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Bradykinin B_1 receptors in the rabbit urinary bladder: induction of responses, smooth muscle contraction, and phosphatidylinositol hydrolysis

Semal K. Butt, Lee G. Dawson & [1]Judith M. Hall

Pharmacology Group, Biomedical Sciences Division, King's College London, Chelsea, London SW3 6LX

1 The aim of this study was to analyse the pharmacological characteristics, and second-messenger coupling-mechanisms, of bradykinin B_1 receptors in an intact tissue, the rabbit urinary bladder; and to investigate the influence of inhibition of endogenous peptidases on kinin activities.

2 In preparations of rabbit mucosa-free urinary bladder, at 90 min after mounting of the preparations, bradykinin (1 nM–10 μM) evoked contractile responses. In contrast, the B_1 receptor-selective agonist [des-Arg^9]-BK (10 nM–10 μM) was only weakly active at this time. Contractile responses to [des-Arg^9]-BK increased with time of tissue incubation in the organ bath, reaching a maximum after 3 h, when the pD_2 estimates were 6.4 ± 0.3 for bradykinin, and 6.9 ± 0.2 for [des-Arg^9]-BK.

3 Once stabilized, responses to [des-Arg^9]-BK in the bladder were competitively antagonized by the B_1 receptor-selective antagonists [Leu^8,des-Arg^9]-BK and D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8,des-Arg^9]-BK ([des-Arg^{10}]-Hoe140) (pK_B estimates were 6.1 ± 0.1 and 7.1 ± 0.1, respectively; $n = 17-21$), but responses were unaffected by the B_2 receptor-selective antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Hoe140) (100 nM; $n = 4$). Contractile responses to bradykinin itself were partially, but significantly, inhibited by the B_1 receptor-selective antagonist, [Leu^8,des-Arg^9]-BK (10 μM) ($P < 0.05$), or by the B_2 receptor-selective antagonist Hoe140 (100 nM) ($P < 0.005$) alone, and were largely blocked by a combination of the two antagonists ($P < 0.0001$).

4 The combined presence of the carboxypeptidase inhibitor DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (mergetpa; 10 μM), the neutral endopeptidase inhibitor, phosphoramidon (1 μM), and the angiotensin-converting enzyme inhibitor, enalaprilat (1 μM) increased the potency of bradykinin 17 fold ($P < 0.001$), but that of [des-Arg^9]-BK was unchanged ($P > 0.05$): pD_2 estimates were 7.6 ± 0.1 and 6.8 ± 0.1 for bradykinin and [des-Arg^9]-BK, respectively, in treated preparations. In the presence of peptidase inhibitors, the affinities of the antagonists [Leu^8,des-Arg^9]-BK and [des-Arg^{10}]-Hoe140 were unchanged as compared with those determined in the absence of peptidase inhibitors ($P > 0.05$). [Leu^8,des-Arg^9]-BK inhibited responses to bradykinin under these conditions ($n = 4$).

5 In endothelium-denuded preparations of the rabbit isolated aorta, an archetypal B_1 receptor preparation, contractile responses to the B_1 receptor-selective agonist [des-Arg^9]-BK (10 nM–10 μM) (and to bradykinin) increased progressively with time of tissue incubation; and [des-Arg^9]-BK responses were completely antagonized by the B_1 receptor antagonist [Leu^8,des-Arg^9]-BK (pK_B 6.3 ± 0.2; $n = 13$).

6 In experiments measuring stimulation of hydrolysis of phosphatidylinositol in rabbit urinary bladder, [des-Arg^9]-BK (10 μM–1 mM), and bradykinin (100 μM) significantly increased accumulation of inositol phosphates ($P < 0.0001$). The increase in accumulation of inositol phosphates evoked by [des-Arg^9]-BK (10 μM–1 mM) was significantly inhibited by [des-Arg^{10}]-Hoe140 (10 μM) ($P < 0.01$).

7 We conclude that in the mucosa-free rabbit urinary bladder, [des-Arg^9]-BK evokes contraction largely via activation of B_1 receptors which have similar properties, including time-dependent induction, to B_1 receptors in the rabbit isolated aorta. Bradykinin evokes contraction via stimulation of both B_1 and B_2 receptors, but does not require conversion by peptidases in order to activate B_1 receptors. We demonstrate, for the first time, B_1 receptor-coupling to phosphatidylinositol hydrolysis in an intact tissue preparation.

