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Detection of bradykinin B_1 receptors in rat aortic smooth muscle cells

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Abstract

The tritiated bradykinin B_1 receptor agonist [^{3}H]des-Arg^{10}-kallidin bound to a single class of high-affinity binding sites (K_d = 0.5 ± 0.16 nM; B_{max} = 15,000 ± 8,000 sites/cell) on cultured rat aortic smooth muscle cells. [^{3}H]Des-Arg^{10}-kallidin association and dissociation kinetics were monoexponential, making it possible to determine the association and dissociation rate constants (k_{+1} = 1.5 10^5 M^{-1} sec^{-1}; k_{-1} = 4.2 10^{-5} sec^{-1}). [^{3}H]Des-Arg^{10}-kallidin binding was inhibited by specific ligands of bradykinin B_1 and B_2 receptors with a rank order of potency consistent with that known for bradykinin B_1 receptors in other species (des-Arg^9-[Leu^8]bradykinin = des-Arg^{10}-kallidin = des-Arg^9-bradykinin = des-Arg^{10}-[Leu^9]kallidin > des-Arg^{10}-HOE-140≫bradykinin≫HOE-140). Bradykinin B_1 receptor mRNA was also detected in these cells. Des-Arg^{10}-kallidin increased cytosolic free Ca^{2+} levels, phosphoinositide turnover, and arachidonic acid release at nanomolar concentrations (respective EC_{50} values: 16 ± 2, 4 ± 2.7, 6 ± 2 nM). These functional effects of des-Arg^{10}-kallidin could be blocked by the bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin, but were not sensitive to bradykinin B_2 receptor antagonists. These results therefore show that rat aortic smooth muscle cells in culture express functional bradykinin B_1 receptors.

Keywords: Smooth muscle cells; Bradykinin; B1; Rat

1. Introduction

The nonapeptide bradykinin and its close analogue Lys-bradykinin (kallidin) are major inflammatory mediators implicated in a variety of pathological situations [1]. Whereas most of the effects of these kinins can be ascribed to bradykinin B_2 receptor activation and are blocked by specific antagonists of B_2 receptors such as HOE-140, it has long been known that some of the effects of bradykinin are due to its kininase II-generated degradation product des-Arg^9-bradykinin acting on B_1 receptors [2]. Bradykinin B_1 receptors were first characterised pharmacologically in the rabbit aorta by their low sensitivity to bradykinin, high responsiveness to des-Arg^9-bradykinin, and their gradual appearance as a function of incubation time [3]. These *in vitro* data have been substantiated by *in vivo* experiments showing that des-Arg^9-bradykinin injection produces marked hypotension in bacterial lipopolysaccharide-treated rabbits, and that these effects are blocked by the specific bradykinin B_1 receptor antagonist des-Arg^{10}-[Leu^9]kallidin [4,5]. Molecular cloning of human [6], rabbit [7], mouse [8], and rat [9,10] B_1 receptors has shown that in most species bradykinin B_1 receptors exhibit a low homology with B_2 receptors, and that B_1 receptor mRNA is expressed to a very small extent or not at all in normal tissue, but can be induced to a considerable extent by inflammatory stimuli [8]. This notion has been confirmed by recent experiments on cultured rabbit aortic or mesenteric smooth muscle cells, which also show an induction of bradykinin B_1 receptor expression by inflammatory cytokines or growth factors likely to be released in the vicinity of smooth muscle cells during inflammatory processes [11–13].

Although bradykinin B_1 receptors have been best characterized in the rabbit, there is considerable evidence for the implication of inducible bradykinin B_1 receptors in different inflammatory responses in the rat. The use of des-Arg^9-bradykinin and the antagonist des-Arg^9-[Leu^8]bradykinin in the rat has shown that bradykinin B_1 receptors are implicated in phenomena as diverse as arthritis [14], hyperalgesia

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Abbreviations: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} levels; IL-1β, interleukin-1β; PSS, physiological salt solution; and RT–PCR, reverse transcriptase–polymerase chain reaction.

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[15,16], fever [17], paw oedema [18,19], cardiac action potential prolongation [20], and intestinal motility [21,22] or colonic epithelial secretion [23]. However, although hypotensive responses to des-Arg^9-bradykinin have been reported in lipopolysaccharide-treated rats [24,25], the rat aorta *in vitro* does not seem to respond to des-Arg^9-bradykinin [2]. This suggests that the bradykinin B_1 receptor may not be significantly expressed in the rat aorta, although bradykinin B_1 receptor mRNA has been detected *ex vivo* in the aorta of lipopolysaccharide-treated rats [26] and functional effects ascribed to bradykinin B_1 receptors have been described in a rat smooth muscle cell line [27]. In an attempt to determine whether functional bradykinin B_1 receptors are indeed expressed in rat aortic cells, we studied bradykinin B_1 receptor expression in rat aortic smooth muscle cells by binding experiments and functional responses.

2. Materials and methods

2.1. Materials

[^{3}H]Des-Arg^{10}-kallidin (80 Ci/mmol) was from NEN, and *myo*-[^{3}H]inositol (100 Ci/mmol) and [^{3}H]arachidonic acid (200 Ci/mmol) were from Amersham. All peptides were either from BACHEM Inc. or Neosystem. Fura-2 was from Molecular Probes. Collagenase (CLS II) was from Worthington.

