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Characterisation of bradykinin receptors from juvenile pig coronary artery

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Abstract

Coronary artery rings from juvenile male farm pigs were incubated for 6 h and precontracted with U46619. The rings relaxed in response to des-Arg^9-bradykinin (pD_2, 7.78 ± 0.13; E_{max}, $87.4 \pm 4.3\%$) and to bradykinin (pD_2, 8.69 ± 0.30; E_{max}, $104.2 \pm 4.4\%$). These responses were abolished by endothelium removal and unaffected by indomethacin whilst N^G-nitro-L-arginine reduced the relaxation due to des-Arg^9-bradykinin only. Preincubation with cycloheximide or actinomycin had no effect against relaxations mediated by kinins whilst the protein trafficking inhibitor, brefeldin A, reduced by 52% the maximum response to des-Arg^9-bradykinin. The bradykinin receptor antagonists, des-Arg^9-[Leu^8]bradykinin, Hoe 140 (D-Arg-[Hyp^3, Thi^5, D-Tic^7,Oic^8]bradykinin) and NPC 567 (D-Arg-[Hyp^3,D-Phe^7]bradykinin) antagonized competitively the response to des-Arg^9-bradykinin, giving respective pA_2 values of 6.82 ± 0.34, 6.63 ± 0.28 and 6.48 ± 0.41 whereas the non-peptide bradykinin B_2 receptor antagonist, WIN 64338 (phosphonium, [[4-[[2-[[*bis*(cyclohexylamino)methylene]amino]-3-(2-naphtalenyl) 1-oxopropyl]amino]-phenyl]-methyl]tributyl chloride, monohydrochloride), was inactive. Hoe 140 and WIN 64338 but not des-Arg^9[Leu^8]bradykinin behaved as competitive antagonists towards the relaxation due to bradykinin. In conclusion, both bradykinin B_2 and B_1 receptors are present on the endothelium of large coronary arteries from juvenile pig. The bradykinin B_1 receptor subtype appears partly inducible and is coupled to the synthesis of nitric oxide.

Keywords: Bradykinin; Bradykinin receptor; Coronary artery; (Pig)

1. Introduction

Based on structure-activity pharmacological studies, receptors for kinins have been primarily divided into B_1 and B_2 types (Regoli and Barabé, 1980). This division has been confirmed recently by cloning and molecular characterization of both human bradykinin B_1 and B_2 receptors (Hess et al., 1992; Mencke et al., 1994). In the vasculature, most of the effects mediated by kinins are ascribed to the stimulation of bradykinin B_2 receptors which are usually found on the endothelium where they mediate the release of nitric oxide (NO) and/or prostacyclin (Pelc et al., 1991; Hall, 1992). The natural agonists, bradykinin and kallidin, bind with high affinity to the bradykinin B_2 receptor whereas the kinin fragments lacking the C-terminal arginine residue such as des-Arg^9-bradykinin and des-Arg^{10}-kallidin selectively stimulate the bradykinin B_1 receptor (Regoli and Barabé, 1980; Hall, 1992).

Pig coronary arteries relax in response to bradykinin in an endothelium-dependent manner (Richard et al., 1990; Myers et al., 1992). However, it is not clear if the response in this species is mediated by the release of NO, since NO-synthase inhibitors failed to block the response (Richard et al., 1990; Kauser and Rubanyi, 1992). A contractile response to des-Arg^9-bradykinin was described in isolated pig coronary arteries (Beny et al., 1987) while infusion of live *E. Coli* was shown to result in a hypotensive response to des-Arg^9-bradykinin in weaned piglets (Siebeck et al., 1989). Careful analysis revealed that des-Arg^9-bradykinin lowered arterial blood pressure by 10% in intact pigs, suggesting the presence of constitutive bradykinin B_1 receptors in this species (Siebeck et al., 1989).

The present study was undertaken to clarify the type, distribution and function of bradykinin receptors of pig left coronary artery. Changes of tension in

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response to des-Arg^9-bradykinin and bradykinin were obtained in vascular preparations incubated 6 h with and without endothelium and in the absence and presence of specific antagonists of NO or prostanoid formation. The phenomenon of bradykinin B_1 receptor induction was also addressed by incubating tissues in the presence of a protein synthesis inhibitor (cycloheximide), an inhibitor of transcription (actinomycin D) or a blocker of protein trafficking (brefeldin A). To further characterize the type of bradykinin receptors of the pig coronary artery, the effects of various bradykinin receptor antagonists were examined against responses to des-Arg^9-bradykinin and bradykinin.

2. Materials and methods

2.1. Vessel preparation

7–9-week-old male Large White pigs (25–35 kg) were anaesthetised and killed by an overdose of sodium pentobarbital. The heart was removed and placed in Krebs solution of the following composition (in mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylenediaminetetracetic acid (EDTA) 0.026, glucose 5.5, and bubbled with 95% O_2 plus 5% CO_2. After a proximal segment of the left anterior descending coronary artery was isolated, 3-mm-long rings were prepared and suspended on triangular stainless-steel wires (0.3 mm in diameter) in 20 ml jacketed organ baths maintained at 37°C. In some rings the endothelium was rubbed off using a polyethylene catheter (No. 5, Biotrol, Paris, France) which was introduced into the lumen and gently moved back and forth several times. One hook was suspended from a Gould-Statham UC_2 or UTC_2 transducer, and the other was fixed to a plastic support leg. Changes in isometric tension were recorded continuously on two-channel recorders (Gould BS272 or Linseis Type 7025). The rings were left unstretched for 30 min and were then stretched in a stepwise fashion to a passive force of 4 g.