Keywords: Kinins; bradykinin; B_1 receptor; bradykinin receptor; urinary bladder (rabbit); phosphatidylinositol; receptor induction; peptidases; [des-Arg^9]-BK; [des-Arg^{10}]-Hoe140

Introduction

The recent introduction of receptor-selective agonist and antagonist kinin analogues has allowed confirmation of the long-standing proposal by Regoli and colleagues of the existence of two types of bradykinin receptor, termed B_1 and B_2 (see: Regoli & Barabé, 1980; Burch *et al.*, 1990; Hall, 1992). Further, the availability of active and stable B_2 receptor antagonist analogues of bradykinin has resulted in extensive study of the pharmacology and biological roles of the B_2 receptor type, and has led to proposals of the existence of further bradykinin receptor types (e.g. Farmer *et al.*, 1989; Farmer & DeSiato, 1994) and, additionally or alternatively, the existence of B_2 receptor species-homologues (Field *et al.*, 1992; Hall, 1992; Hall *et al.*, 1993). In contrast, there has been little study of the pharmacology of B_1 receptors, despite important recent proposals of a role for this receptor type in chronic hyperalgesia (Perkins & Kelly, 1993). This lack of research interest may be attributed, in part at least, to the paucity of reliable bioassay preparations for determining B_1 receptor characteristics and second-messenger coupling-mechanisms; and also the lack of selective, high specific activity, radiolabels for the B_1 receptor site.

This present study set out to identify a suitable non-vascular, functional (non-cell line) tissue preparation for the study of B_1 receptors characteristics; in particular second-messenger coupling-mechanisms. To this end, we chose the

[1] Author for correspondence.

rabbit isolated urinary bladder preparation which we found has the advantage of allowing measurement of B_1 receptor-coupling to phosphatidylinositol hydrolysis in an intact tissue. B_1 receptor-coupling to phosphatidylinositol hydrolysis has previously been demonstrated only in vascular cell lines in culture (Levesque *et al.*, 1993; Tropea *et al.*, 1993; Schneck *et al.*, 1994). In some experiments, peptidase inhibitors, including the carboxypeptidase inhibitor DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid (mergetpa), were used to investigate the influence of endogenous peptidases on agonist potency and antagonist affinity estimates (Hall *et al.*, 1990). In particular, we used the carboxypeptidase inhibitor, mergetpa, to investigate whether bradykinin itself required conversion (e.g. to the C-terminal-arginine deleted fragment, [des-Arg^9]-BK), by enzymes sensitive to this inhibitor, prior to its interaction with B_1 receptors.

Preliminary accounts of this work have been communicated to the Bayliss and Starling Meeting, Southampton, July 1994 (Butt *et al.*, 1994a) and the British Pharmacological Society Meeting, Sunderland, September, 1994 (Butt *et al.*, 1994b).

Methods

Tissue preparation and solutions

Male New Zealand albino rabbits (2.5–3.0 kg) were killed by an i.v. overdose of pentobarbitone sodium (Sagatal), and the urinary bladder and thoracic aorta were removed and placed in oxygenated Krebs solution (composition below). In all experiments, the urinary bladder and aorta were stored overnight in the refrigerator (4°C). Experiments were carried out in Krebs solution of composition (mM): NaCl 118, KH_2PO_4 1.17, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.95, $CaCl_2$ 2.5 and glucose 11. The Krebs solution, at 37°C, was maintained at pH 7.4 by constant bubbling with 95% O_2:5% CO_2.

Isolated tissue studies

The preparations were attached to isometric Grass FT03B force-displacement transducers and suspended in 2.5 ml silanised organ baths. Mechanical activity was recorded with Grass model 7E polygraphs. The urinary bladder was cleared of mucosa and connective tissue and divided longitudinally into four preparations (length 10 mm) and set up under 2 g initial tension. The aorta was divided into strips (10 mm length) and set up under 2 g initial tension. Preparations were left to equilibrate for 60 min followed by determination of the maximum response to carbachol (bladder) or phenylephrine (aorta).

Urinary bladder Thirty minutes after determination of the maximal response to carbachol, cumulative concentration-response curves were obtained for bradykinin (1 nM–10 μM) or [des-Arg^9]-BK (10 nM–10 μM), and thereafter at 30 min intervals until consistent responses were obtained. Preliminary experiments had been carried out to determine antagonist incubation times resulting in optimum inhibition of submaximal kinin agonist responses (see Results). Once responses had stabilized, the antagonists (Hoe140, [Leu^8,des-Arg^9]-BK or [des-Arg^{10}]-Hoe140) were tested by applying the antagonist 5 min prior to determination of cumulative concentration-response curves in two preparations, whilst the other two preparations acted as concurrent time-controls (no antagonist). Each test preparation received one agonist and one antagonist only, with up to four antagonist concentrations tested in a single preparation. Some experiments were carried out in the additional continual presence (30 min pre-incubation) of the peptidase inhibitors mergetpa (10 μM), phosphoramidon (1 μM) and enalaprilat (1 μM). In a separate series of experiments, the effect of [Leu^8,des-Arg^9]-BK and Hoe140, alone and in combination, were tested against single submaximal responses to bradykinin (10 μM). After determination of control responses to bradykinin, one half of the preparations were re-tested following 5 min incubation with [Leu^8,des-Arg^9]-BK (10 μM), the other half following 5 min incubation with Hoe140 (100 nM). All preparations were then tested in the combined presence of [Leu^8,des-Arg^9]-BK (10 μM) and Hoe140 (100 nM). In some experiments, the effect of [Leu^8,des-Arg^9]-BK was tested against responses to bradykinin in the presence of peptidase inhibitors.

