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Characterization of des-Arg^9-bradykinin-induced contraction in guinea-pig gallbladder in vitro

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Abstract

We have reported that bradykinin induces graded contraction in guinea-pig gallbladder in vitro through activation of bradykinin B_2 receptors and prostanoid release, while des-Arg^9-bradykinin, a selective bradykinin B_1 receptor agonist, causes only a weak contraction, suggesting the presence of badykinin B_1 receptors in this tissue. In the present study, we attempted to characterise the receptor subtype and the possible mechanism by which des-Arg^9-bradykinin induces contraction in this preparation. Contractions induced by des-Arg^9-bradykinin in guinea-pig gallbladder (1 pM to 1 μM) increased significantly as a function of time elapsed after setting up of the preparation, reaching the maximum after 6 h of equilibration (EC_{50} 16.4 pM and E_{max} 0.6 ± 0.08 g). Des-Arg^9-bradykinin-induced contraction in guinea-pig gallbladder was totally prevented by cycloheximide (70 μM, an inhibitor of protein synthesis), indomethacin (3 μM), ibuprofen (30 μM), phenidone (30 μM) or Ca^{2+}-free medium plus EGTA, and was partially antagonised by MK 571 ((3-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl ((3-dimethyl amino-3-oxo-propyl) thio) methyl) propanoic acid, 0.1 μM) or by nicardipine (1 μM), but was not affected by dazoxiben (30 μM), staurosporine (100 nM) or L 655,240 (240 (3-[1-(4-clorobenzil)-5-fluoro-3-metilhyindol-2il] 2,2-dimetilpropanoic acid, 1 μM). Unexpectedly, des-Arg^9-bradykinin-induced contraction was unaffected by the selective bradykinin B_1 receptor antagonists, des-Arg^9-[Leu^8]-bradykinin and des-Arg^9-NPC 17761 (des-Arg^0-D-Arg [Hip^3, D-HipE (transtiofenil)^7, Oic^8]-des-Arg^9-bradykinin). However, the selective bradykinin B_2 receptor antagonists, HOE 140 (D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) and NPC 17731 (D-Arg^0 [Hyp^3, DHypE (transpropyl)^7, Oic^8]-bradykinin), completely blocked des-Arg^9-bradykinin-mediated contraction. Pre-treatment of the animals with *Escherichia coli* endotoxin (lipopolysaccharide, 30 μg/animal, i.v., 24 h) did not significantly change the response to des-Arg^9-bradykinin induction. It is concluded that des-Arg^9-bradykinin-induced contractions in guinea-pig gallbladder are mediated primarily by the release of proinflammatory eicosanoid(s) derived from the cyclo-oxygenase pathway. These effects are unrelated to thromboxane A_2 and do not seem to be coupled to activation of a protein kinase C-dependent mechanism. Response to des-Arg^9-bradykinin increases as a function of the equilibration period of the preparation by a mechanism dependent on protein synthesis and seems to be mediated by activation of bradykinin B_2 (but not B_1) receptors. Finally, in contrast to that observed for bradykinin, the contraction induced by des-Arg^9-bradykinin in guinea-pig galbladder is fully dependent on the influx of extracellular Ca^{2+}, partially through L-type Ca^{2+} channels.

Keywords: Des-Arg^9-bradykinin; Gallbladder, guinea pig; Bradykinin B_1 receptor-selective antagonist; Bradykinin B_2 receptor-selective antagonist; Prostanoid; Ca^{2+}

1. Introduction

Kinin action is mediated by the activation of two membrane receptors, denoted B_1 and B_2. Bradykinin preferentially acts through stimulation of constitutive B_2 receptors which are widely distributed in both peripheral and central nervous systems (Hall, 1992). On the other hand, the kinins without the C-terminal arginine des-Arg^9-bradykinin or des-Arg^{10}-lys-bradykinin, two kininase I active metabolites, exhibit higher affinities for the kinin B_1 than B_2 receptor (Marceau, 1995). Much less is known about the bradykinin B_1 receptors than for the B_2 receptors. Bradykinin B_1 receptors are rarely expressed in non-traumatized tissues and are up-regulated following in vitro incubation for long periods, after tissue trauma or infection or in vivo following treatment of animals with bacterial lipopolyssacharide endotoxin (Regoli and Barabé, 1980; Bhoola et al., 1992; Burch et al., 1993; Marceau, 1995).