2.2. Smooth muscle cell cultures

Smooth muscle cells were isolated from the thoracic aorta of male Sprague–Dawley rats (160–180 g, Iffa-Credo) by enzymatic digestion with collagenase (1.5 mg/mL) (Worthington, Type II) in DMEM (Dulbecco's modified Eagle's medium) at 37° for 16 hr as described previously [28]. At the end of the incubation period, cells were centrifuged at 400 × *g* for 7 min, washed, and resuspended in DMEM containing heat-inactivated foetal bovine serum (10%), penicillin (100 IU/mL), streptomycin sulphate (100 μg/mL), and glutamine (4 mM). The cell pellet was then dispersed by repeated pipetting, and cells were seeded in 75-cm^2 culture flasks which were incubated in a 95% air, 5% CO_2 humidified atmosphere at 37°. Culture medium was removed every other day and aortic smooth muscle cells were subcultured by treatment with trypsin 0.05%, EDTA 0.02%. Cells were used between the first and tenth passage.

2.3. [^{3}H]Des-Arg^{10}-kallidin binding experiments

Smooth muscle cells grown in 225-cm^2 flasks and stimulated overnight with IL-1β (10 ng/mL) were detached with a trypsin/EDTA solution, centrifuged, and resuspended in PSS (composition: NaCl 145 mM, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 1 mM, glucose 5.6 mM, HEPES/NaOH 5 mM pH 7.4) containing BSA (1 mg/mL), captopril (20 μM), bacitracine (140 mg/mL), and dithiothreitol (DTT) (100 μM). Cells (1–5 10^6 cells/mL) were then incubated with [^{3}H]des-Arg^{10}-kallidin for 4 hr at 4° in a final volume of 500 μL. IL-1β preincubation was used to increase the level of [^{3}H]des-Arg^{10}-kallidin binding. Preliminary experiments showed that although this increase (*circa* 25%) did not reach statistical significance, it clearly improved the quality of the binding data. Preliminary experiments also showed that specific binding of [^{3}H]des-Arg^{10}-kallidin increased linearly with cell number up to 5 10^6 cells/mL. At the end of the incubation period, 2 mL of ice-cold washing solution (composition: NaCl 137 mM, BSA 1 mg/mL, HEPES 20 mM pH 7.4) was added and cell suspensions were rapidly filtered on polyethylene (0.5%)-treated Whatman GF/C glass fiber filters, using Millipore filtration manifolds. Filters were then washed twice with 5 mL washing solution and the remaining radioactivity determined in a liquid scintillation counter.

2.4. Detection of bradykinin B_1 receptor mRNA

Total RNA from rat smooth muscle stimulated overnight with interleukin-1β (10 ng/mL) or from untreated cells was extracted with Trizol (GIBCO BRL) and treated with DNAse (GIBCO BRL) according to the manufacturer's instructions. Reverse transcription (RT) was performed on 2.5 μg RNA with 200 U of reverse transcriptase (Super-Script II, GIBCO BRL) in a mixture containing 0.5 mM dNTP, 25 μg/mL of oligo dT, 10 mM DTT, 2.5 mM $MgCl_2$ 50 mM KCl, 20 mM Tris–HCl pH 8.4, 20 μL final volume. Samples were incubated for 50 min at 42° then heated for 15 min at 70° and chilled on ice. After treatment with 2 U of RNase H (20 min, 37°), cDNA amplification of a specific sequence from BK1 gene (AF009899) was performed by PCR with the following primers [1]: sense primer 5′ GGAC-CGCTACAGGCTCCTGGTATAC 3′, antisense primer 5′ AGCAGTCCTGGATCACTCTTAC 3′, amplifying a 454-bp fragment from position 446 to 900 in the rat BK1 gene (AF0098990). RT sample aliquots (5 μL) were added to a mixture containing 1.5 mM $MgCl_2$, 300 μM dNTP, 2 μg/mL of each primer, 50 U/mL of Taq DNA polymerase (GIBCO BRL) in 20 mM Tris–HCl pH 8.4, 100 μL final volume. PCR was performed on samples submitted or not to reverse transcription according to the following protocol: 4 min at 94°, then 29 cycles 35 sec at 94°/35 sec at 56°/35 sec at 72°, and finally 10 min at 72°. Aliquots (25 μL) from each sample were analysed on a 20% TBE (Tris/borate/EDTA) polyacrylamide gel (Novex) and stained by ethidium bromide.

2.5. Measurement of $[Ca^{2+}]_i$

Smooth muscle cells cultured in 75-cm^2 flasks and incubated for 18 hr with IL-1β (10 ng/mL) were detached with

[1] Bader, M., personal communication. Cited with permission.

a trypsin/EDTA solution, centrifuged, and resuspended in PSS containing fura-2/AM (acetoxymethyl ester of fura-2, 1 μM) and incubated at 37° for 30 min. The cell suspension was then diluted five times with PSS and incubated for a further 60 min at 37°. After two washes with PSS to remove extracellular fura-2, cells were resuspended in PSS and kept in the dark at room temperature. Experiments were carried out under constant stirring in a PTI spectrofluorometer using approximately 3 10^5 cells in 3-mL fluorescence cuvettes at 37°. $[Ca^{2+}]_i$ was calculated from the fluorescence ratio $R = F_{340}/F_{380}$ (where F_{340} and F_{380} are the fluorescence intensities of fura-2 measured at 510 nm after excitation at 340 nm and 380 nm, respectively) as described by Grynkiewicz *et al*. [29].

