Bradykinin B₂ receptors and coupling mechanisms in the smooth muscle of the guinea-pig taenia caeci

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1 In the smooth muscle of the guinea-pig taenia caeci, bradykinin produces a relaxation followed by a contraction. In the presence of hexamethonium and guanethidine, both these phases of the response were insensitive to tetrodotoxin (100 nM), ω -conotoxin GVIA (100 nM) and ibuprofen (1 μ M), suggesting that they are due to a direct action on the smooth muscle.

2 The B₁ receptor-selective agonist, [des-Arg⁹]-BK (1–100 μ M), was inactive in the taenia caeci, and the B₁ receptor-selective antagonist, [Leu⁸,des-Arg⁹]-BK (1–10 μ M), did not inhibit either phase of the bradykinin-induced response. The B₂ receptor-selective antagonist, D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Hoe 140) (30–300 nM), inhibited both the bradykinin-induced relaxation and contraction with a similar affinity (apparent pK_B estimates of 8.5 $\pm$ 0.1 and 8.4 $\pm$ 0.1 respectively).

3 In a depolarizing high-K⁺-solution, bradykinin produced concentration-related contractions, though of diminished magnitude; but no relaxation was observed in such media. In Krebs solution, the Ca²⁺-activated K⁺-channel blocker, apamin (10 nM), abolished relaxant responses. These observations suggest that contraction results both from membrane potential-dependent, and membrane potential-independent, mechanisms; whereas relaxant responses result entirely from membrane potential-dependent mechanisms. Contractile responses obtained in the high K⁺-solution were inhibited by D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK with an apparent pK_B value of 8.4 $\pm$ 0.1.

4 In a Ca²⁺-free, EGTA-containing medium, relatively high concentrations of bradykinin (> 100 nM) produced transient contractions, suggesting that a component of the contractile response results from release of Ca²⁺ from an intracellular store. This intracellular Ca²⁺ store could be refilled in the presence of extracellular Ca²⁺. The B₁ receptor antagonist, [Leu⁸,des-Arg⁹]-BK (10 μ M), did not inhibit this bradykinin-induced contraction, whereas the B₂ receptor antagonist, D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (100 nM) markedly attenuated it (P < 0.001; n = 6).

5 Bradykinin (10 nM–100 μ M) significantly elevated tissue levels of total [³H]-inositol phosphates in the presence of Li⁺, after incubation with *myo*-[³H]-inositol. The B₁ receptor-selective agonist, [des-Arg⁹]-BK (100 μ M) did not stimulate [³H]-inositol phosphate formation, and the B₁ receptor-selective antagonist, [Leu⁸,des-Arg⁹]-BK, did not inhibit the formation of [³H]-inositol phosphates in response to a sub-maximal concentration of bradykinin (10 μ M; P > 0.05). Two B₂ receptor antagonists, D-Arg-[Hyp³,D-Phe⁷]-BK and D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK, inhibited bradykinin-induced accumulation of total [³H]-inositol phosphates with apparent pK_B estimates of 5.4 $\pm$ 0.3 and 8.4 $\pm$ 0.1, respectively.

6 These data suggest that in the guinea-pig taenia caeci, the five aspects of the action of bradykinin studied (the relaxant and the contractile elements of the biphasic mechanical response, the contractile response in a depolarizing high-K⁺ solution medium and zero-Ca²⁺ media, and stimulation of phosphatidylinositol turnover), all result from activation of B₂ receptors. A possible causal relationship is suggested between these B₂ receptor-mediated membrane potential-dependent, and -independent events, and their roles in excitation contraction coupling.

Keywords: Bradykinin receptors; bradykinin B₂ receptors; bradykinin receptor antagonists; bradykinin receptor mechanisms; taenia caeci (guinea-pig); electromechanical coupling; pharmacomechanical coupling; phosphatidylinositol turnover; Ca²⁺-activated K⁺-channel; apamin

Introduction

Bradykinin receptors have been divided into two types, designated B₁ and B₂ (see Regoli & Barabé, 1980; see Hall, 1992). In isolated smooth muscle preparations, activation of these receptors leads to relaxation and/or contraction; and these responses may result from a direct action on the smooth muscle, or involve the release of other mediators (see Hall, 1992). Two signal transduction pathways have been linked to bradykinin receptor activation in smooth muscle, phosphatidylinositol hydrolysis (see Hall, 1992; Marsh & Hill, 1992; Ransom *et al.*, 1992) and eicosanoid biosynthesis (Bramley *et al.*, 1990; see Burch, 1990; Farmer & Burch, 1992 and Hall, 1992).