2.2. Response of coronary artery to kinins

After 4-h equilibration at 4-g resting force or at the end of the experiment, the arteries were contracted with an isotonic maximally depolarizing high-K^+ Krebs salt solution in which all the NaCl of normal Krebs was replaced by KCl. Previous experiments have shown that the contractions produced by high-K^+ Krebs salt solution were constant over a 7-h period. At the plateau of contraction, the tissues were washed twice with normal Krebs and allowed to return to their resting tone. After 2 h or 6 h of total equilibration period, artery rings were contracted to approximately 50% of their maximum contraction induced by high-K^+ Krebs salt solution with U46619 (0.01–0.10 μM). At the plateau of contraction, the responses to cumulative concentrations of des-Arg^9-bradykinin, des-Arg^{10}-kallidin or bradykinin were obtained. Responses to des-Arg^9-bradykinin and bradykinin were also obtained in tissues without endothelium. In a separate set of experiments, intact artery rings were treated with either indomethacin (3 μM), N^G-nitro-L-arginine (L-NOARG, 30 μM), or a combination of L-NOARG (30 μM) and L-arginine (100 mM). Some other preparations were continuously exposed either to cycloheximide (50 or 100 μM), actinomycin D (2 or 4 μM) or brefeldin A (20 μM) for 6 h before addition of des-Arg^9-bradykinin or bradykinin. In 6-h incubated vessels, a series of antagonists was evaluated against des-Arg^9-bradykinin: des-Arg^9-[Leu^8]bradykinin (0.3–3 μM), D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (Hoe 140, 0.3–3 μM), D-Arg-[Hyp^3, D-Phe^7]bradykinin (NPC 567, 0.3–3 μM) and WIN 64338 (1 and 10 μM). The antagonists des-Arg^9-[Leu^8]bradykinin (3 μM), Hoe 140 and WIN 64338 were also tested against a bradykinin-induced relaxation curve. In order to block degradation of NPC 567 by carboxypeptidases, DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (Mergetpa, 8 μM) was used in corresponding experiments.

It was assumed that, in arteries without endothelium, absence of relaxation in response to substance P (0.001 μM) or to the Ca^{2+} ionophore, A23187 (0.01 μM), indicates complete endothelium removal. Sodium nitroprusside was also added at the end of the experiment in some endothelium-denuded vessels to evaluate their capacity to relax.

2.3. Drugs

Acetylcholine chloride, A 23187 free base, L-arginine, actinomycin D, bradykinin acetate, brefeldin A, cycloheximide, des-Arg^9-bradykinin acetate, des-Arg^9-[Leu^8]bradykinin acetate, D-Arg-[Hyp^3,D-Phe^7]bradykinin acetate (NPC 567), indomethacin, N^G-nitro-L-arginine, sodium nitroprusside, substance P and U46619 (1,5,5-hydroxy-11,9-(epoxymethano)prosta-5*z*,13*E*-dienoic acid) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Mergetpa (DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid) was purchased from Calbiochem Corp. (La Jolla, CA, USA). Neosystem (Strasbourg, France) provided des-Arg^{10}-kallidin acetate. Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) was a generous gift from Dr K. Wirth (Hoechst AG, Frankfurt, Germany). WIN 64338 (phosphonium, [[4-[[2-[[*bis*(cyclohexylamino)methylene]amino]-3-(2-naphtalenyl) 1-oxopropyl]amino]-phenyl]-methyl]tributyl chloride, monohydrochloride) was synthetized by Dr P. Dodey (Laboratoires Fournier, Daix, France). All drugs were made up in distilled

Table 1
Values of pD_2 and E_{max} of des-Arg^9-bradykinin, des-Arg^{10}-kallidin and bradykinin for the relaxation of precontracted pig coronary artery incubated 2 h or 6 h

	2-h incubation		6-h incubation	
	pD_2	E_{max} (%)	pD_2	E_{max} (%)
Des-Arg^9-bradykinin	6.35 ± 0.52 [a]	50.6 ± 13.3 [a]	7.78 ± 0.13	87.4 ± 4.3
Des-Arg^{10}-kallidin	ND	ND	8.77 ± 0.14	92.0 ± 3.4
Bradykinin	8.73 ± 0.37	86.3 ± 7.7	8.69 ± 0.30	104.2 ± 4.4

After 2 h or 6 h of incubation, the artery rings were precontracted with U46619 to approximately 50% of the maximal contractile response to high-K^+ Krebs salt solution. Values are means ± S.E.M. [a] $P < 0.05$ compared with 6 h of incubation. The number of animals/group was 6–12. ND, not determined.

water or dimethylsulfoxide (maximal concentration of 0.5% in the organ bath) and kept on ice and protected from light.

2.4. Statistical analysis

Data are given as means ± S.E.M. The negative logarithm of the concentration of agonist (des-Arg^9-bradykinin, des-Arg^{10}-kallidin, bradykinin) needed to reach 50% (EC_{50}) of the maximal response to this agonist (pD_2) was calculated by least-square regression analysis (Tallarida and Murray, 1981). After the testing for linearity and parallelism, pD_2 values for relaxation and maximal responses (E_{max}) were compared by means of Student's t-test. Values of pA_2 for antagonists were obtained from the EC_{50} ratios in the presence and absence of antagonist as described by Arunlakshana and Schild (1959). Values of pD_2 and pA_2 are given ± intervals at 95% confidence limits. E_{max} values are expressed as % relaxation. n indicates the number of animals. The level of significance was $P < 0.05$.

