diversion of arachidonic acid to the lipoxygenase pathway with the production of vasoconstrictor metabolites. For instance, the 15-lipoxygenase metabolite of arachidonic acid, 15-hydroperoxyeicosatetroenoic acid (15-HPETE) and its hydroxyderivative (15-HETE) can evoke contraction of canine isolated saphenous veins (Van Diest *et al*., 1986; 1991; 1993). Thus, the potentiation of the response to bradykinin in the presence of indomethacin involves two

components: (a) inhibition of the release of vasodilator prostanoids; and (b) production of lipoxygenase compounds that facilitate the direct contraction caused by the peptide. Moreover, since contractions were observed in the presence of both indomethacin and NDGA, the contractile response of the canine isolated saphenous vein to bradykinin is mediated in part by a pathway independent of the metabolism of eicosanoids. This conclusion is supported by the finding that in cultured smooth muscle of the canine saphenous vein, bradykinin increases in a concentration-dependent manner the production of myo-inositol triphosphates (unpublished data).

Bradykinin receptors have been subdivided mainly into B_1 and B_2 subtypes (Regoli & Barabe, 1980; Burch & Kyle, 1992), although a B_3 receptor subtype has been proposed (Farmer *et al*., 1989). In the present study, the log concentration-response curve to bradykinin was biphasic, suggesting the possible involvement of two different mechanisms or receptor subtypes. Taking into consideration the affinity of bradykinin for B_1 and B_2 receptor subtypes, the first phase could theoretically be mediated by the B_2, and the second one by the B_1 receptor subtype. The observation that the B_1-selective agonist [des-Arg^9]-bradykinin induces, at high concentrations, contractions in the presence of indomethacin tends to reinforce the interpretation that B_2 receptors mediate the first and B_1 receptors the second phase of the contraction. However, the latter is sensitive to Hoe 140, is not prevented by the B_1 receptor antagonist [des-Arg^9,Leu^8]-bradykinin, and therefore is probably mediated by a B_2 receptor subtype. Since [des-Arg^9]-bradykinin causes contractions which are significantly smaller than those obtained with bradykinin, it must be a weak B_2 agonist. No differences were observed in the contraction to bradykinin under basal conditions and in the presence of the B_1 receptor antagonist. By contrast, the concentration-response curve to bradykinin was shifted to the right by Hoe 140 and the B_2 receptor antagonist of the [D-Phe^7]-bradykinin series. The pA_2 values obtained from the Schild plot analysis are consistent with the expression of B_2 receptors. Since the B_1 receptor subtype can be induced in a large variety of tissues (Regoli *et al*., 1981; Bouthillier *et al*., 1987; Campos & Calixto, 1994), the concentration-response curve to [des-Arg^9]-bradykinin was repeated 6 h after the preparations had been set up. No differences were observed (data not shown). The two phases of the contraction could represent the presence of two isoforms of B_2 receptors. Indeed, data obtained in isolated blood vessels indicate the existence of different B_2 receptor subtypes not only in different species but also within the same preparation (Regoli *et al*., 1994; Féletou *et al*., 1994). The putative B_2 receptor subtypes present in the canine saphenous vein may exhibit a different sensitivity to the antagonists tested.

In conscious dogs, the local infusion of bradykinin into the saphenous vein evokes a concentration-dependent reduction in compliance, indicating venoconstriction. This response is abolished by the intravenous administration of the thromboxane A_2 synthesis inhibitor dazoxiben and reduced by the competitive B_1 receptor antagonist [des-Arg^9,Leu^8]-bradykinin (Müller-Schweinitzer, 1988). In isolated veins, bradykinin evokes concentration-dependent contractions that are mediated mainly through the formation of vasoconstrictor leukotrienes (Müller-Schweinitzer, 1989). By contrast to the present experiments, these studies were performed on rings with endothelium. The present study did not investigate the potential release of endothelium-derived contracting factors such as endoperoxides, thromboxane A_2 or leukotrienes by bradykinin.

Although bradykinin causes vasodilatation at the precapillary level, it is a venoconstrictor. The present study demonstrates that bradykinin causes contraction by activating B_2 receptors. This contraction was curtailed by the enhanced production by the smooth muscle of dilator prostanoids, particularly prostaglandin E_2 and prostacyclin.

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