In the guinea-pig taenia caeci, bradykinin produces a biphasic response consisting of a fast relaxation followed by a slow contraction (Carter *et al.*, 1986). This is in contrast to the fast monophasic contractions seen in this preparation

with a number of spasmogens including carbachol, substance P and histamine (Hall *et al.*, 1986; Hall & Morton, 1991a). Stimulation of one bradykinin receptor type could cause both phases of the response; or alternatively, the two phases could result from activation of two distinct receptor types. Additionally, one or both phases may follow release of neuronally-derived mediators or cyclo-oxygenase products. The aim of this study was to examine these possibilities by determining the involvement of cyclo-oxygenase and neuronal-derived mediators and the nature of the receptors and coupling-mechanisms involved in each component of the biphasic response to bradykinin in the guinea-pig taenia caeci. To this end, experiments included functional tissue-contraction studies, measurement of phosphatidylinositol hydrolysis, and determination of the affinities of the B₁ and B₂ bradykinin receptor antagonists, [Leu⁸,des-Arg⁹]-BK (Regoli & Barabé, 1980) and D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Hock *et al.*, 1991), respectively.

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Preliminary accounts of these results have been communicated to the British Pharmacological Society (Hall *et al.*, 1991; Field *et al.*, 1992a).

Methods

Tissue preparation

Two of the three available taenia caeci longitudinal muscle strips were dissected from the caecum of male, Dunkin-Hartley guinea-pigs (450–800 g), killed by stunning and exsanguination.

Isolated tissue studies

Segments of taenia caeci were mounted in 2 ml organ baths, by use of a micrometer-controlled isometric assembly that allowed precise length adjustment, with an initial resting tension of 500 mg. In taenia caeci preparations which developed spontaneous tone, a biphasic response to bradykinin was observed; only such tissues were used in this study. Tension was measured by means of Grass FT03B force-displacement transducers coupled to JJ Instruments CR342 potentiometric flat-bed recorders following d.c. amplification, or Grass model 7E polygraphs. Responses to bradykinin were normally compared with carbachol, as a control, in the same preparation; so allowing normalization of tension in terms of maximum individual tensions developed with carbachol.

All experiments, unless otherwise stated, were carried out in Krebs solution (composition (mM): NaCl 118, KCl 4.6, MgSO₄ 1.15, NaHCO₃ 25, CaCl₂ 2.5, KH₂PO₄ 1.2, glucose 10), maintained at 37°C and oxygenated with 95% O₂:5% CO₂. The Krebs solution contained atropine, mepyramine, cimetidine, guanethidine, (all at 1 µM) and hexamethonium (10 µM). In experiments to test for the involvement of neuronal activity or production of prostanoids in the responses to bradykinin, full concentration-response curves were compared in the presence and absence of tetrodotoxin (100 nM), ω -conotoxin GVIA (100 nM) or ibuprofen (1 µM). In experiments with apamin, tissues were incubated with this channel-blocker (10 nM) for at least 10 min.

Experiments in high-K⁺ media

In experiments in which responses in the absence of membrane potential were studied, after equilibration in Krebs solution, tissues were transferred to a high-K⁺ medium (composition (mM): K₂SO₄ 107, Na₂SO₄ 2.5, MgCl₂ 1.15, KHCO₃ 16, CaCl₂ 5, KCl 5, Na phosphate buffer 0.4, glucose 10; see Jenkinson & Morton, 1967a,b), maintained at 37°C and oxygenated with 95% O₂:5% CO₂. Bradykinin responses were compared, throughout, with those to carbachol in the same preparations. This high-K⁺ medium, based on impermeant SO₄²⁻, has been shown in the taenia caeci preparation to prevent swelling with time (Durbin & Jenkinson, 1961) which is otherwise observed with KCl media, and allows responses and changes in ion permeability to be studied over relatively long periods (Jenkinson & Morton, 1967a,b). However, for comparison, some control experiments were also made testing bradykinin responses at various times after exposure (5, 10, 15 and 20 min) to a high-K⁺ medium with 30 mM KCl substituted for NaCl in the Krebs solution, where there was an initial contracture and subsequent fall in tension.