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Both kinin B_1 and B_2 receptors have been cloned in most animal species and they possesses high homology in the amino acid sequence (Mceachern et al., 1991; Hess et al., 1992; Mcintyre et al., 1993; Hess et al., 1994; Pesquero et al., 1996).

In a recent study, we demonstrated that bradykinin and related kinins, after 2 h of equilibration of guinea-pig gallbladder, caused in this preparation graded contractions characterized by two distinct phases: high-affinity (0.1 pM to 1 nM) and low-affinity (3 nM to 3 μM) through activation of bradykinin B_2 receptors (Cabrini et al., 1995). In addition, des-Arg^9-bradykinin, the selective B_1 receptor agonist, caused a weak contraction compared to bradykinin, suggesting the possible presence of B_1 receptors in this preparations. The present study was therefore aimed at investigating the receptor subtype and also the mechanisms by which des-Arg^9-bradykinin induces contraction in the guinea-pig gallbladder in vitro.

2. Materials and methods

2.1. Tissue preparation

Guinea pigs of either sex (300–400 g), maintained on a 12 h light–dark cycle at 23 ± 2°C and fed with standard commercial diet, were killed by stunning and exsanguination. The abdomen was opened and the gallbladder was removed. After washout in Krebs solution (see composition below), usually 4 strips of each gallbladder (3–4 mm wide and 10–15 mm long) were set up in individual 5 ml organ baths containing Krebs solution of the following composition (mM): NaCl, 113; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; $MgSO_4$, 1.1; KH_2PO_4, 0.9 and glucose, 11, maintained at 37°C and pH of 7.4, continuously gassed with 95% of O_2 and 5% of CO_2, as described previously (Cabrini et al., 1995). Preparations were submitted to a basal tension equivalent to a 0.5 g load and were allowed to equilibrate for at least 2 h before drug addition, during which the bath solution was renewed every 15 min. Isometric tension changes were recorded by means of an F-60 force-transducer (Narco Biosystem, Huston, TX, USA). All experiments were carried out in presence of captopril (3 μM) to avoid the action of kininase II. The des-Arg^9-bradykinin-induced contractile responses are expressed as means ± S.E.M. in g of tension.

2.2. Concentration–response curves for des-Arg^9-bradykinin

Following the equilibration period of 2 h and in order to confirm the viability of the tissues, preparations were exposed to a high potassium concentration (KCl 80 mM, prepared by equimolar replacement of 74.4 mM NaCl by KCl) as a standard stimulus. After washout replacement with normal medium and return to the original baseline, concentration–response curves were obtained for des-Arg^9-bradykinin (1 pM to 1 μM). The concentration response curves for the agonist were made by means of the cumulative method (Van Rossum, 1963). Each concentration of the agonist was added when the effect of the preceding addition had reached its maximum. No more than two complete concentration–response curves for des-Arg^9-bradykinin were carried out on the same strip. To establish the influence of the equilibrium period on the action of the bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, complete cumulative concentration–response curves were obtained at 3, 4, 5, 6 and 7 h after setting up the preparation. To assess the possible involvement of protein synthesis in the time-dependent contraction induced by des-Arg^9-bradykinin, preparations were pre-incubated, in a separate series of experiments, with cycloheximide (inhibitor of protein synthesis, 70 μM) for the entire equilibration period for stabilization of the preparation; the response to the peptide was also obtained in its presence.