2.6. Measurement of phosphoinositide turnover

The medium of smooth muscle cells grown as confluent monolayers in 35-mm dishes was changed to low-serum (0.5%) culture medium 48 hr before the experiment. Eighteen hours before the experiment, 5 μCi/mL of *myo*-[^{3}H]inositol and IL-1β (10 ng/mL) were added to the culture medium. At the beginning of the experiment, the medium was aspirated and the cell monolayers washed twice with PBS and incubated for 30 min with PSS containing 20 mM LiCl and antagonists or vehicle. Cells were then stimulated in the same medium with different concentrations of des-Arg^{10}-kallidin and antagonists for an additional 30 min at 37°. At the end of the incubation period, buffer was aspirated and the cells were extracted with an ice-cold methanol/HCl 0.1 N (50/50) solution for 30 min. Extracts were then neutralised with 1 M Na_2CO_3 and [^{3}H]inositol monophosphate separated as described by Berridge *et al*. [30] using columns containing 1 mL of AG1-X8 resin (Bio-Rad).

2.7. Measurement of arachidonic acid release

Smooth muscle cells grown as confluent monolayers in 24-well cluster plates were incubated for 18 hr in low-serum medium (0.5% foetal bovine serum) containing IL-1β (10 ng/mL) and [^{3}H]arachidonic acid (0.5 μCi/mL). Cell monolayers were then washed 4 times with PSS containing fatty acid-free BSA (0.2%) and incubated in this medium (0.5 mL) containing the antagonists or the vehicle for 6 min at 37°. Cells were then stimulated with 0.5 mL of the same medium containing the agonists and incubated for 30 min at 37°. At the end of the incubation period, an aliquot of the supernatant was recovered and the amount of radioactivity determined in a liquid scintillation counter.

3. Results

[^{3}H]des-Arg^{10}-kallidin binding was studied in rat aortic smooth muscle cells which had been cultured overnight in the presence of IL-1β (10 ng/mL). As evidenced by the linear Scatchard plot, [^{3}H]des-Arg^{10}-kallidin bound to a single class of saturable high-affinity binding sites (Fig. 1). From several experiments, the dissociation constant (K_d) was consistently observed to be subnanomolar (K_d = 0.5 ± 0.16 nM, N = 5) (Fig. 1, inset), whereas the density of binding sites was found to be more variable (B_{max} = 15,000 ± 8,000 sites/cell).

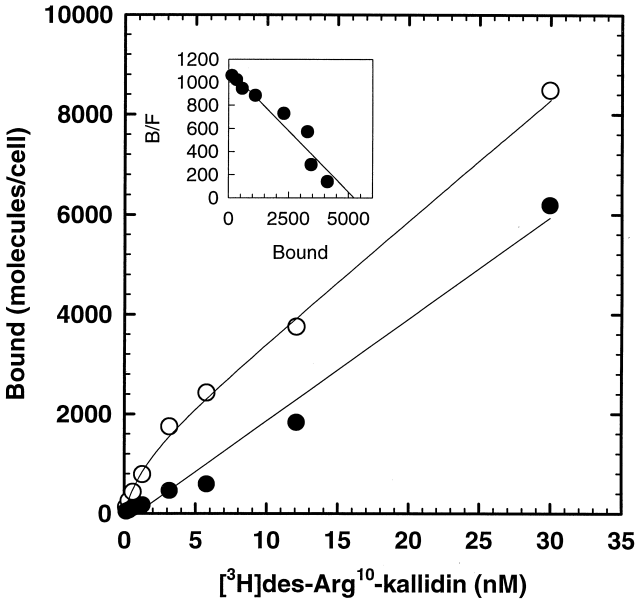

Fig. 1. [^{3}H]Des-Arg^{10}-kallidin binding to rat aortic smooth muscle cells. Cells (4 10^6 cells/mL) were incubated for 4 hr at 4° with different concentrations of [^{3}H]des-Arg^{10}-kallidin (total binding, ○) or [^{3}H]des-Arg^{10}-kallidin and unlabelled des-Arg^{10}-kallidin (1 μM, non-specific binding, ●). Cell-bound ligand was separated by rapid filtration. *Inset:* Scatchard representation of specific binding calculated as the difference between total binding and non-specific binding. Data are from one experiment representative of a total of five experiments.

Both the association and dissociation of [^{3}H]des-Arg^{10}-kallidin at 4° were slow, equilibrium being reached after 4 hr of incubation. Both association and dissociation reactions proceeded with a monoexponential time course (Fig. 2), enabling us to calculate the association rate constant k_{+1} = 1.5 10^5 M^{-1} sec^{-1} and dissociation rate constant k_{-1} = 4.2 $10^{-5}sec^{-1}$. The dissociation constant $K_d = k_{-1}/k_{+1}$ = 0.3 nM calculated from these kinetic experiments was very similar to the dissociation constant determined at equilibrium.

In order to determine the specificity of the [^{3}H]des-Arg^{10}-kallidin binding sites, the effect of different agonists and antagonists of the bradykinin B_1 and B_2 receptors on [^{3}H]des-Arg^{10}-kallidin binding was then studied. As clearly appears from Fig. 3, the different compounds inhibited [^{3}H]des-Arg^{10}-kallidin binding with an order of potency consistent with that known for bradykinin B_1 receptors [2] (IC_{50} ± SEM, nM, N = 2–3): des-Arg^9-[Leu^8]bradykinin (1 ± 0.2), des-Arg^{10}-kallidin (1.1 ± 0.5), des-Arg^9-bradykinin (1.6 ± 0.7), des-Arg^{10}-[Leu^9]kallidin (6 ± 3.9), des-Arg^{10}-HOE-140 (30 ± 3.6), bradykinin (300 ± 94), HOE-140 (>10,000).