3. Results

3.1. Vascular relaxation in response to des-Arg^9-bradykinin, des-Arg^{10}-kallidin and bradykinin

After 2 h or 6 h of incubation, arterial rings were contracted with U46619 (0.036 ± 0.004 μM) to approximately 50% of the maximal contractile response to high-K^+ Krebs salt solution (Fig. 1B). Des-Arg^9-bradykinin and bradykinin relaxed precontracted arteries in a concentration-dependent manner (see representative tracings obtained after 6 h of incubation in Fig. 1A). Des-Arg^9-bradykinin relaxed arteries incubated for 6 h more potently ($P < 0.05$) than arteries incubated for 2 h (Table 1 and Fig. 1B). The bradykinin B_2 receptor agonist, bradykinin, caused similar relaxation of coronary artery rings incubated for 2 h or 6 h (Table 1 and Fig. 1B). Des-Arg^{10}-kallidin also behaved as a relaxing agent after 6 h of incubation but was about 10 × more potent than des-Arg^9-bradykinin (Table 1).

3.2. Effect of metabolic inhibitors

Pretreatment with cycloheximide (50 or 100 μM) or actinomycin D (2 or 4 μM) had no significant inhibitory effect on the relaxation curves for des-Arg^9-bradykinin and bradykinin (Fig. 2). In contrast, brefeldin A significantly inhibited ($P < 0.05$) by 52% the maximum relaxation due to des-Arg^9-bradykinin whilst it did not affect the response to bradykinin (Fig. 2).

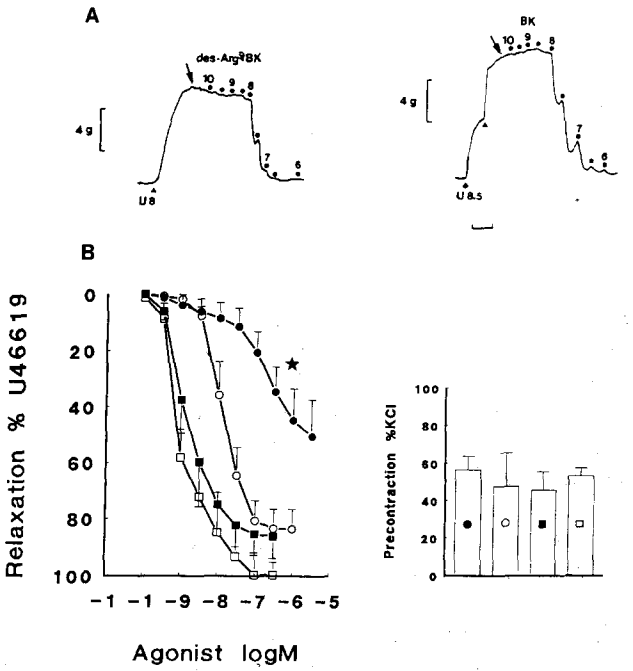

Fig. 1. (A) Original chart recordings showing the effects of cumulative concentrations (−log M) of des-Arg^9-bradykinin (des-Arg^9-BK) and bradykinin (BK) on ring segments of pig coronary artery incubated for 6 h. The horizontal calibration bar represents 10 min and 3 min before and after the arrow, respectively. (B) Group data showing relaxation curves for des-Arg^9-bradykinin (circles) and bradykinin (squares) after incubation of the arteries for 2 h (closed symbols) or 6 h (open symbols). Arteries were precontracted with U46619 to approximately 50% of the contraction with high-K^+ Krebs salt solution. The symbol (⋆) indicates a significant difference with 6-h incubation for des-Arg^9-bradykinin. Values (means ± 1 S.E.M. from 6–12 animals/point) are expressed as percentage relaxation.