2.3. Influence of Ca^{2+} and protein kinase C

To assess the contribution of extracellular Ca^{2+} to the des-Arg^9-bradykinin-induced contraction of guinea-pig gallbladder, once equilibration was reached, cumulative concentration–response curves for des-Arg^9-bradykinin were obtained, using normal Krebs or Ca^{2+}-free solution plus EGTA (0.1 mM), for 20 min, during which the bath solution was renewed every 5 min. In another set of experiments, the preparations were allowed to equilibrate in normal medium and a concentration–response curve was obtained for des-Arg^9-bradykinin in either the absence or the presence of nicardipine (L type Ca^{2+} channel blocker, 100 nM) which was incubated for 20 min. To evaluate the influence of protein kinase C on the contraction induced by des-Arg^9-bradykinin, the preparations were incubated for 20 min with the inhibitor of this enzyme, staurosporine (100 nM) and a new concentration–response was obtained for des-Arg^9-bradykinin in its presence.

2.4. Effect of prostanoids

In order to investigate whether prostanoids were involved in the contractile response elicited by des-Arg^9-bradykinin, following the equilibration period, complete concentration response curves were obtained for des-Arg^9-bradykinin (1 pM to 1 μM), in the absence or presence of the following drugs added 20 min before des-Arg^9-bradykinin: indomethacin (3 μM) or ibuprofen (30 μM) (both cyclo-oxygenase inhibitors), phenidone (lipooxygenase inhibitor, 30 μM), dazoxiben (inhibitor of tromboxane A_2 synthase, 30 μM), L 655,240 (antagonist of tromboxane A_2 receptor, 10 nM) or MK 571 (leukotriene D_4 and E_4 selective receptor antagonist, 100 nM).

2.5. Effect of selective bradykinin B_1 and B_2 receptor antagonists

To examine the kinin receptor subtype involved in the response induced by des-Arg^9-bradykinin in the guinea-pig gallbladder, following the equilibration period, complete cumulative concentration–response curves were obtained for this peptide in the absence or in the presence of two selective kinin B_2 receptor antagonists, HOE 140 or NPC 17731 (1 μM), or kinin B_1 receptor antagonists, des-Arg^9-[Leu^8]-bradykinin or des-Arg^9-NPC 17761 (1 μM). All antagonists were added to the preparations at least 10 min before challenge with the agonist. Control experiments for des-Arg^9-bradykinin were always carried out in parallel in the presence of phosphate-buffered solution (PBS), in order to correct for any time-dependent changes in the responsiveness to des-Arg^9-bradykinin.

2.6. Influence of treatment of animals with endotoxin of Escherichia coli

To analyze the possible de novo synthesis of bradykinin B_1 receptors involved in the contractile response of des-Arg^9-bradykinin in the guinea-pig gallbladder, a new set of experimental animals were slightly anaesthetised with ether and treated with of *Escherichia coli* endotoxin (lipopolysaccharide, 30 μg/animal, i.v.) 24 h before experiments (Cabrini et al., 1996). Preparations were made and set up as described before and following the equilibration period complete cumulative concentration–response curves were obtained for des-Arg^9-bradykinin and were then compared to those from control responses (animals treated with the vehicle-PBS).

2.7. Statistical analysis

Most of the data are presented as mean ± S.E.M. The EC_{50} values (i.e. the concentration of the agonist necessary to produced 50% of maximal response) are given as geometric means accompanied by their 95% confidence limits. Tests for statistical significance were performed with either paired or unpaired Student's *t*-test. $P < 0.05$ or less was considered as indicative of significance. The contractiles response for des-Arg^9-bradykinin are expressed in absolute tension (g) developed per preparation. The EC_{50} values were calculated from individual experiments, from complete agonist concentration–response curves, using a test-square regression analysis. The contractile responses induced by des-Arg^9-bradykinin (10 μM), the highest concentration used, were also compared.