The presence of bradykinin B_1 receptors in these cells

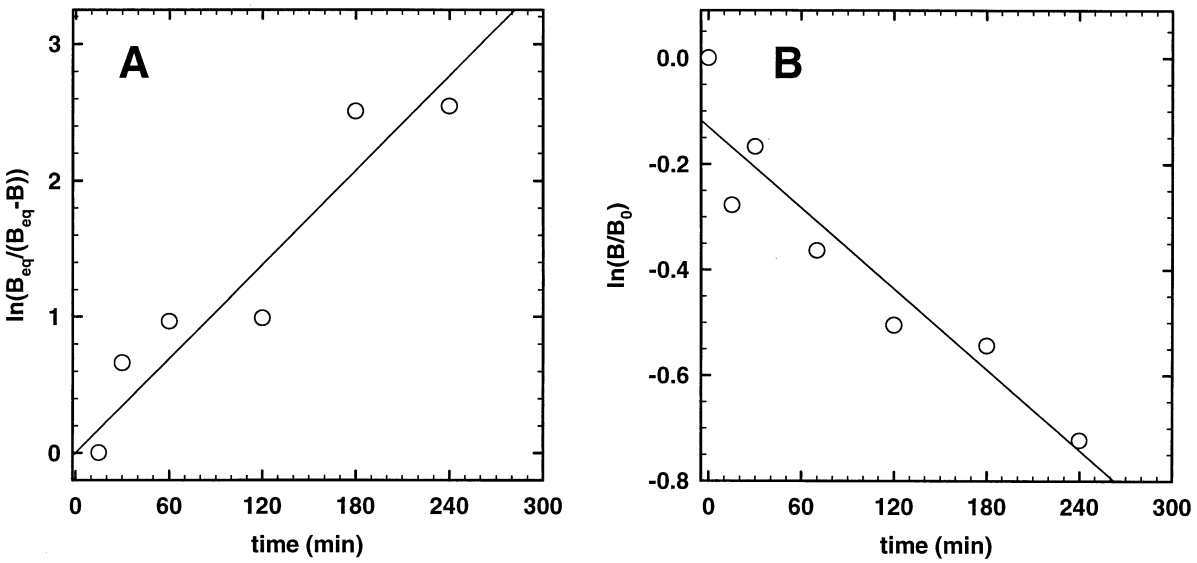

Fig. 2. Association and dissociation kinetics of [^{3}H]des-Arg^{10}-kallidin binding to rat smooth muscle cells. Cells were incubated at 4° with [^{3}H]des-Arg^{10}-kallidin (1 nM) in the absence (total binding) and presence (non-specific binding) of unlabelled des-Arg^{10}-kallidin (1 μM). The reaction was terminated at different time points by rapid filtration. Data are represented as specific binding determined as the difference between total binding and non-specific binding at each time point and are the means of three determinations. (a) Association kinetics. B = [^{3}H]des-Arg^{10}-kallidin bound, B_{eq} = [^{3}H]des-Arg^{10}-kallidin bound at equilibrium. (b) Dissociation kinetics. [^{3}H]Des-Arg^{10}-kallidin dissociation was induced by unlabelled des-Arg^{10}-kallidin (1 μM).

was confirmed after RT–PCR amplification of the bradykinin B_1 receptor mRNA, which showed only a single 454-bp band after electrophoresis (Fig. 4). This band was identified as a bradykinin B_1 receptor mRNA-derived amplicon by Southern blotting, whereas no amplification products could be detected in the blank samples. Interestingly, there was no significant difference in mRNA levels between cells cultured in normal medium and cells that had been exposed to IL-1β, suggesting that culture conditions led to bradykinin B_1 receptor induction. This was also confirmed by further binding experiments that showed only a small, non-signif-

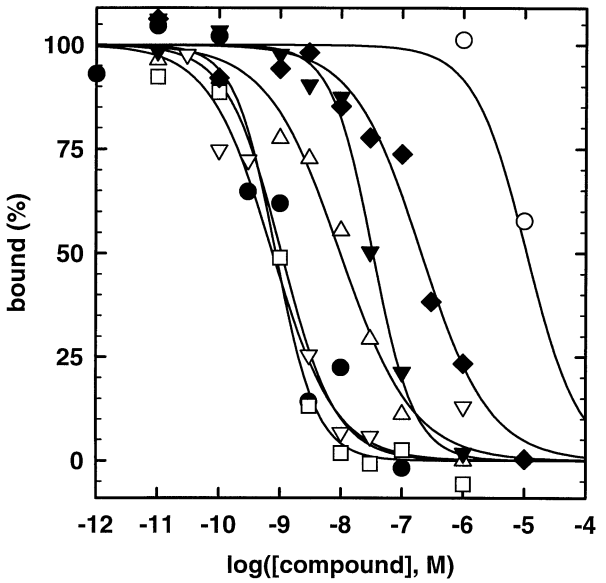

Fig. 3. Effect of specific ligands of bradykinin B_1 and B_2 receptors on [^{3}H]des-Arg^{10}-kallidin binding to rat aortic smooth muscle cells. Cells were incubated for 4 hr at 4° with [^{3}H]des-Arg^{10}-kallidin (1 nM) and different compounds: des-Arg^9-[Leu^8]bradykinin (▽), des-Arg^{10}-kallidin (●), des-Arg^9-bradykinin (□), des-Arg^{10}-[Leu^9]kallidin (△), des-Arg^{10}-HOE-140 (▼), bradykinin (◆), HOE-140 (○). The solid line represents a fit of the logistic equation to the data. Results are expressed as a percentage of specific binding in the absence of inhibitors and are representative of two to three experiments.