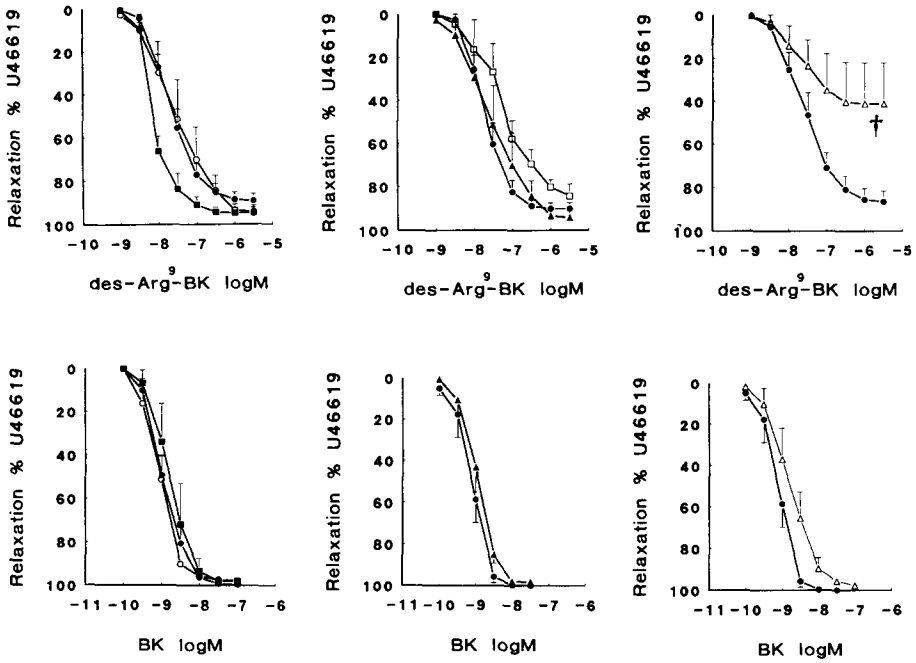

Fig. 2. Concentration-relaxation curves for des-Arg^9-bradykinin and bradykinin in pig coronary artery rings incubated for 6 h in the absence (●) or presence of cycloheximide (50 μM, ○; 100 μM, ■), actinomycin D (2 μM, ▲; 4 μM, □) or brefeldin A (20 μM, △). Cycloheximide, actinomycin D or brefeldin A were added to the organ baths at the beginning of the experiment. The symbol (†) indicates significant differences for E_{max} values of des-Arg^9-bradykinin in the presence and absence of brefeldin A. Values (means ± 1 S.E.M. from 4–7 animals/point) are expressed as percentage relaxation.

3.3. Endothelium-dependent relaxation in response to des-Arg^9-bradykinin and bradykinin

Mechanical removal of the endothelium abolished the relaxation due to des-Arg^9-bradykinin and to bradykinin (Fig. 3). Relaxation curves for des-Arg^9-bradykinin and bradykinin were unaffected by indomethacin (3 μM) (Fig. 3). In contrast, the NO-synthase inhibitor, L-NOARG (30 μM), markedly reduced the response to des-Arg^9-bradykinin and had no effect on the bradykinin relaxation curve (Fig. 3). L-Arginine (100 mM) reversed the blockade by L-NOARG of the des-Arg^9-bradykinin-induced relaxation (Fig. 3).

3.4. Pharmacological characterisation of endothelial bradykinin receptors

The bradykinin B_1 receptor antagonist, des-Arg^9-[Leu^8]bradykinin, caused a concentration-dependent parallel rightward shift of the relaxation curve for des-Arg^9-bradykinin without affecting E_{max} (Fig. 4A). Schild plot analysis gave a linear regression with a slope not significantly different from unity and a pA_2 of 6.82 ± 0.34 (Fig. 4B). Both the bradykinin B_2 receptor antagonist, Hoe 140, and the mixed bradykinin B_1/B_2 antagonist, NPC 567, also produced a rightward and parallel shift of the relaxation curve for des-Arg^9-bradykinin (Fig. 5A). Schild plot analyses gave slopes of the regression lines that did not differ from 1 and pA_2 values of 6.63 ± 0.28 and 6.48 ± 0.41 for Hoe 140 and NPC 567, respectively (Fig. 5B). In contrast, the non-peptide bradykinin B_2 receptor antagonist, WIN 64338 (1 and 10 μM), had no effect against the relaxation curve for des-Arg^9-bradykinin (Fig. 6).

The relaxation curve for bradykinin was not inhibited by des-Arg^9-[Leu^8]bradykinin (3 μM; $n = 6$) (data not shown) but was shifted to the right by Hoe 140 in a competitive manner (Fig. 7A). A corresponding pA_2 value of 9.28 ± 0.40 was calculated from Schild plot analysis (Fig. 7B). WIN 64338 also produced a rightward shift of the concentration-relaxation curve for bradykinin (Fig. 7A). The Schild plot slope was 1.33 ± 0.22 (not significantly different from unity) and the pA_2 value was 5.50 ± 0.25 (Fig. 7B).

4. Discussion

This study demonstrated that the porcine left anterior descending coronary artery contains two types of bradykinin receptor, both located on the endothelium. One fulfills the pharmacological characteristics of a bradykinin B_2 receptor and the other appears to be a bradykinin B_1 receptor.

Bradykinin B_1 receptors, thought to be normally absent from blood vessels, have been shown to be expressed after in vitro incubation (Regoli et al., 1981; Pruneau and Bélichard, 1993), treatment with

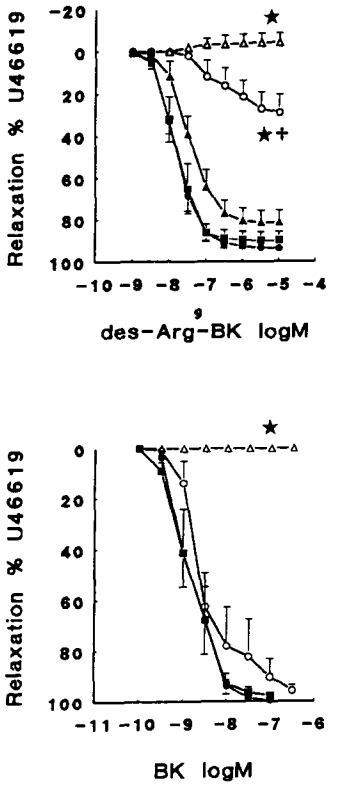

Fig. 3. Effect of indomethacin (3 μM, ■), L-NOARG (30 μM, ○), L-NOARG (30 μM) in the presence of L-arginine (100 mM, ▲) and endothelium removal (△) on responses to des-Arg^9-bradykinin and bradykinin in pig coronary artery. Control responses are given as (●). Changes in tone are expressed as percentages of precontraction level obtained with U46619. The symbol (⋆) indicates significant differences for pD_2 values and E_{max} values between endothelium-denuded or L-NOARG-treated groups and control group. The symbol (†) indicates a significant difference for pD_2 values and E_{max} values between L-NOARG alone and L-NOARG in the presence of L-arginine. Values (means ± 1 S.E.M. from 6–10 animals/point) are expressed as percentage relaxation.