Aorta Cumulative concentration-response curves were obtained for bradykinin or [des-Arg^9]-BK (10 nM–10 μM) at 60 min intervals until consistent responses were obtained. The B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK, was then applied 15 min prior to determination of the agonist responses in two preparations, whilst the other two preparations acted as concurrent time-control preparations. Each test preparation received one agonist and antagonist only, with up to three antagonist concentrations tested in a single preparation.

Phosphatidylinositol hydrolysis measurement

The methodology was adapted from Berridge *et al.* (1982) and Bristow *et al.* (1987). Briefly, cross-chopped tissues (350 × 350 μm, McIllwain tissue chopper) was washed three times at 37°C and then 25 μl aliquots added to tubes containing 2 μCi *myo*-[2-^{3}H]-inositol (844 GBq mol^{-1}) and 195 μl Krebs solution. Tubes were oxygenated with 95% O_2:5% CO_2 and placed in a shaking water bath for 60 min for prelabelling of inositol phospholipids. Following prelabelling, 20 μl lithium chloride (150 mM), and drug or drug-combination (to make up 250 μl total volume), was added to each tube; with each drug or drug-combination carried out in triplicate. Tubes were returned to the shaking water-bath following oxygenation, and incubated for a further 45 min at 37°C. Reaction was terminated by addition of 1.0 ml chloroform:methanol:hydrochloric acid (1 M) (100:200:1. v.v.v.), and total [^{3}H]-inositol phosphates extracted as described by Berridge *et al.* (1982). Distilled water (0.30 ml) and chloroform (0.31 ml) were added, the samples centrifuged (1000 *g* for 5 min). The aqueous phase (0.75 ml) was diluted with 2 ml deionised water and added, following 8 ml deionised water (pH 7.0), to a Dowex formate anion-exchange column (AG 1-X8 resin, 100–200 mesh). Total inositol phosphates were eluted with 6 ml ammonium formate:formic acid (800:100 mM) after removing [^{3}H]-inositol and [^{3}H]-glycerophosphoinositol by an 8 ml wash with sodium tetraborate:ammonium formate (5:60 mM). The lipid (0.40 ml) was removed and left overnight to evaporate off the chloroform. Samples were counted by liquid scintillation spectrometry to 2% error (Beckman LS1701) after addition of Liquiscint scintillation fluid and vortexing.

Preliminary experiments were carried out to determine concentrations of agonists (bradykinin, [des-Arg^9]-BK, and carbachol) producing submaximal increases in accumulation of total inositol phosphates (total-[^{3}H]-IPs). The effect of [des-Arg^{10}]-Hoe140 (10 μM) on submaximal increases in accumulation of total-[^{3}H]-IPs evoked by [des-Arg^9]-BK (10 μM and 1 mM) was investigated by co-incubation of agonist and antagonist in the same tubes.

Source of agents

Agents were obtained as follows: sodium pentobarbitone (Sagatal, Rhône Mérieux Limited, Essex), carbamylcholine chloride (carbachol), phosphoramidon (Sigma, Dorset), bradykinin, [des-Arg^9]-BK, [Leu^8,des-Arg^9]-BK (Bachem, Essex), D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Hoe140) (gift Sandoz Institute for Medical Research, London), D-Arg-[Hyp^3,Thi^5, D-Tic^7,Oic^8,des-Arg^9]-BK ([des-Arg^{10}]-Hoe140) (Peninsula Laboratories Europe, Cheshire).

DL-2-Mercaptomethyl-3-guanidino-ethylthiopropanoic acid

(mergetpa) (Calbiochem, CA, U.S.A.), enalaprilat (Merck, Sharp & Dohme, New Jersey, U.S.A.), *myo*-[2-^{3}H]-inositol (Amersham International plc, Buckinghamshire). All inorganic salts were of analytical grade and obtained from B.D.H., Essex. Dowex AG 1-X8 (100–200 mesh, formate form) was obtained from Biorad Laboratories, California, U.S.A., and Liquiscint from National Diagnostics, Buckinghamshire. All agents were dissolved in distilled water and peptides were stored at −20°C.