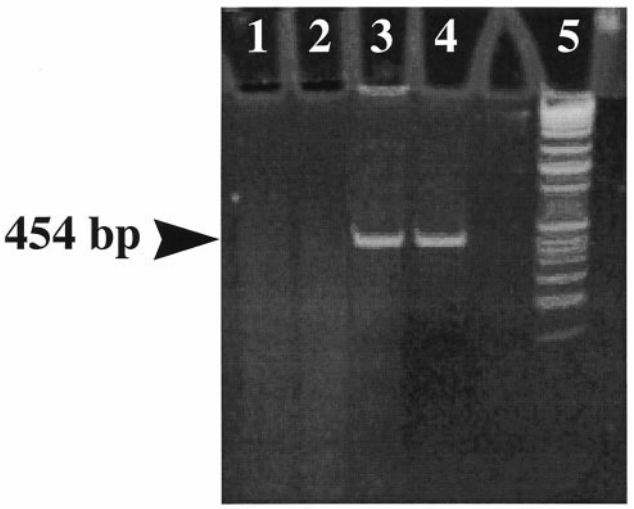

Fig. 4. Detection of bradykinin B_1 receptor mRNA in vascular smooth muscle cells. Smooth muscle cell mRNA was prepared as described under Materials and Methods. RT–PCR products were run on a 20% TBE (Tris/borate/EDTA) polyacrylamide gel and stained with ethidium bromide. Lanes: (1) non-reverse-transcribed RNA samples from rat smooth muscle cells treated with IL-1β (10 ng/mL) or (2) untreated, (3) reverse-transcribed RNA samples from cells treated with IL-1β, or (4) untreated and (5) size standards.

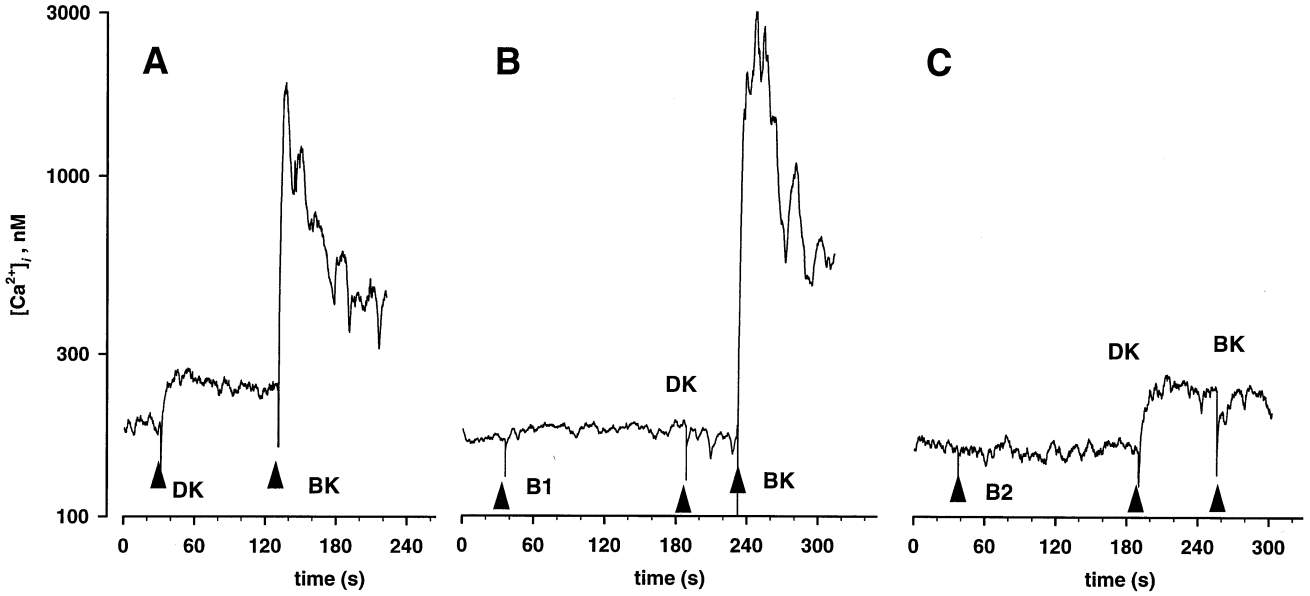

Fig. 5. Effect of des-Arg^{10}-kallidin and bradykinin on $[Ca^{2+}]_i$ levels in rat aortic smooth muscle cells. (a) Fura-2-loaded cells were stimulated with des-Arg^{10}-kallidin (DK, 1 μM) and bradykinin (BK, 100 nM) in sequence. (b) Effect of preincubation with the B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin (B1, 1 μM) on the $[Ca^{2+}]_i$ increase induced by des-Arg^{10}-kallidin (DK, 1 μM) and bradykinin (BK, 100 nM). (c) Effect of preincubation with the B_2 receptor antagonist [D-Arg^0,Hyp^3,D-Tic^7,Oic^8] bradykinin (B2, 1 μM) on the $[Ca^{2+}]_i$ increase induced by des-Arg^{10}-kallidin (DK, 1 μM) and bradykinin (BK, 100 nM).

icant increase in [^{3}H]des-Arg^{10}-kallidin binding in IL-1β-exposed cell cultures as compared to control cell populations (not shown).