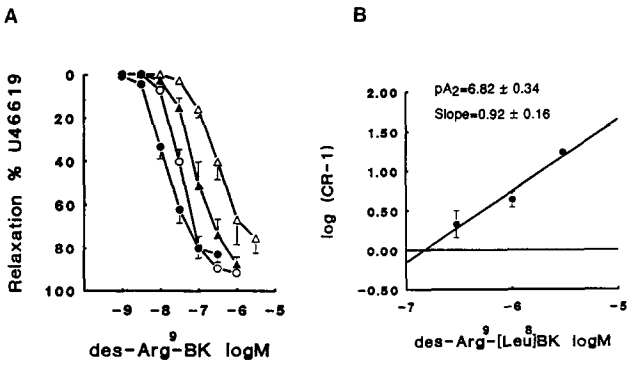

Fig. 4. (A) Relaxation curves for des-Arg^9-bradykinin alone (●; $n = 14$) or in the presence of des-Arg^9-[Leu^8]bradykinin 0.3 μM (○; $n = 6$), 1 μM (▲; $n = 6$) and 3 μM (△; $n = 6$). Values which are means ± 1 S.E.M. (vertical bars) are expressed as percentages relaxation from U46619-induced contractions. (B) Schild plot analysis demonstrating competitive antagonism by des-Arg^9-[Leu^8]bradykinin of the response to des-Arg^9-bradykinin. Concentration ratios were determined from pD_2 values. The value of pA_2 is given ± intervals at 95% confidence limits.

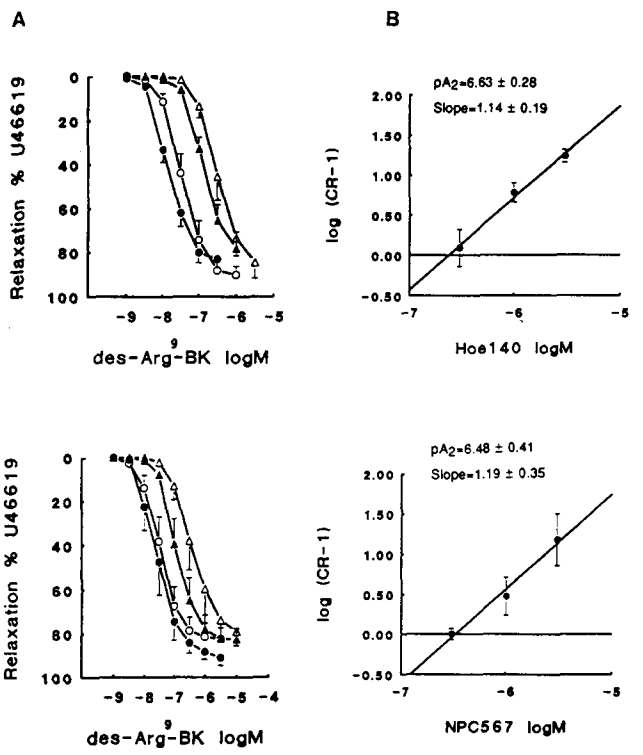

Fig. 5. (A) Relaxation curves for des-Arg^9-bradykinin alone (●; $n = 5$ to 14) or in the presence of Hoe 140 (upper part) 0.3 μM (○; $n = 6$), 1 μM (▲; $n = 6$) and 3 μM (△; $n = 6$) or D-Arg-[Hyp^3, D-Phe^7]bradykinin (NPC 567) (lower part) 0.3 μM (○; $n = 5$), 1 μM (▲; $n = 5$) and 3 μM (△; $n = 5$). Values which are means ± 1 S.E.M. (vertical bars) are expressed as percentages of relaxation from U46619-induced contractions. (B) Respective Schild plot demonstrating competitive antagonism by Hoe 140 or NPC 567 of the response to des-Arg^9-bradykinin. Concentration ratios were determined from pD_2 values. Values of pA_2 are given ± intervals at 95% confidence limits.

lipopolysaccharides (Marceau et al., 1980; Regoli et al., 1981; Bouthillier et al., 1987) or vascular trauma (Pruneau et al., 1994). However, it was recently reported that administration of the bradykinin B_1 recep-

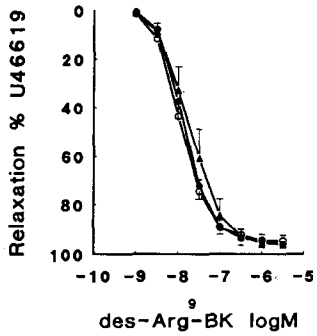

Fig. 6. Lack of effect of WIN 64338 at 1 μM (○) and 10 μM (▲) on the relaxation curve for des-Arg^9-bradykinin (●) in pig coronary artery. Values, which are means ± 1 S.E.M. (vertical bars), are expressed as percentages relaxation from U46619-induced contractions. $n = 7$ animals/point.