2.8. Drugs

Drugs used were: des-Arg^9-bradykinin, captopril, staurosporine, indomethacin, ibuprofen, phenidone, nicardipine, EGTA, PBS (concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffered 10 mM), lipopolysaccharide (fragment 0111, L = 2630), cycloheximide (all from Sigma, St. Louis, MO, USA), MK-571 (3-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl ((3-dimethyl amino-3-oxo-propyl) thio) methyl) propanoic acid (kindly provided by Merck Frost, Canada), HOE 140 (D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin (supplied by Hoechst, Frankfurt am Main, Germany), NPC 17731 (D-Arg^0 [Hyp^3, D-HypE (transpropyl)^7, Oic^8]-bradykinin (supplied by Scios Nova, Baltimore, MD, USA), des-Arg^9-[Leu^8]-bradykinin and L 655,240 (gifts from Dr. P. D'Orleans-Juste and Dr. Pierre Sirois, Department of Pharmacology, University of Sherbrooke, Sherbrooke, Canada), dazoxiben (Pfizer, Kent, UK) and des-Arg^9-NPC 17761 (Scios Nova). The stock solutions for all peptides used was prepared in PBS (1–10 mM) and kept in siliconized plastic tubes, maintained in a freezer at −18°C. Stock solutions for indomethacin, nicardipine, staurosporine and L 655,240 were made in absolute ethanol. All other drugs were dissolved in PBS to the desired concentration just before use. The final bath concentration of ethanol did not exceed 0.05% and did not significantly affect the concentration–response curves for des-Arg^9-bradykinin.

3. Results

3.1. Contraction induced by des-Arg^9-bradykinin

The results in Fig. 1A show that the addition of des-Arg^9-bradykinin (1 pM to 1 μM) caused a concentration-dependent contractile response in guinea-pig gallbladder. The results in Fig. 1A also show the concentration–response curves for des-Arg^9-bradykinin obtained 3, 4, 5, 6 and 7 h after the equilibration period. As may be observed, the concentration–response curves elicited by des-Arg^9-bradykinin increased significantly as a function of time elapsed after setting up the preparation, reaching the maximum after 6 h of equilibration. The EC_{50} value after 3 h of equilibrium was 2.4 (0.8–6.0) pM and the maximal response was 0.3 ± 0.02 g, while after 6 h of equilibrium the EC_{50} was 16.4 (1.6–160.0) pM and the maximal response was 0.6 ± 0.08 g ($P < 0.05$). Thus, a 6 h period of equilibration was used throughout. As shown in Fig. 1B, the des-Arg^9-bradykinin response after 6 h of equilibrium was completely blocked by the protein synthesis inhibitor cycloheximide (70 μM), incubated with the preparations during the time (6 h) that the preparation remained at equilibrium.

3.2. Influence of extracellular Ca^{2+} and protein kinase C

Pre-incubation of the strips of guinea-pig gallbladder with nicardipine (1 μM), 20 min beforehand, significantly

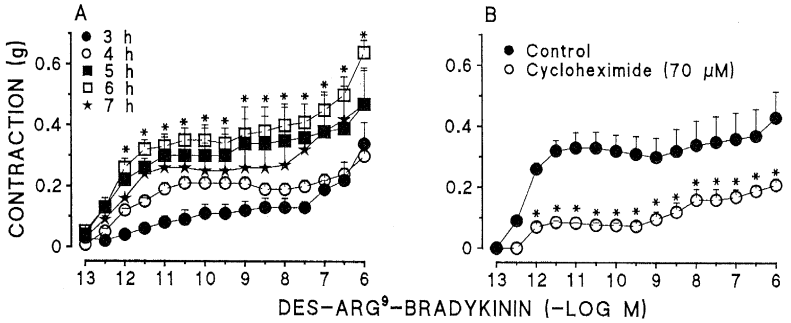

Fig. 1. (A) Mean concentration–response curves for des-Arg^9-bradykinin in guinea-pig gallbladder 3 (●), 4 (○), 5 (■), 6 (□) and 7 (★) h of incubation. (B) Mean concentration–response curves for des-Arg^9-bradykinin in guinea-pig gallbladder, after 6 h of equilibration period, in the absence (●) or in the presence (○) of cycloheximide. Each point represents the mean of six to eight experiments and the vertical bars indicate the S.E.M. Differs significantly from control group: * $P < 0.05$.