Expression of results and statistical analysis

The pD_2 (± s.e.mean) estimates were obtained from individual log concentration-response curves, and are defined as the $\log_{10}$ of the concentration producing 50% of the maximal response to the kinin receptor agonist. The pK_B estimates and their s.e.mean were obtained from individual dose-ratio estimates (x) by calculation from the Gaddum-Schild equation, $pK_B = \log_{10}(x\text{-}1) - \log_{10}[A]$, where [A] is the applied antagonist concentration (M), where Schild regressions do not differ from unity. In phosphatidylinositol hydrolysis experiments, increases in accumulation of total [^{3}H]-IPs were calculated and expressed as % over basal. Tests for significant differences were carried out using paired or unpaired Student's *t* tests, as appropriate. Analysis of Schild plots, and tests for unity and linearity of Schild regressions, were according to MacKay (1978); using conventional regression analysis (MINITAB 8.2, PA, U.S.A.) to estimate the coefficients of linear regression (b) and linear correlation (r). Numbers (*n*) shown are for individual estimates of pD_2 or pK_B of preparations (all other experiments); and experimental data are taken from at least 3 animals.

Results

Isolated tissue studies

Induction of agonist responses with time When tested after 90 min organ bath incubation, [des-Arg^9]-BK (10 nM–10 μM) evoked only small contractile responses in the isolated urinary bladder preparation (Figure 1), whereas responses to bradykinin were near the eventual maximum (not shown). Contractile responses to [des-Arg^9]-BK increased progressively with time of incubation of the preparation in the organ bath (Figure 1), but those to bradykinin showed little or no increase over this time period (not shown). Responses to both peptides were stable after 3 h of tissue incubation, and once stabilized, remained constant throughout the remainder of the experiment (a further 2–3 h). At this time, pD_2 estimates for bradykinin and [des-Arg^9]-BK were 6.4 ± 0.3, and 6.9 ± 0.2, respectively (Table 1). The presence of the peptidase inhibitors phosphoramidon (1 μM), enalaprilat (1 μM) and mergetpa (10 μM) resulted in a significant ($P<0.001$) 17 fold leftward-shift of the log concentration-responses curves for bradykinin, with no significant change ($P>0.05$) for [des-Arg^9]-BK (Figure 2; Table 1). In the presence of these inhibitors, pD_2 estimates for bradykinin and [des-Arg^9]-BK were 7.6 ± 0.1 and 6.8 ± 0.1, respectively (Table 1). There was little difference in the rate of induction of responses in peptidase inhibitor-treated preparations (data not shown). In the rabbit aorta, an archetypal B_1 receptor preparation, in the absence of peptidase inhibitors, B_1 response-induction was seen both with [des-Arg^9]-BK (data not shown) and with bradykinin (unpublished data).

Bradykinin receptor antagonist studies Preliminary studies in the bladder showed that maximal inhibition of agonist responses was obtained after 5 min antagonist incubation times for [Leu^8,des-Arg^9]-BK, [des-Arg^{10}]-Hoe140, or Hoe140. Increasing antagonist incubation time (to up to 15 min), in the absence of peptidase inhibitors, resulted in an apparent decrease in the affinities of [Leu^8,des-Arg^9]-BK and [des-