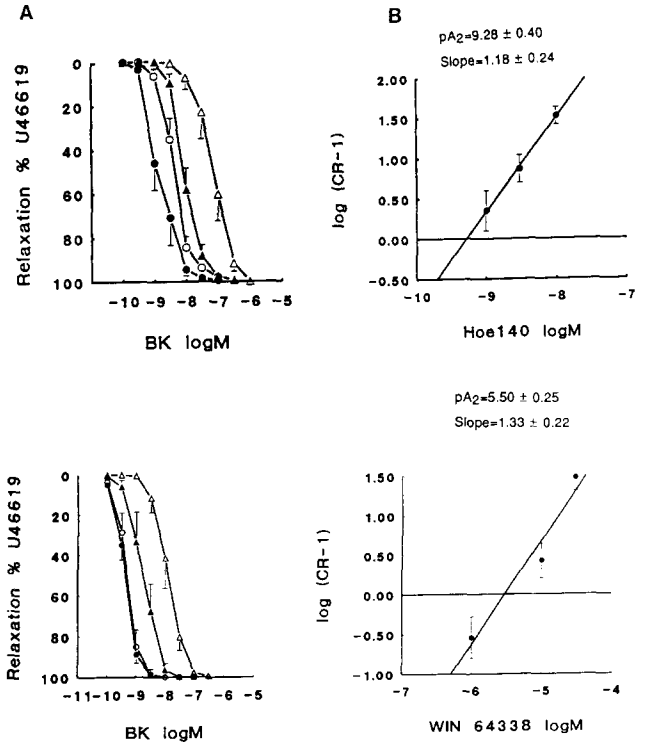

Fig. 7. (A) Relaxation curves for bradykinin alone (●; $n = 8$) or in the presence of Hoe 140 (upper part) 1 nM (○; $n = 6$), 3 nM (▲; $n = 6$) and 10 nM (△; $n = 6$) or WIN 64338 (lower part) 1 μM (○; $n = 6$), 10 μM (▲; $n = 6$) and 30 μM (△; $n = 6$). Values, which are means ± 1 S.E.M. (vertical bars), are expressed as percentages relaxation from U46619-induced contractions. (B) Schild plot analysis demonstrating competitive antagonism by Hoe 140 and WIN 64338 of the response to bradykinin. Concentration ratios were determined from pD_2 values. Value of pA_2 is given ± intervals at 95% confidence limits.

tor agonist, des-Arg^9-bradykinin, produced a marked dose-related hypotensive response in the anesthetized dog, a result suggesting the presence of constitutive bradykinin B_1 receptors in the dog vasculature (Nakhostine et al., 1993). Inducible bradykinin B_1 receptors were primarily described in the isolated rabbit aorta (Regoli and Barabé, 1980). Following in vitro incubation or lipopolysaccharide treatment, a contractile response of aortic rings to des-Arg^9-bradykinin was obtained which was specifically antagonized by des-Arg^9-[Leu^8]bradykinin (Regoli and Barabé, 1980). The response to des-Arg^9-bradykinin was prevented by cycloheximide, indicating that it was dependent on the de novo synthesis of proteins (Regoli and Barabé, 1980; Deblois et al., 1989), and further experiments showed that some cytokines such as tumor necrosis factor α and interleukine-1β were potent inducers of bradykinin B_1 receptors in vascular preparations (Deblois et al., 1989, 1991). In other rabbit vessels such as coeliac and mesenteric arteries, des-Arg^9-bradykinin was shown to produce a relaxation which was endothelium-independent and mediated by cyclooxygenase products (Deblois and Marceau, 1987; Ritter et al., 1989) whereas in the carotid artery, the relaxation was NO-dependent (Pruneau and Bélichard, 1993). Using the isolated pig coronary artery, Beny et al. (1987) reported a contractile action of des-Arg^9-bradykinin which was not dependent on the endothelium and appeared to be mediated through direct activation of bradykinin B_1 receptors in the smooth muscle.

Under our experimental conditions, we found consistent and reproducible relaxation in response to bradykinin B_1 and B_2 receptor agonists in intact arterial rings. However, the relaxation due to des-Arg^9-bradykinin was significantly more pronounced after 6 h than 2 h of incubation, a result suggesting a time-dependent induction of bradykinin B_1 receptors. Surprisingly and in contrast with previous reports (Deblois et al., 1989; Audet et al., 1994), we found that neither cycloheximide, a protein synthesis inhibitor, nor actinomycin D, an inhibitor of protein transcription, prevented the development of the response to des-Arg^9-bradykinin. However, brefeldin A which acts by inhibiting the migration of vesicles from the endoplasmic reticulum to the Golgi apparatus and thus blocks protein trafficking (Helms and Rothman, 1992) was found to inhibit partially the response to des-Arg^9-bradykinin at 6 h. Under the same conditions, cycloheximide, actinomycin D and brefeldin A had no effect against bradykinin-mediated relaxations. An attractive hypothesis to explain these results is that a population of additional bradykinin B_1 receptors or G-proteins was recruited from an intracellular pool of endothelial cells during the incubation. Such a phenomenon has recently been suggested for the synaptosomal AMPA receptor, a G-protein-coupled receptor, that could be recruited during long-term potentiation (Henley, 1995).

Relaxations in response to des-Arg^9-bradykinin and bradykinin were abolished by endothelium removal. In the absence of endothelium, des-Arg^9-bradykinin and bradykinin did not cause contraction, indicating that, in contrast with the rabbit aorta (Regoli and Barabé, 1980), in vitro incubation of the pig coronary artery for 6 h did not result in the induction of bradykinin receptors on smooth muscle cells. Treatment with L-NOARG, a NO-synthase inhibitor, blocked by 75% the relaxation due to des-Arg^9-bradykinin whereas indomethacin had no effect. Furthermore, the inhibition by L-NOARG was reversed by L-arginine, indicating that the relaxation in response to des-Arg^9-bradykinin was essentially dependent on endothelium-derived NO. However, the 25% remaining relaxation obtained in the presence of L-NOARG might indicate that a so far unknown factor, such as the endothelium-derived hyperpolarising factor, might also participate in the relaxation caused by des-Arg^9-bradykinin. In accordance with previous studies (Richard et al., 1990; Kauser and Rubanyi, 1992), we found that the response to bradykinin was not altered by indomethacin or by

L-NOARG. Taken together, these results suggest that the activation of endothelial bradykinin B_2 receptors from the pig large coronary arteries resulted in the production of a relaxing factor other than NO or a prostanoid.