Experiments in reduced-Na⁺ medium

Taenia preparations were mounted in Krebs solution, and then contractility was tested in a modified Krebs medium containing 75, 50 and 25 mM Na⁺; where reduced Na⁺ was replaced with equimolar tris-HCl buffer (Brading *et al.*,

1980). In one series, full concentration-response curves to bradykinin were obtained; and in another, comparison was made between matched submaximal concentrations of bradykinin (1 µM), carbachol (100 nM), and KCl (30 mM).

Antagonists pK_B estimates for responses in Krebs and high-K⁺ media

In both normal Krebs and high-K⁺ media, concentration-response curves to bradykinin were obtained using sequential dosing in a randomized block-design, with responses compared with those obtained in the presence of bradykinin receptor antagonist (test preparation), or no antagonist (concurrent-control preparation). A 12 min bradykinin dose-cycle was used in conjunction with a 10 min antagonist pre-test incubation period. In studies with high-K⁺ medium, tissues were first placed in Krebs solution and responses obtained to bradykinin and carbachol. Responses were normalized in each preparation in terms of the maximal carbachol contraction obtained in Krebs solution, and the estimates are shown as means $\pm$ s.e.mean.

Experiments in Ca²⁺-free medium

Submaximal contractile responses to bradykinin (10 µM) were obtained in Krebs solution, and in a modified-Krebs Ca²⁺-free medium (Ca²⁺ replaced by equimolar Mg²⁺, with 1 mM EGTA) for 3 min. To refill Ca²⁺-stores, tissues were placed in a modified Krebs depolarizing-medium containing 5 mM Ca²⁺ and 30 mM K⁺, for 15 min. In experiments to determine the receptor type involved in these contractions, individual preparations were tested with single applications of bradykinin (10 µM) in Ca²⁺-free Krebs medium, each challenge after a 15 min period in the modified Krebs Ca²⁺-containing medium to refill stores. After stabilization of responses, preparations were re-tested with bradykinin (10 µM) in the presence of either the B₁ receptor antagonist [Leu⁸,des-Arg⁹]-BK (10 µM) or the B₂ receptor antagonist D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (100 nM); or no antagonist (controls).

Phosphatidylinositol hydrolysis

The methodology was adapted from Berridge *et al.* (1982) (see Hall & Morton, 1991a). Briefly, cross-chopped (350 $\times$ 350 µm) tissues were washed twice in Krebs solution (composition as above, but omitting atropine) at 37°C, then incubated under 95% O₂:5% CO₂ with cleaned myo-[2-³H]-inositol (844 GBq mol⁻¹) for 1 h at 37°C in a shaking water-bath, followed by washing and resuspension in Krebs solution. Tissue aliquots (40 µl) were transferred to vials containing Li⁺ (12 mM) and agonist, with or without antagonist, in Krebs solution to a final volume of 250 µl under 95% O₂:5% CO₂. Tubes were incubated at 37°C for 30 min in a shaking water-bath. The reaction was terminated by addition of 940 µl of chloroform:methanol:HCl (100:200:2,v:v:v)(0.1 M HCl), followed by 300 µl each of chloroform and distilled water and centrifugation (1000 g for 5 min). A 800 µl aliquot of the aqueous phase was applied with 8 ml of water to a Dowex-formate anion-exchange column (AG1-X8 resin, Biorad Laboratories, California, U.S.A.). The inositol phosphates were eluted with 6 ml 800 mM ammonium formate: 100 mM formic acid after removal of [³H]-inositol and other labelled impurities, by 2 $\times$ 8 ml washes with 5 mM sodium tetraborate: 60 mM ammonium formate. The [³H]-inositol phosphate eluate was collected and the samples counted by liquid scintillation spectrometry after addition of Liquescent (National Diagnostics, U.K.) and vortexing. No attempt was made to separate the inositol phosphates and therefore results are described as total [³H]-inositol phosphates ([³H]-IPs). The lipid (400 µl) was removed and left overnight before counting these samples as above. To allow for variation in absolute amounts of tissue samples, results were normalized

with respect to sample lipid content, and are expressed as the % maximal carbachol-induced accumulation of total [^3H]-inositol phosphates (see Hall & Morton, 1991a). This normalization procedure was valid only if the agonists do not increase incorporation of ^3H into lipids, and under these conditions neither carbachol (Hall & Morton, 1991a), nor bradykinin (bradykinin $118 \pm 26\%$ of basal control 100% ; $P > 0.05$; $n = 8$), increased ^3H incorporation into the lipid fraction.