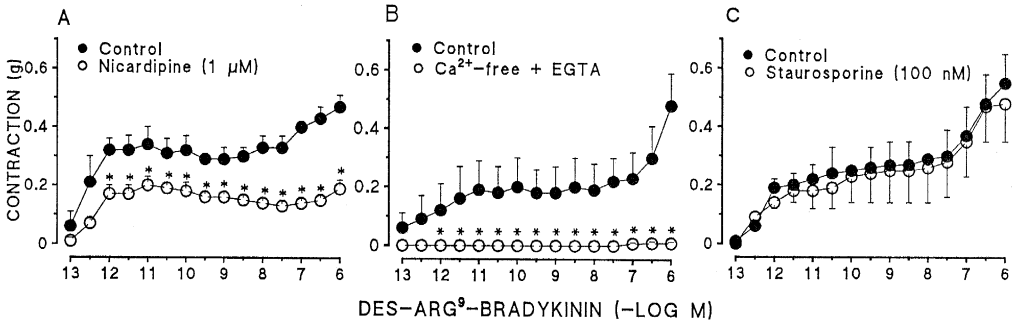

Fig. 2. Mean concentration–response curves for des-Arg^9-bradykinin in guinea-pig gallbladder, after 6 h of equilibration, in the absence (●) or in the presence (○) of (A) nicardipine, (B) in Ca^{2+}-free medium plus EGTA or (C) staurosporine. Each point represents the mean of six to seven experiments and the vertical bars indicate the S.E.M. Differs significantly from control group: * $P < 0.05$.

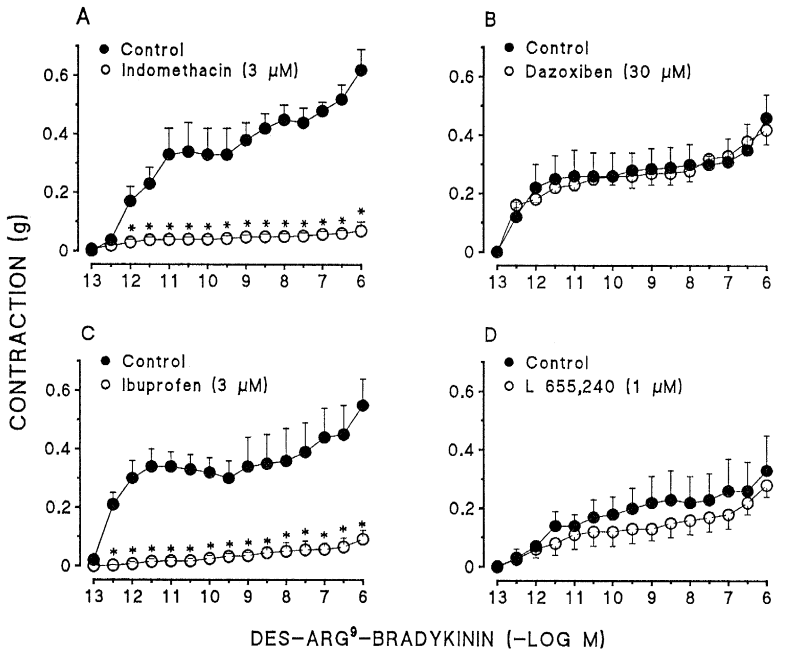

Fig. 3. Contraction response obtained for des-Arg^9-bradykinin in the absence (●) or in the presence (○) of (A) indomethacin, (B) dazoxiben, (C) ibuprofen or (D) L 655,240 in strips of guinea-pig gallbladder, after 6 h of equilibration. Each point represents the mean of six to seven experiments and the vertical bars indicate the S.E.M. Differs significantly from control group: * $P < 0.05$.