In order to determine whether these des-Arg^{10}-kallidin binding sites represent functional bradykinin B_1 receptors, the effect of des-Arg^{10}-kallidin on $[Ca^{2+}]_i$ in fura-2-loaded smooth muscle cell suspensions was then assessed. As shown in Fig. 5A, des-Arg^{10}-kallidin induced a small but persistent increase in $[Ca^{2+}]_i$, whereas bradykinin induced a very strong, transient increase in $[Ca^{2+}]_i$. Furthermore, whereas bradykinin acted in the low nanomolar range (EC_{50} = 3 ± 0.5 nM), significantly higher concentrations of des-Arg^{10}-kallidin (EC_{50} = 16 ± 2 nM) and des-Arg^9-bradykinin (EC_{50} = 24 ± 6 nM) were necessary to induce a $[Ca^{2+}]_i$ increase (Fig. 6). The effect of des-Arg^{10}-kallidin was not due to low-level bradykinin B_2 receptor activation, because preincubation with the bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin abolished the $[Ca^{2+}]_i$ response of des-Arg^{10}-kallidin, but was ineffective on the bradykinin response (Figs. 5B and 6 [inset]). Conversely, the bradykinin B_2 antagonist [D-Arg^0,Hyp^3,D-Tic^7,Oic^8] bradykinin [31] did not decrease the effect of des-Arg^{10}-kallidin, but did abolish the response to bradykinin (Figs. 5C and 6 [inset]). Neither des-Arg^9-[Leu^8]bradykinin nor [D-Arg^0,Hyp^3,D-Tic^7,Oic^8]bradykinin showed any agonist activity.

Des-Arg^{10}-kallidin also induced an increase in phosphoinositide turnover in rat aortic smooth muscle cell monolayers (Fig. 7), with a half-maximal effect reached at concentrations very similar to those inducing a $[Ca^{2+}]_i$ increase in cell suspensions (EC_{50} = 4 ± 2.7 nM). As expected, the bradykinin B_2 receptor antagonist [D-Arg^0,Hyp^3,D-Tic^7,Oic^8]bradykinin had no effect on these responses of des-Arg^{10}-kallidin. The bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin strongly inhibited des-Arg^{10}-kallidin-induced responses (around 60% inhibition at 1 μM, Fig. 7).

An increase in arachidonic acid release is a hallmark of bradykinin B_1 receptor activation in several different tissues [2]. As could be expected, low concentrations of des-Arg^{10}-kallidin also induced the release of arachidonic acid from rat smooth muscle cells in culture (EC_{50} = 6 ± 2 nM, Fig. 8A). This effect was not inhibited by the bradykinin B_2 receptor antagonist [D-Arg^0,Hyp^3,D-Tic^7,Oic^8]bradykinin at high concentrations, but was abolished by des-Arg^9-[Leu^8]bradykinin (Fig. 8B).

Discussion

Bradykinin B_1 receptors have been extensively characterised in rabbit vascular smooth muscle cells in culture [11–13,32]. This paper shows that rat aorta smooth muscle cells in culture also express bradykinin B_1 receptors. The rat bradykinin B_1 receptors described in this paper show many characteristics of the previously described rabbit and human B_1 receptors [6,7]. Thus, [^{3}H]des-Arg^{10}-kallidin binding was potently inhibited by the classical B_1 agonists des-Arg^9-bradykinin and des-Arg^{10}-kallidin, as well as the antagonists des-Arg^9-[Leu^8]bradykinin, des-Arg^{10}-[Leu^9]kallidin, and des-Arg^{10}-HOE-140, and was insensitive to the bradykinin B_2 receptor antagonist HOE-140.

Some differences between responses to B_1 agonists in

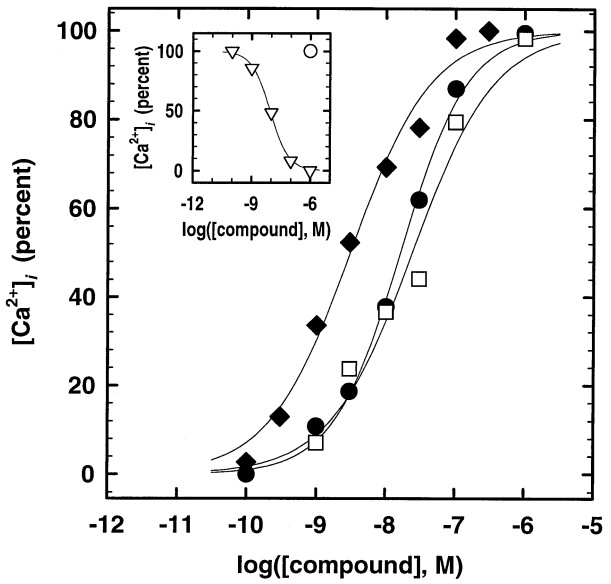

Fig. 6. Concentration–effect relationships of B_1 and B_2 agonist-induced $[Ca^{2+}]_i$ increase in rat aortic smooth muscle cells. The peak increase in $[Ca^{2+}]_i$ induced by bradykinin (◆), des-Arg^{10}-kallidin (●), and des-Arg^{9}-bradykinin (□) was determined at each concentration and then expressed as a percentage of the maximal effect induced by each compound. *Inset:* Inhibitory effect of des-Arg^{9}-[Leu^{8}]bradykinin (▽) and [D-Arg^{0},Hyp^{3},D-Tic^{7},Oic^{8}]bradykinin (○) after 3 min of preincubation on des-Arg^{10}-kallidin (1 μM)-induced $[Ca^{2+}]_i$ increase. Results are expressed as a percentage of the control response in the absence of antagonist and are the means of two to three determinations.