Materials

Agents were obtained as follows: carbamylcholine chloride (carbachol), atropine sulphate, tetrodotoxin, ω -conotoxin GVIA, ibuprofen and hexamethonium bromide (Sigma, UK), mepyramine maleate (May & Baker, UK), cimetidine (Smith, Kline & French, UK), guanethidine sulphate (Ciba, UK), bradykinin, D-Arg-[Hyp ^{3} ,D-Phe ^{7}]-BK (Bachem, UK). D-Arg-[Hyp ^{3} ,Thi ^{5} ,D-Tic ^{7} ,Oic ^{8}]-BK (Hoe 140) was a kind gift from Dr A. Hallett, Sandoz Institute for Medical Research, London. All salts were of analytical grade and were obtained from B.D.H., U.K. All drugs were dissolved in distilled water (except ibuprofen in 5% Na_2CO_3), and peptides were stored deep frozen.

Expression of results and statistical analysis

In all experiments, apparent pK_B estimates and their s.e.mean were obtained from individual dose-ratio estimates (x) between responses in test and control preparations, by calculation from the Gaddum-Schild equation, $\text{pK}_B = -\log_{10} K_B = \log_{10} (x-1) - \log_{10} [A]$, where $[A]$ is the antagonist concentration. In functional experiments in Krebs solution, some depression of the maximal contractile response to bradykinin was observed with high concentrations of D-Arg-[Hyp ^{3} ,Thi ^{5} ,D-Tic ^{7} ,Oic ^{8}]-BK, so affinity estimates were taken from individual dose-ratios only where no depression was evident (see Field *et al.*, 1992b). Affinity estimates are referred to throughout as apparent pK_B values, since there was no full Schild analysis to test for competition. Tests for statistical significance were made using Student's t test for two independent-samples, or paired-samples, as appropriate. EC_{50} estimates are shown with 95% confidence limits.

Results

Functional studies

The taenia caeci preparations used in this study had a moderate degree of natural tone where, as shown in Figure 1, a typical response to bradykinin is bisphasic with an immediate initial relaxant phase (EC_{50} 1.3 (0.8–2.0 μM); followed by a delayed second contractile phase (EC_{50} 0.1 (0.05–0.2) μM) which is markedly slow as compared with the fast monophasic contractile responses to carbachol in the same tissue. In preparations with greater or lesser degrees of tone, the relaxant or contractile phases, respectively, are relatively enhanced (data not shown). Both the relaxant and contractile components of the response to bradykinin appear to be mediated through a direct action on smooth muscle cells since the responses were unaffected by tetrodotoxin, ω -conotoxin (both 100 nM) or ibuprofen (1 μM) ($n = 4-6$; data not shown). The Ca^{2+} -activated K^+ -channel blocker, apamin (10 nM), completely blocked the relaxant phase without affecting the contractile phase (data not shown; Carter *et al.*, 1986).

The B_1 receptor-selective agonist, [des-Arg ^{9}]-BK (1–100 μM), was inactive and the B_1 receptor-selective antagonist, [Leu ^{8} ,des-Arg ^{9}]-BK (1–10 μM), did not inhibit either the relaxation or contraction in response to bradykinin (1 nM–10 μM ; data not shown). The B_2 receptor antagonist, D-Arg-[Hyp ^{3} ,Thi ^{5} ,D-Tic ^{7} ,Oic ^{8}]-BK (30–300 nM), potently

antagonized both the relaxant and contractile phases of the biphasic response with apparent pK_B estimates of 8.4 ± 0.2 and 8.4 ± 0.1 ($n = 12$ for both phases; Figure 2, Table 1).