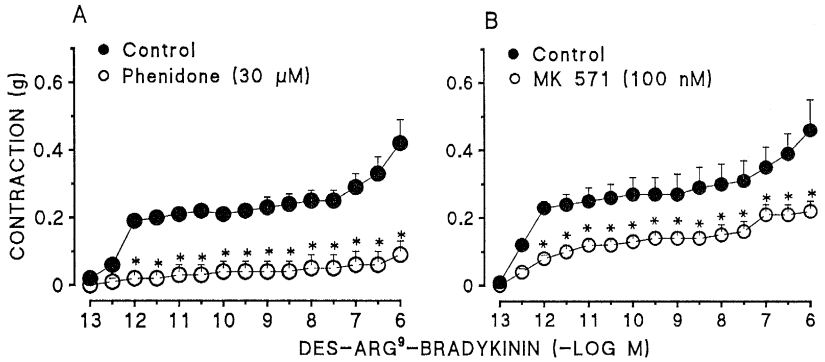

Fig. 4. Contraction response obtained for des-Arg^9-bradykinin in the absence (●) or in the presence (○) of (A) phenidone and (B) MK 571 in strips of guinea-pig gallbladder, after 6 h of equilibration. Each point represents the mean of six to seven experiments and the vertical bars indicate the S.E.M. Differs significantly from control group: * $P < 0.05$.

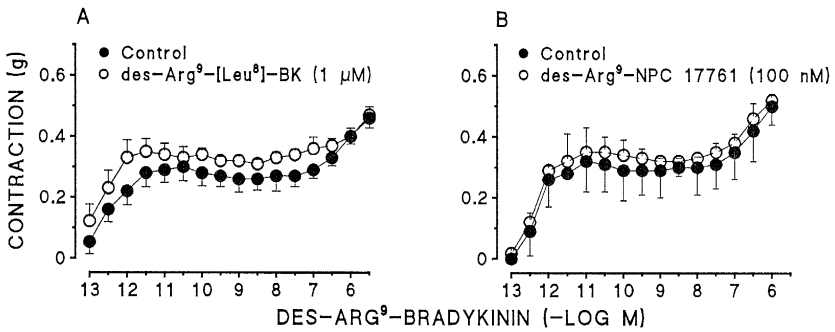

Fig. 5. Concentration–response curves for des-Arg^9-bradykinin-induced contraction in guinea-pig gallbladder, after 6 h of equilibration, in the absence (●) or in the presence (○) of (A) des-Arg^9-[Leu^8]-bradykinin or (B) des-Arg^9-NPC 17761. Each point represents the mean of seven to eight experiments and the vertical bars indicate the S.E.M.

reduced the contraction induced by des-Arg^9-bradykinin (Fig. 2A). When strips were bathed in Ca^{2+}-free medium containing EGTA (0.1 mM), the contractile response caused by des-Arg^9-bradykinin was completely abolished (Fig. 2B). Pre-incubation of the strips with the protein kinase C inhibitor, staurosporine (100 nM), did not change

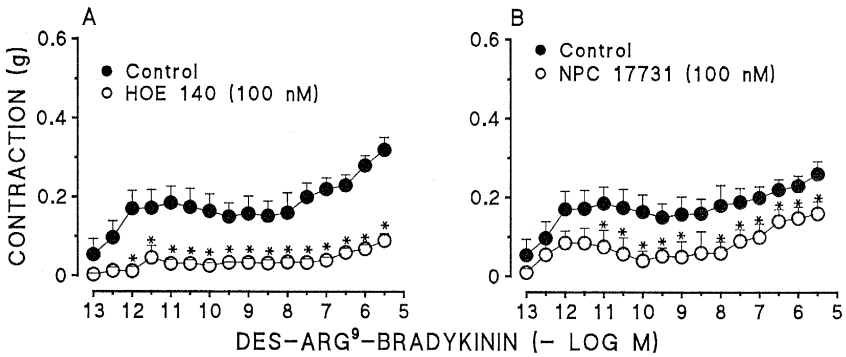

Fig. 6. Mean concentration–response curves for des-Arg^9-bradykinin in guinea-pig gallbladder, after 6 h of equilibration, in the absence (●) or the presence (○) of (A) HOE 140 or (B) NPC 17731. Each point represents the mean of six to eight experiments and the vertical bars indicate the S.E.M. Differs significantly from control group: * $P < 0.05$.

the tone of the preparation and also failed to affect des-Arg^9-bradykinin-induced contraction (Fig. 2C).