In an attempt to characterize the type of receptors involved in the response to des-Arg^9-bradykinin we tested another bradykinin B_1 receptor agonist, des-Arg^{10}-kallidin. This compound was found to be 10 times more potent than des-Arg^9-bradykinin, in agreement with the previously reported order of potency for bradykinin B_1 receptor agonists (Regoli et al., 1990). When testing bradykinin receptor antagonists, we found that des-Arg^9-[Leu^8]bradykinin inhibited the response to des-Arg^9-bradykinin in a competitive fashion. Surprisingly, Hoe 140 also behaved as a competitive antagonist of the des-Arg^9-bradykinin-induced response giving a pA_2 value of 6.63 ± 0.28. In addition, the mixed bradykinin B_2/B_1 receptor antagonist, NPC 567, was also found to be a competitive antagonist. Hoe 140 has been shown previously to be a selective bradykinin B_2 receptor antagonist with no effect against the bradykinin B_1 receptor-mediated response in the rabbit aorta at a concentration of 0.1 μM (Hock et al., 1991). We extended these findings by showing no effect of Hoe 140 at 1 μM against des-Arg^9-bradykinin-induced contractions of the rabbit aorta (Pruneau and Luccarini, unpublished data). In the present study, Hoe 140 inhibited bradykinin-induced relaxation with a pA_2 value of 9.28 ± 0.40 thus confirming that Hoe 140 is a potent antagonist at bradykinin B_2 receptors (Hock et al., 1991). However, a previous report showed that 0.1 μM Hoe 140 was able to inhibit cGMP production in response to bradykinin B_1 receptor stimulation in cultured bovine aortic endothelial cells (Wiemer and Wirth, 1992). In addition, we have also found in binding experiments with human IMR-90 cells that Hoe 140 bound to the native bradykinin B_1 receptor with a K_i value of 103.0 ± 2.8 nM (Bastian et al., 1995). Thus, we suggest that, in addition to bradykinin B_2 receptors, the endothelium of porcine coronary arteries contains a bradykinin B_1 receptor which exhibits a pharmacological profile different from that of the rabbit bradykinin B_1 receptor (Regoli et al., 1990; Bouthillier et al., 1987). WIN 64338 has been recently described as a non-peptide bradykinin B_2 receptor antagonist (Sawutz et al., 1994). In accordance with a previous study (Marceau et al., 1994), we have found that this compound did not affect bradykinin B_1 receptors but inhibited bradykinin B_2 receptor-mediated responses although with a weak potency. Interestingly and in contrast with the present findings, it was recently reported that WIN 64338 potently inhibited the production of cGMP in response to des-Arg^9-bradykinin but not to bradykinin in bovine aortic endothelial cells (Wirth et al., 1994). These results both suggest that WIN 64338 discriminates between bradykinin receptor subtypes in different ways according to tissues and species and highlight the possible existence of different bradykinin B_1 receptor subtypes.

In conclusion, we have provided pharmacological evidence to support the view that the bradykinin B_1 receptor from the porcine coronary artery differs from the one previously described for the rabbit aorta. We suggest that this receptor is a species isoform and cloning of this receptor, which is underway in our laboratory, should help to clarify this question.

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References

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative use of drug antagonists, Br. J. Pharmacol. 14, 48.

Audet, R., E. Peticlerc, G. Drapeau, F. Rioux and F. Marceau, 1994, Further analysis of the upregulation of bradykinin B_1 receptors in isolated rabbit aorta by using metabolic inhibitors, Eur. J. Pharmacol. 271, 551.

Bastian, S., J.L. Paquet and D. Pruneau, 1995, Pharmacological characterization of a human B_1 receptor on IMR-90 cells, Arch. Mal. Coeur Vaisseaux (in press).

Beny, J.L., P. Brunet and H. Huggel, 1987, Interaction of bradykinin and des-Arg^9-bradykinin with isolated pig coronary arteries: mechanical and electrophysiological events, Reg. Pept. 17, 181.

Bouthillier, J., D. Deblois and F. Marceau, 1987, Studies on the induction of pharmacological responses to des-Arg^9-bradykinin in vitro and in vivo, Br. J. Pharmacol. 92, 257.

Deblois, D. and F. Marceau, 1987, The ability of des-Arg^9-bradykinin to relax rabbit isolated mesenteric arteries is acquired during in vitro incubation, Eur. J. Pharmacol. 142, 141.

Deblois, D., J. Bouthillier and F. Marceau, 1989, Pharmacological modulation of the up-regulated responses to des-Arg^9-bradykinin in vivo and in vitro, Immunopharmacology 17, 187.

Deblois, D., J. Bouthillier and F. Marceau, 1991, Pulse exposure to protein synthesis inhibitors enhances tissue response to des-Arg^9-bradykinin: possible role of interleukin-1, Br. J. Pharmacol. 103, 1057.

Hall, J.M., 1992, Bradykinin receptors: pharmacological properties and biological roles, Pharmacol. Ther. 56, 131.