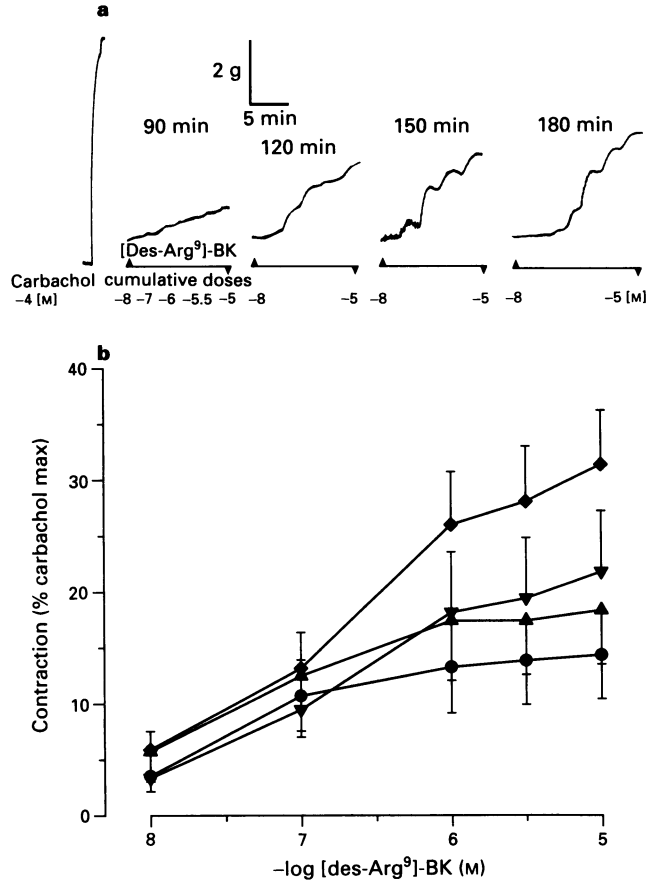

Figure 1 Time-dependent induction of contractile responses of rabbit isolated urinary bladder to [des-Arg^9]-BK. Contractile responses to [des-Arg^9]-BK were obtained by cumulative addition of the agonist. The first concentration-response curve was obtained 90 min following mounting the tissue, and subsequent concentration-response curves determined every 30 min thereafter until no further increase in contractile response was obtained (180 min). The upper panel (a) shows a representative original trace with addition of [des-Arg^9]-BK indicated by the arrows, and shown as the logarithm of the concentration. The lower panel (b) shows averaged data, expressed as % maximum response to carbachol obtained at the start of experiments, with each point representing the mean ± s.e.mean of experiments carried out in 8 preparations (times: ● 90 min; ▲ 120 min; ▼ 150 min; ◆ 180 min).

Table 1 Activity estimates for agonists and antagonists in the rabbit bladder

Contractile potency estimates for agonists (pD_2 ± s.e.mean)	*Control*	*+ Peptidase inhibitors*
Bradykinin	6.35 ± 0.30	7.63 ± 0.14**
[Des-Arg^9]-BK	6.94 ± 0.22	6.82 ± 0.09°
Affinity estimates for antagonists vs. [des-Arg^9]-BK (pK_B ± s.e.mean; *n*)	*Control*	*+ Peptidase inhibitors*
[Leu^8,des-Arg^9]-BK	6.06 ± 0.10	6.12 ± 0.09°
[Des-Arg^{10}]-Hoe 140	7.08 ± 0.13	7.09 ± 0.06°

Potencies of agonists are shown in terms of pD_2 ($-\log_{10}EC_{50}$) estimates ($n = 4$–29). Affinity estimates are given as pK_B values, since Schild slopes do not differ significantly ($P>0.05$; $n = 17$–57) from unity over the range used in analysis (see Figure 3 and Results). Significance differences between controls, and estimates in the presence of peptidase inhibitors phosphoramidon (1 μM), enalaprilat (1 μM) and DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (mergetpa; 10 μM), are shown as: **$P<0.001$; °$P>0.05$.

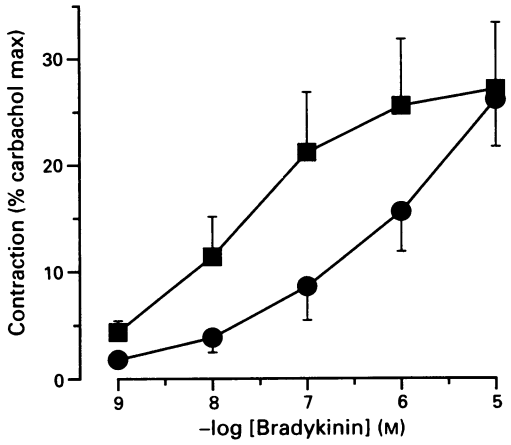

Figure 2 Peptidase inhibitors potentiate contractile responses to bradykinin in rabbit urinary bladder. Control log concentration-response curves (●) are compared to those obtained in the presence of the peptidase inhibitors enalaprilat (1 μM), phosphoramidon (1 μM) and DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid (mergetpa; 10 μM) (■). Contractile responses to bradykinin are expressed as % maximal response to carbachol, and are the mean ± s.e.mean of data obtained in 4–16 preparations.

Arg[10]-Hoe140 which was not prevented by the presence of peptidase inhibitors (data not shown). In concurrent time-control preparations, the lateral position and maximum response to [des-Arg[9]]-BK did not change with time (data not shown). Using a 5 min incubation time, the B_1 receptor antagonists [Leu[8],des-Arg[9]]-BK, and [des-Arg[10]]-Hoe140, showed competitive antagonism of contractile responses to the B_1 receptor-selective agonist [des-Arg[9]]-BK in the urinary bladder: pK_B estimates were 6.1 ± 0.1 and 7.1 ± 0.1, respectively (Figure 3 and Table 1). In these experiments under control conditions [Leu[8],des-Arg[9]]-BK gave a linear Schild regression of unity (slope 1.03 ± 0.22, correlation 0.8, $n = 17$). In contrast, [des-Arg[10]]-Hoe140 showed some flattening of the Schild regression at the highest concentration studied (10 μM), but when these data were excluded from analysis, the remaining range of concentrations (0.1–3 μM) gave satisfactory parameter estimates (slope 1.01 ± 0.24, correlation 0.7, $n = 21$). The reason for this behaviour of the antagonist at high concentration was not investigated further. In the rabbit isolated aorta preparation, [Leu[8],des-Arg[9]]-BK competitively antagonized responses to [des-Arg[9]]-BK (pK_B 6.3 ± 0.2, slope 1.04 ± 0.25, correlation 0.7, $n = 13$; Figure 3). The B_2 receptor-selective antagonist Hoe140 (100 nM) did not inhibit contractile responses to [des-Arg[9]]-BK in either preparation ($n = 4$, $P > 0.05$; data not shown). In the presence of the peptidase inhibitors phosphoramidon (1 μM), enalaprilat (1 μM) and mergetpa (10 μM), the affinities in the urinary bladder of [Leu[8],des-Arg[9]]-BK and [des-Arg[10]]-Hoe140 in antagonizing [des-Arg[9]]-BK were no different ($P > 0.05$) from those determined in the absence of peptidase inhibitors, with pK_B estimates of 6.1 ± 0.1 ($n = 20$) and 7.1 ± 0.1 ($n = 57$), respectively (Table 1). Contractile responses evoked by bradykinin in the urinary bladder were significantly inhibited by the B_1 receptor antagonists [Leu[8],des-Arg[9]]-BK (10 μM; $P < 0.05$), or by the B_2 receptor antagonist Hoe140 (100 nM; $P < 0.005$) (Figure 3) alone; and to a marked extent when used in combination at these concentrations, when bradykinin responses were reduced to 7.0% of control; $P < 0.0001$) (Figure 3). In the presence of the carboxypeptidase inhibitor mergetpa, responses to bradykinin were still inhibited by [Leu[8],des-Arg[9]]-BK (1–30 μM; $n = 6$; data not shown).