Experiments in Ca^{2+} -free medium

Bradykinin (10 μM) produced a transient monophasic contractile response in Ca^{2+} -free EGTA-containing medium which, on first challenge, was $37.5 \pm 5.2\%$ ($n = 20$) of the control bradykinin contractile response obtained in normal Krebs solution (see Figure 3a). This contractile response decreased with repeated applications of bradykinin in the Ca^{2+} -free medium (Figure 3a), but could be restored if tissues were incubated in a modified depolarizing (30 mM K^+) Ca^{2+} -containing (5 mM) Krebs medium to refill stores (Figure 3a). In experiments to determine the type of bradykinin receptor involved in contractile responses that are independent of external- Ca^{2+} , preparations were tested in the absence, and then the presence, of the B_1 receptor-selective

Figure 1 Typical biphasic concentration-related isometric tension records for bradykinin (BK) in the guinea-pig isolated taenia caeci preparation, compared to the fast monophasic contractile response to carbachol (CCh). The preparation was mounted in Krebs solution at 37°C . This figure is representative of >12 experiments.

Figure 2 Log concentration-response curves for the biphasic responses to bradykinin in the guinea-pig taenia caeci and their antagonism by D-Arg-[Hyp ^{3} ,Thi ^{5} ,D-Tic ^{7} ,Oic ^{8}]-BK. The upper section represents bradykinin-induced contraction, and the lower, bradykinin-induced relaxation; with both responses shown in terms of normalized tension, as % of maximal responses to carbachol in individual preparations. Curves are control ($\bullet$), or in the presence of antagonist at 30 nM ($\blacktriangle$), 100 nM ($\blacksquare$), and 300 nM ($\blacktriangledown$). Each point is the mean ($\pm$ s.e.mean) from 9–12 preparations.

Table 1 Affinity estimates for D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK against bradykinin

System	Response	Medium	pK_B (s.e.mean; n)
Functional	Contraction	Krebs medium	8.4 (0.1; 12)
Functional	Relaxation	Krebs medium	8.5 (0.1; 12)
Functional	Contraction	High-K ⁺ medium	8.4 (0.1; 7)
Biochemical	Phosphatidylinositol hydrolysis	Krebs medium	8.4 (0.1; 4)

Figure 3 Mechanical responses to bradykinin (10 μ M) were tested in a zero-Ca²⁺ medium. In (a), typical responses with isometric recording at 37°C, are shown in normal Krebs solution, followed by responses to bradykinin in the Ca²⁺-free medium (see Methods) that diminish on re-challenge; and then a restored response after a third challenge in the Ca²⁺-free medium, following 15 min incubation in a Ca²⁺-store refilling-medium (5 mM Ca²⁺, 30 mM K⁺; see methods). In (b) a similar protocol is shown, except after Ca²⁺-store refilling a contracture is induced with substance P (SP, 10 μ M) before addition of bradykinin (10 μ M) in order to demonstrate a relaxant phase in the action of the latter. In (c) the receptor type involved in contractions in the Ca²⁺-free medium is tested by including a B₁ receptor-selective antagonist ([Leu⁸,des-Arg⁹]-BK; 10 μ M) or a B₂ receptor-selective antagonist (D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (100 nM) in the Ca²⁺-free medium during re-challenge after Ca²⁺-store refilling, with a third group acting as control: there was a significant attenuation of bradykinin responses (compared with the control level of 100%) after blockade of B₂ (** P < 0.001; n = 6), but not B₁, receptors.

antagonist, [Leu⁸,des-Arg⁹]-BK (10 μ M) or the B₂ receptor-selective antagonist, D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (100 nM), and compared with appropriate concurrent controls (Figure 3c). The B₁ receptor antagonist was without effect (P > 0.05), whereas the B₂ receptor antagonist markedly attenuated these responses (P < 0.001); controls showed no significant change in response (P > 0.05) on re-testing. No relaxant responses were seen in this Ca²⁺-free medium, where there is no natural tone; but when transient contractions were induced by application of substance P (10 μ M), relaxant responses could be demonstrated (Figure 3b).