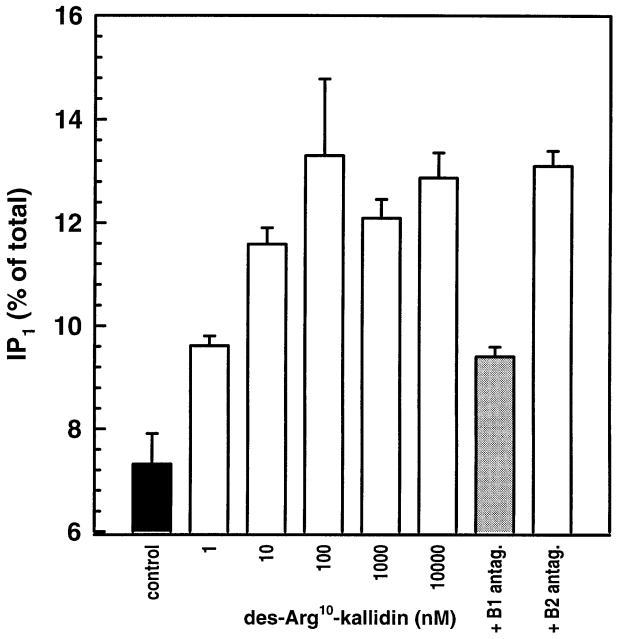

Fig. 7. Effect of the B_1 receptor agonist des-Arg^{10}-kallidin and antagonists on phosphoinositide turnover in rat aortic smooth muscle cell monolayers. Cell monolayers were stimulated for 30 min at 37° with different concentrations of des-Arg^{10}-kallidin. Antagonists (des-Arg^{9}-[Leu^{8}]bradykinin, +B1. antag, 1 μM and [D-Arg^{0},Hyp^{3},D-Tic^{7},Oic^{8}]bradykinin, +B2 antag. 1 μM) were present during the 30-min μM LiCl preincubation period and during the 30-min incubation period with des-Arg^{10}-kallidin. Inositol monophosphate levels are expressed as percent of total phosphoinositides and are representative of two experiments performed in triplicate. Error bars represent the SEM.

rabbit and rat smooth muscle cells should be noted, however. Bradykinin was able to inhibit [^{3}H]des-Arg^{10}-kallidin binding at submicromolar concentrations, and des-Arg^{9}-bradykinin and des-Arg^{9}-[Leu^{8}]bradykinin were as potent as des-Arg^{10}-kallidin and des-Arg^{10}-[Leu^{9}]kallidin as inhibitors of [^{3}H]des-Arg^{10}-kallidin binding. This is very different from the results reported on human and rabbit bradykinin B_1 receptors, which have very low affinity for bradykinin, and where the Lys-bradykinin (kallidin) compounds are much more active (10–100 times) than the des-Arg^{9}-bradykinin compounds [33]. In this respect, rat smooth muscle bradykinin B_1 receptors are very similar to mouse bradykinin B_1 receptors [33], probably because the Lys-bradykinin (kallidin) compounds are not natural agonists in the rat and mouse [9,10,33]. This is also consistent with the greater homology between the rat and mouse bradykinin B_1 receptor sequences as compared to rat and human or rabbit receptor sequences [34].

The activation of $[Ca^{2+}]_i$ increase, phosphoinositide turnover, and arachidonic acid release by des-Arg^{10}-kallidin was very similar to what has been reported in rabbit smooth muscle cells in culture [11–13,32]. All these functional responses were insensitive to B_2 receptor antagonists, showing that they were not mediated by B_2 receptor activation and were abolished or inhibited to a significant degree by des-Arg^{9}-[Leu^{8}]bradykinin, confirming that they implicate B_1 receptor activation.

The density of bradykinin B_1 receptors found in this study was in the same range as the receptor density reported in rabbit aortic smooth muscle cells in culture, but was lower than that found in rabbit mesenteric artery smooth muscle cells [12]. Interestingly, $[Ca^{2+}]_i$ responses to the B_2 agonist bradykinin and the B_1 agonist des-Arg^{9}-bradykinin reported in rabbit mesenteric artery cells were of the same magnitude [35], whereas in smooth muscle cells used in this work, bradykinin responses were found to be much stronger than those induced by des-Arg^{10}-kallidin and des-Arg^{9}-bradykinin. The difference in the $[Ca^{2+}]_i$ response of these preparations may thus reflect the difference in receptor density observed on these cells. In this context, it should be noted that similar differences between bradykinin B_1 and B_2 receptor-mediated responses have been reported in other rat smooth muscle preparations such as rat mesenteric artery smooth muscle cells [36], and to a lesser extent in rat mesangial cells [37].