Phosphatidylinositol hydrolysis studies

Bradykinin (100 μM) and [des-Arg[9]]-BK (10 μM–1 mM) significantly increased accumulation of total-[^{3}H]-IPs ($P <$ 0.0001) (Figure 4). Submaximal increases in accumulation of total-[^{3}H]-IPs evoked by [des-Arg[9]]-BK were significantly inhibited by co-incubation with [des-Arg[10]]-Hoe140 (10 μM; $P < 0.01$, $n = 11–15$) (Figure 4). The antagonist [des-Arg[10]]-Hoe140 (10 μM) had no action in its own right (Figure 4).

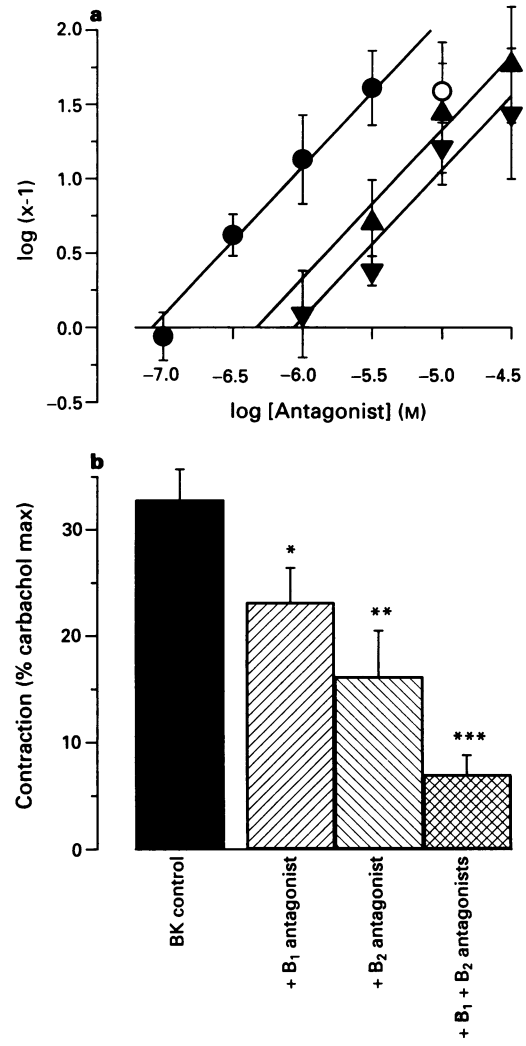

Figure 3 Antagonism of contractile responses to [des-Arg[9]]-BK and bradykinin in rabbit isolated urinary bladder by bradykinin receptor antagonists. In (a), Schild regressions are shown for antagonism of responses to [des-Arg[9]]-BK by [des-Arg[10]]-Hoe140 (●) and [Leu[8],des-Arg[9]]-BK (▼) in the urinary bladder; and for comparison, by [Leu[8],des-Arg[9]]-BK (▲) in the aorta. The lines fitted are of unity slope since the Schild regressions did not differ significantly ($P > 0.05$) from b = 1.0 over the range analysed and the correlation (r) was reasonably high. Estimates for [Leu[8],des-Arg[9]]-BK in the bladder were $pK_B = 6.06 \pm 0.10$, b = 1.03 ± 0.22, $r = 0.8$, $n = 17$; and in the aorta $pK_B = 6.33 \pm 0.24$, b = 1.04 ± 0.25, $r = 0.7$, $n = 13$. For [des-Arg[10]]-Hoe140 in the bladder, the estimates were $pK_B = 7.08 \pm 0.13$, b = 1.01 ± 0.24, $r = 0.7$, $n = 21$; analysed discounting the data at 10 μM (○) (see Results). The pK_B estimates are summarized in Table 1. In (b), submaximal control responses to bradykinin (10 μM; solid column) in the rabbit urinary bladder are significantly inhibited by the B_1 receptor antagonist [Leu[8],des-Arg[9]]-BK (10 μM; left-hatched column) and by the B_2 receptor antagonist Hoe140 (100 nM; right-hatched column), and further inhibited by a combination of the two antagonists (cross-hatched column). Data are shown as mean ± s.e.mean and represent data obtained from experiments carried out in 6–19 preparations. Significance is denoted: $*P < 0.05$, $**P < 0.005$, $***P < 0.0001$.