Helms, J.B. and J.E. Rothman, 1992, Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF, Nature 360, 352.

Henley, J.M., 1995, Subcellular localization and molecular pharmacology of distinct populations of [^{3}H]-AMPA binding sites in rat hippocampus, Br. J. Pharmacol. 115, 295.

Hess, J.F., J.A. Borkowski, G.S. Young, C.D. Strader and R.W. Ransom, 1992, Cloning and pharmacological characterization of a human bradykinin (bradykinin-2) receptor, Biochem. Biophys. Res. Commun. 184, 260.

Hock, F.J., K. Wirth, U. Albus, W. Linz, H.J. Gerhards, G. Wiemer, St. Henke, G. Breipohl, W. König, J. Knolle and B.A. Schölkens, 1991, Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies, Br. J. Pharmacol. 102, 769.

Kauser, K. and G.M. Rubanyi, 1992, Bradykinin-induced, N^{ω}-nitro-L-arginine-insensitive endothelium-dependent relaxation of porcine coronary arteries is not mediated by bioassayable relaxing substances, J. Cardiovasc. Pharmacol. 20 (Suppl. 12), S101.

Marceau, F., J. Barabé, S. St-Pierre and D. Regoli, 1980, Bradykinin receptors in experimental inflammation, Can. J. Physiol. Pharmacol. 58, 536.

Marceau, F., L. Levesque, G. Drapeau, F. Rioux, J.M. Salvino, H.R. Wolfe, P.R. Seoane and D.G. Sawutz, 1994, Effects of peptide and nonpeptide antagonists of bradykinin B_2 receptors on the venoconstrictor action of bradikinin, J. Pharmacol. Exp. Ther. 269, 1136.

Mencke, J.G., J.A. Borkowski, K.K. Bierilo, T. MacNeil, A.W. Derrick, K.A. Schneck, R.W. Ransom, C.D. Strader, D.L. Linemeyer and J.F. Hess, 1994, Expression cloning of a human B_1 bradykinin receptor, J. Biol. Chem. 269, 21583.

Myers, P.R., R. Guerra, Jr. and D.G. Harrison, 1992, Release of multiple endothelium-derived relaxing factors from porcine coronary arteries, J. Cardiovasc. Pharmacol. 20, 392.

Nakhostine, N., Ribuot, D. Lamontagne, R. Nadeau and R. Couture, 1993, Mediation by B_1 and B_2 receptors of vasodepressor responses to intravenously administered kinins in anaesthetized dogs, Br. J. Pharmacol. 110, 71.

Pelc, L.R., G.J. Gross and D.C. Warltier, 1991, Mechanism of coronary vasodilation produced by bradykinin, Circulation 83, 2048.

Pruneau, D. and P. Bélichard, 1993, Induction of bradykinin B_1 receptor-mediated relaxation in the isolated rabbit carotid artery, Eur. J. Pharmacol. 239, 63.

Pruneau, D., J.M. Luccarini, C. Robert and P. Bélichard, 1994, Induction of bradykinin B_1 receptor-dependent vasoconstriction following balloon catheter injury to the rabbit carotid artery, Br. J. Pharmacol. 111, 1029.

Regoli, D. and J. Barabé, 1980, Pharmacology of bradykinin and related kinins, Pharmacol. Rev. 32, 1.

Regoli, D., F. Marceau and J. Lavigne, 1981, Induction of B_1-receptors for kinins in the rabbit by a bacterial lipopolysaccharide, Eur. J. Pharmacol. 71, 105.

Regoli, D., N.-E. Rhaleb, G. Drapeau and S. Dion, 1990, Bradykinin receptor subtypes, J. Cardiovasc. Pharmacol. 15 (Suppl. 6), S30.

Richard, V., F.C. Tanner, M. Tschudi and T.F. Luscher, 1990, Different activation of L-arginine pathway by bradykinin, serotonin and clonidine in coronary arteries, Am. J. Physiol. 259, H1433.

Ritter, J.M., H.S. Doktor and E.J. Cagoe, 1989, Actions of bradykinin and related peptides on rabbit coeliac artery rings, Br. J. Pharmacol. 96, 23.

Sawutz, D.G., J.M. Salvino, R.E. Dolle, F. Casiano, S.J. Ward, W.T. Houck, D.M. Faunce, B.D. Douty, E. Baizman, M.M.A. Awad, F. Marceau and P.R. Seoane, 1994, The nonpeptide WIN 64338 is a bradykinin B_2 receptor antagonist, Proc. Natl. Acad. Sci. USA 91, 4693.

Siebeck, M., E.T. Whalley, H. Hoffman, J. Weipert and H. Fritz, 1989, The hypotensive response to des-Arg^9-bradykinin increases during *E. Coli* septicemia in the pig, Adv. Exp. Med. Biol. 247, 389.

Tallarida, R.J. and R.B. Murray, 1981, Computational procedures, in: Manual of Pharmacologic Calculations with Computer Programs, ed. R.J. Tallarida and R.B. Murray (Springer Verlag, New York) p. 3.

Wiemer, G. and K. Wirth, 1992, Production of cyclic GMP via activation of B_1 and B_2 bradykinin receptors in cultured bovine aortic endothelial cells, J. Pharmacol. Exp. Ther. 262, 729.

Wirth, K.J., B.A. Schölkens and G. Wiemer, 1994, The bradykinin B_2 receptor antagonist WIN 64338 inhibits the effect of des-Arg^9-bradykinin in endothelial cells, Eur. J. Pharmacol. 288, R1.