The much lower response of des-Arg^{10}-kallidin and the fact that the difference between bradykinin and des-Arg^{10}-kallidin has now been reported in smooth muscle cells from three different rat tissues may have a physiological significance. Thus, des-Arg^{9}-bradykinin injection induces potent hypotensive effects in rabbits, but is inactive in the rat except in some subgroups (young Brown–Norway [24]) or if rendered protease-resistant by amino acid substitution

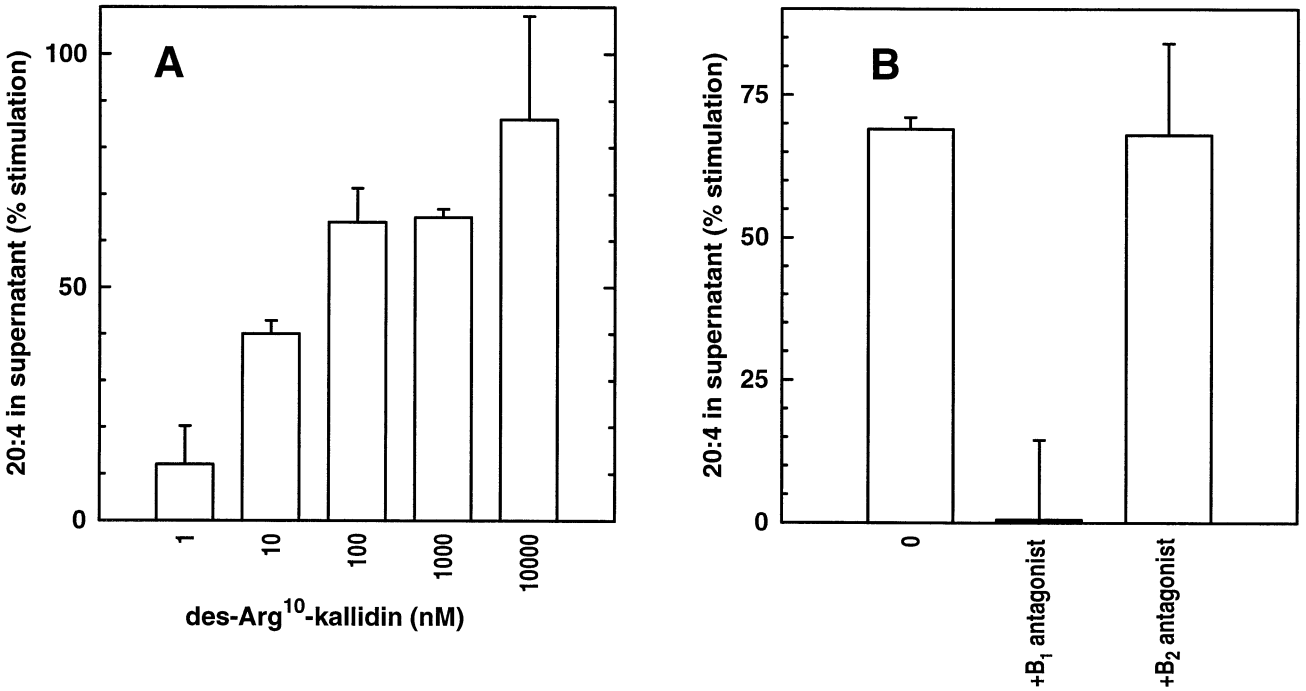

Fig. 8. Effect of the B_1 receptor agonist des-Arg^{10}-kallidin and antagonists on arachidonic acid release from rat aortic smooth muscle cell monolayers. (a) Cells were incubated for 30 min with des-Arg^{10}-kallidin. Results are expressed as percent stimulation of basal release and are representative of two experiments performed in triplicate. (b) Cells were preincubated for 6 min with the B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin (+B1 antagonist) (1 μM) or the B_2 receptor antagonist [D-Arg^0,Hyp^3,D-Tic^7,Oic^8]bradykinin (+B2 antagonist) (10 μM) and then stimulated for 30 min with des-Arg^{10}-kallidin (100 nM). Results are expressed as percent stimulation of basal release and are representative of two experiments performed in triplicate. Error bars represent the SEM.

[25]. Furthermore, rat aorta *in vitro* is insensitive to des-Arg^9-bradykinin even after bacterial lipopolysaccharide pretreatment ([2] and[2]). This paper clearly shows that rat aortic smooth muscle cells have the potential to express B_1 receptors, but the expression level may not be high enough to make contractile responses to B_1 agonists detectable in the isolated rat aorta. Furthermore, culture conditions in heterologous serum may increase the number of bradykinin B_1 receptors in cultured smooth muscle cells as compared to the *in vivo* situation, making B_1 responses easier to detect, although they are still considerably smaller than responses induced by bradykinin. Rat aorta may thus well express bradykinin B_1 receptors, but in numbers insufficient to induce a detectable functional response. The small functional effects of bradykinin B_1 receptor activation may also be partly explained by the absence of significant stimulation of bradykinin B_1 receptor mRNA expression by IL-1β, even though IL-1β has been reported to increase responses mediated by bradykinin B_2 receptors [12], which are not normally inducible [2].

In conclusion, this paper shows for the first time that rat aortic smooth muscle cells in culture express functional bradykinin B_1 receptors. Activation of B_1 receptors induces an increase in $[Ca^{2+}]_i$, stimulation of phosphoinositide turnover, and arachidonic acid release. B_1 receptor-mediated responses are notably smaller than those obtained through B_2 receptor activation, which may explain the finding that B_1 agonists are unable to induce the contraction of the rat aorta.

[2] Dol, F. and Schaeffer, P., unpublished observations.

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