Discussion

This study demonstrates, in the mucosa-free rabbit urinary bladder preparation, that [des-Arg[9]]-BK causes contraction

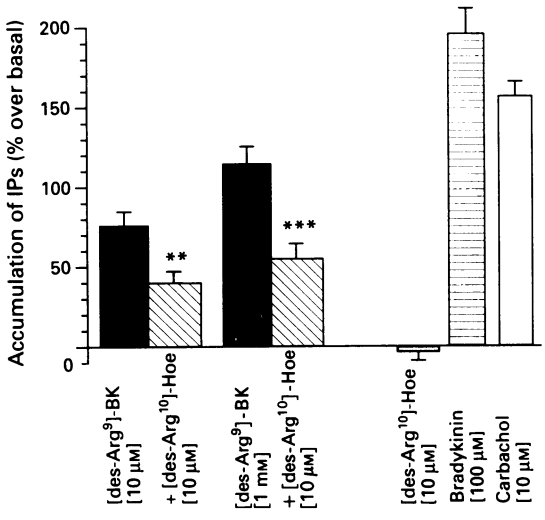

Figure 4 Effect of [des-Arg^{10}]-Hoe140 on [des-Arg^9]-BK-evoked accumulation of total-[^{3}H]-inositol phosphates (total-[^{3}H]-IPs). The filled columns show control increases in total-[^{3}H]-IPs evoked by [des-Arg^9]-BK (10 μM and 1 mM), the right-hatched columns show [des-Arg^9]-BK-evoked increases in total-[^{3}H]-IPs obtained in the presence of B_1 receptor antagonist [des-Arg^{10}]-Hoe140 (10 μM); the stippled column represents the effect of [des-Arg^{10}]-Hoe140 (10 μM) alone, on basal accumulation of total-[^{3}H]-IPs. Bradykinin-evoked (100 μM) and carbachol-evoked (10 mM) increases in total-[^{3}H]-IPs are shown as horizontal-fill and open columns, respectively. Data are shown as mean ± s.e.mean for at least 5 experiments carried out in triplicate. All agonists significantly increased accumulation of total-[^{3}H]-IPs ($P<0.0001$), and the increase evoked by [des-Arg^9]-BK was significantly inhibited by [des-Arg^{10}]-Hoe140 ($P<0.01$). [des-Arg^{10}]-Hoe140 did not significantly affect basal accumulation of total-[^{3}H]-IPs ($P>0.05$) in its own right.

largely via B_1 receptor-activation, whereas bradykinin contracts the urinary bladder via an interaction with both B_1 and B_2 receptors. The B_1 receptors in the urinary bladder have similar recognition properties and response characteristics, including time-dependent induction, to those in an archetypal B_1 receptor preparation, the rabbit isolated aorta. Metabolism of kinins by endogenous peptidases sensitive to the peptidase inhibitors used in this study does not influence B_1 receptor response-induction characteristics or antagonist affinities in this preparation, though does have the effect of decreasing the apparent potency of bradykinin (but not that of [des-Arg^9]-BK). Contractile responses to bradykinin are still obtained in the presence of mergetpa, and are in part inhibited by B_2 receptor antagonists (as well as by B_1 receptor antagonists). This suggests that conversion of bradykinin to [des-Arg^9]-BK by mergetpa-sensitive peptidases is not necessary for the interaction of bradykinin with B_1 receptors. We demonstrate, for the first time, B_1 receptor-coupling to phosphatidylinositol hydrolysis in a functional (non-cell line) preparation.