3.3. Influence of prostanoids

The data in Fig. 3A and C shows that the contractile response induced by des-Arg^9-bradykinin in the guinea-pig gallbladder was markedly antagonised by indomethacin (3 μM) or by ibuprofen (30 μM). However, the des-Arg^9-bradykinin-induced contraction was not affected by dazoxiben (30 nM) or L 655,240 (1 μM) (Fig. 3, B and D). Pre-incubation with phenidone (30 μM) completely inhibited the contraction induced by des-Arg^9-bradykinin, while MK 571 (100 nM) caused only a partial inhibition of the concentration–response curve of des-Arg^9-bradykinin (Fig. 4). Positive control experiments carried out with these drugs showed that they were effective to antagonise their respective agonist-mediated responses (results not shown).

3.4. Effect of B_1 and B_2 selective receptor antagonists

The selective bradykinin B_1 receptor antagonists, des-Arg^9-[Leu^8]-bradykinin and des-Arg^9-NPC 17761 (1 μM), did not affect the concentration–response curve for des-Arg^9-bradykinin (Fig. 5). In contrast, the incubation of guinea-pig gallbladder with HOE 140 or NPC 17731 (both selective bradykinin B_2 receptor antagonists, 1 μM) significantly antagonised the concentration–response curve for des-Arg^9-bradykinin, through in a non-competitive manner (Fig. 6). Neither antagonist displayed residual partial agonistic activity up to the maximal concentration used (results not shown).

3.5. Influence of lipopolysaccharide pretreatment

The results in Fig. 7 show that treatment of animals with lipopolysaccharide (30 μg/animal, i.v.), 24 h previously, failed to significantly modify the des-Arg^9-bradykinin-induced contraction of guinea-pig gallbladder strips, 6 h after setting up the experiment, in comparison with the contraction in PBS-treated animals.

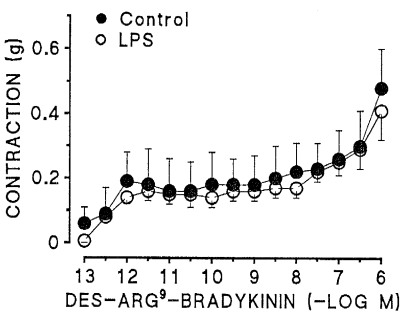

Fig. 7. Influence of pretreatment of animals with PBS (●) or lipopolysaccharide (○), 24 h prior, on des-Arg^9-bradykinin-induced contraction in guinea-pig gallbladder, after 6 h of equilibration. Each point represents the mean of seven experiments and the vertical bars indicate the S.E.M.

4. Discussion

The aim of this study was to investigate the possible presence of B_1 receptors in the guinea-pig galbladder in vitro as we suggested previously (Cabrini et al., 1995). Our results indicate that des-Arg^9-bradykinin induced a time-dependent contraction with high affinity but low efficacy in the guinea-pig gallbladder. The time-dependent increase in the des-Arg^9-bradykinin response was completely inhibited by cycloheximide (an inhibitor of protein synthesis) when it was maintained in contact with the preparation for 6 h. A similar effect has been observed with rabbit aorta and mesenteric arteries, rat urinary bladder, rat portal vein and duodenum, as well as rat paw oedema, where cycloheximide was capable of inhibiting the bradykinin B_1 receptor upregulation (Regoli et al., 1978; Bouthillier et al., 1987; Altinkurt and Ozturk, 1990; Campos and Calixto, 1994; Campos and Calixto, 1995; Campos et al., 1996). Audet et al. (1994) demonstrated that, in rabbit aorta, other drugs acting on protein synthesis, such as actinomicin, brefeldin and tunicamicin, also are capable of inhibiting the spontaneous upregulation of B_1 receptors. Interestingly, our results demonstrated that when the animals were pretreated with lipopolysaccharide, in a dose known to upregulate bradykinin B_1 receptors, no significant change in the responsiveness to des-Arg^9-bradykinin-induced contraction in guinea-pig gallbladder was observed, suggesting that B_1 receptors may not be involved in the action of the peptide.

To further characterize the subtype of kinin receptor mediating des-Arg^9-bradykinin-induced contraction in the guinea-pig gallbladder, we examined the effect of two selective bradykinin B_2 receptor antagonists, HOE 140 and NPC 17731. Surprisingly, both B_2 receptor antagonists, but not the B_1 selective receptor antagonists, des-Arg^9-[Leu^8]-bradykinin (Regoli and Barabé, 1980) and des-Arg^9-NPC 17761 (Cabrini et al., 1996) consistently antagonised the des-Arg^9-bradykinin concentration–response curve in the guinea-pig gallbladder. These results suggest that the des-Arg^9-bradykinin-induced contraction in the guinea-pig gallbladder involves, primarily, the activation of bradykinin B_2 (but not B_1) receptors as usually occurs in des-Arg^9-bradykinin-mediated response (Hall, 1992; Marceau, 1995). Very similar results were described for des-Arg^9-bradykinin in bovine endothelial cells, where HOE 140 was able to antagonise des-Arg^9-bradykinin-induced cyclic GMP production (Weimer and Wirth, 1992).

Consistent with our recent observation for bradykinin in the guinea-pig gallbladder, the des-Arg^9-bradykinin-induced contraction in these preparations was almost totally

inhibited by indomethacin or by ibuprofen, indicating that the cyclo-oxygenase-derived products from arachidonic pathway play an important modulatory role in the des-Arg^9-bradykinin response. A similar effect of des-Arg^9-bradykinin on prostanoids release was observed in the isolated rabbit mesenteric (Tropea et al., 1993) and pig coronary arteries (Beny et al., 1987), rat portal vein (Campos et al., 1996) and in human gengival fibroblasts (Lerner and Modéer, 1991). On the other hand, phenidone, as well as MK 571, which has been demonstrated to be incapable of antagonizing contraction induced by bradykinin in the guinea-pig gallbladder (Cabrini et al., 1995), markedly inhibited the contractile response induced by des-Arg^9-bradykinin in this preparation. These results suggest that the contraction caused by des-Arg^9-bradykinin in the guinea-pig gallbladder involves the release of either cyclo or lipooxygenase-derived metabolites from arachidonic acid, in response to the action of this peptide at the bradykinin B_2 receptor. Therefore, these findings are further support for the evidence showing the absence of expression of bradykinin B_1 receptor in guinea-pig tissues (Hall, 1992; Marceau, 1995). Further confirmation of this view was the fact that the treatment of animals with lipopolysaccharide did not significantly alter the des-Arg^9-bradykinin-induced contraction in the guinea-pig gallbladder. On the other hand, this contraction was completely dependent on extracellular Ca^{2+}, partially through L-type Ca^{2+} channels, an effect which differs somewhat from those reported for bradykinin-induced contraction in the guinea-pig gallbladder (Cabrini et al., 1995).

In summary, the findings of the present study confirm and also extend those we described earlier (Cabrini et al., 1995) and provide strong evidence indicating that des-Arg^9-bradykinin mediation in a concentration and time-dependent way of contraction of the guinea-pig gallbladder in vitro occurs through an unexpected mechanism of action which involves stimulation of bradykinin B_2, but not B_1 receptors which was insensitive to the treatment of animals with lipopolysaccharide. In addition, evidence also suggests that protein synthesis, influx of extracellular Ca^{2+} partly sensitive to L-type Ca^{2+} channels, and the presence of both cyclo and lipooxygenase metabolites derived from arachidonic acid pathways, largely account for the contractile response elicited by des-Arg^9-bradykinin in the guinea-pig gallbladder in vitro.

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