Contractile responses in the rabbit urinary bladder to the B_1 receptor-selective agonist [des-Arg^9]-BK increased progressively during tissue incubation time. Induction of B_1 receptor-responses has previously been described in other preparations including; the rabbit aorta (present study; see Regoli *et al.*, 1977), rabbit mesenteric artery (Regoli *et al.*, 1978; Boutillier *et al.*, 1987) and rat duodenum (Boschcov *et al.*, 1984). Once stabilized, responses to [des-Arg^9]-BK in both the rabbit bladder and aorta preparations were competitively antagonized by B_1 receptor-selective antagonists. The B_1 receptor-selective antagonist, [Leu^8,des-Arg^9]-BK had a similar affinity in both preparations (pK_B estimates of 6.1 and 6.3, respectively). The affinity of [Leu^8,des-Arg^9]-BK in these two preparations is lower than its affinity reported previously in the rabbit aorta (pA_2 6.8; Regoli *et al.*, 1978) and mesenteric artery (pA_2 6.5; Churchill & Ward, 1986). However, this discrepancy does not appear to result from a non-equilibrium state of the antagonist with the receptors, since increasing antagonist incubation time decreased, rather than increased, antagonist apparent affinities. Furthermore, the lower affinities do not seem to be a consequence of antagonist degradation by tissue peptidases, since affinity estimates determined in the presence of inhibitors of neutral endopeptidase (E.C. 3.4.24.11), angiotensin-converting enzyme (E.C. 3.4.15.1) and carboxypeptidases (see Orawski & Simmons, 1989), had no significant effect on antagonist affinity estimates. No appreciable interaction of the B_1 receptor-selective agonist [des-Arg^9]-BK with B_2 receptors seems likely, in as much as [des-Arg^9]-BK is without activity in the rabbit isolated jugular vein, a B_2 mono-receptor preparation (Regoli & Barabé, 1980; Butt *et al.*, 1994a,b), and no complication in interpretation due to differences between species needs to be taken into account (see Hall, 1992; Hall *et al.*, 1993). The novel B_1 receptor antagonist [des-Arg^{10}]-Hoe140 (Wirth *et al.*, 1991) has a ten fold higher affinity than [Leu^8,des-Arg^9]-BK. The relatively high affinity of this antagonist has previously been reported (Wirth *et al.*, 1991): we show here that at concentrations less than 10 μM it acts in a manner compatible with equilibrium competition, though shows a flattened Schild regression at 10 μM. Whether, amongst some plausible explanations, this was due to complications arising from non-equilibrium conditions, non-specific depression of the tissue, or cross-talk of [des-Arg^9]-BK to B_2 receptors at high dose-ratios, is not clear from the present experiments.

With respect to contractile responses to bradykinin itself in the rabbit urinary bladder; in contrast to those to [des-Arg^9]-BK, contractile responses to bradykinin were evident at the start of the experiment, and were little changed with time of tissue incubation. This suggests that bradykinin contracts the rabbit bladder predominantly via stimulation of B_2 receptors. This conclusion was supported by the finding that responses to bradykinin were partially inhibited by the B_2 receptor antagonist Hoe140 (100 nM), though some interaction with B_1 receptors also seems likely since responses to bradykinin were also partially inhibited by the B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK (10 μM). Although bradykinin has been shown to have some pharmacological activity in both B_1 and B_2 receptor preparations (see Hall, 1992), it has not previously been established whether decarboxylation of bradykinin by mergetpa-sensitive peptidases (i.e. to [des-Arg^9]-BK), is required for its interaction with B_1 receptors. In the present study, we showed that, in the presence of peptidase inhibitors including the carboxypeptidase inhibitor mergetpa, the contractile responses evoked by bradykinin were still evident (indeed potentiated); and further, were in part inhibited by the B_1 receptor antagonist [Leu^8,des-Arg^9]-BK, thus suggesting that bradykinin can itself interact with B_1 receptors (although it is not possible to discount N-terminal cleavage by a mergepta-resistant carboxypeptidase).

In the present study, we demonstrated for the first time in an intact functional preparation, that B_1 receptor activation stimulates phosphatidylinositol hydrolysis. Thus, the B_1 receptor-selective agonist [des-Arg^9]-BK evoked a concentration-related increase in accumulation of total-[^{3}H]-IPs, and this increase was significantly inhibited by the B_1 receptor-selective antagonist [des-Arg^{10}]-Hoe140. Recently, B_1 receptor evoked stimulation of phosphatidylinositol hydrolysis was described in rabbit aorta (Schneck *et al.*, 1994), and superior mesenteric artery (Tropea *et al.*, 1993), smooth muscle cells in culture.

We conclude that in the mucosa-free rabbit urinary bladder, [des-Arg^9]-BK causes contraction via B_1 receptor activation, whereas bradykinin itself evokes contraction via B_1 and B_2 receptor activation. Although kinin agonists are degraded by endogenous peptidases, bradykinin does not require conversion by enalaprilat, mergetpa or phosphoramidon-sensitive peptidases in order to activate B_1 receptors in this preparation. The B_1 receptors of the urinary bladder have

similar recognition properties and response characteristics, including time-dependent induction, to those in the rabbit isolated aorta. We demonstrate for the first time, B_1 receptor-coupling to phosphatidylinositol hydrolysis in an intact functioning tissue preparation rather than in a cell line.

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