Experiments in reduced-Na⁺ medium

Contractile responses to bradykinin were attenuated in reduced-Na⁺ media, showing, with 75, 50 and 25 mM Na⁺, a progressive rightward shift of the log concentration-response curves with some depression of maximum (data not shown). In experiments with matched responses to carbachol (100 nM) and KCl (30 mM), responses to bradykinin (1 μ M) were reduced: at these Na⁺ concentrations to 63.6 $\pm$ 7.2%, 48.3 $\pm$ 5.6% and 39.1 $\pm$ 4.3%, respectively, of the bradykinin

Figure 4 Bradykinin-induced contractile responses in Krebs solution and a high-K⁺ medium, and antagonism of responses under the latter conditions by D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK. (a) Shows log concentration-response curves produced by bradykinin in Krebs solution (●), as compared with those after incubation of the preparation in the high-K⁺ depolarizing medium (○); (b) shows, in the high-K⁺ medium, the shift to the right of the responses curves in the presence of antagonist: control (○), and in the presence of 30 nM D-Arg-[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (●). Each point is the mean ($\pm$ s.e.mean) taken from 4–6 experiments. Note rescaling of abscissae in (b).

Figure 5 Phosphatidylinositol (PI) hydrolysis studies in the guinea-pig taenia caeci. Log concentration-response curves for bradykinin-stimulated [^3H]-inositol phosphate formation, in the absence (●), and the presence (◆), of D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK (30 nM). Each point is the mean ($\pm$ s.e.mean) taken from 4 experiments.

control; whereas responses to the control spasmogens were not significantly reduced (data not shown; $P > 0.05$). Preparations in low- Na^+ media showed little tone, and relaxant responses were not seen; but when bradykinin was added during the tonic phase of a carbachol pre-contraction, a relaxant action could then be demonstrated (data not shown).

Experiments in high- K^+ media

In a K_2SO_4 -based depolarizing high- K^+ medium, carbachol and bradykinin (10 nM–100 μM) produced reliable and reproducible concentration-related contractile responses, though of greatly diminished magnitude as compared to that observed in Krebs solution (Figure 4). Under these conditions, the contractions observed with the maximum achievable concentration of bradykinin (100 μM) were reduced to 25.5% of the bradykinin maximum in Krebs solution, or some 19.0% of the carbachol Krebs control (cf. 43% for carbachol, itself, under these conditions; Hall & Morton, 1991a). No relaxation was observed in response to bradykinin, either in this K_2SO_4 -based medium, or a medium where KCl was part-substituted for the NaCl of Krebs medium (30 mM), even when in the initial stage of the K^+ -contracture when there was considerable tone: however, contractions could always be elicited (data not shown). The log concentration-response curve for bradykinin contraction in the K_2SO_4 -based high- K^+ medium was shifted to the right in the presence of D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK (30 nM) (Figure 4), giving an apparent pK_B of 8.4 ± 0.1 ($n = 4$) (Table 1).

Phosphatidylinositol hydrolysis studies

Bradykinin produced a concentration-related increase in tissue levels of total [^3H]-IPs with an EC_{50} value of 0.97 (0.83–1.11) μM ($n = 8$) (Figure 5). The maximum increase in accumulation of [^3H]-IPs by bradykinin (100 μM) was $340 \pm 20\%$ relative to basal levels. For comparison, the maximum accumulation of [^3H]-IPs in response to carbachol (1 mM) was $804 \pm 62\%$ ($n = 8$) relative to basal levels.

The B_1 receptor-selective agonist [des-Arg ^{9}]-BK (1 mM) did not significantly elevate total [^3H]-IPs over basal ($n = 4$; $P > 0.05$), and the B_1 receptor antagonist [Leu ^{8} , des-Arg ^{9}]-BK (3 μM) failed to inhibit total [^3H]-IPs accumulation stimulated by a sub-maximal concentration (10 μM) of bradykinin ($P > 0.05$; $n = 4$) (not shown). In contrast, the B_2 receptor

antagonists D-Arg-[Hyp ^{3} , D-Phe ^{7}]-BK and D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK produced parallel rightward shifts of the bradykinin log concentration-response curve, with apparent pK_B estimates of 5.4 ± 0.3 and 8.4 ± 0.1 ($n = 4$), respectively (Table 1). The inhibitory effect of D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK was selective for bradykinin, since the antagonist (1 μM) did not inhibit basal, nor (100 μM) carbachol-induced, [^3H]-IPs accumulation ($P > 0.05$, data not shown).

Discussion

This study provides evidence that bradykinin receptors with indistinguishable recognition properties mediate the relaxant and contractile phases of the bradykinin-induced response in the guinea-pig taenia caeci under normal conditions: further, these same properties are shown by the receptors mediating bradykinin-induced hydrolysis of inositol phospholipids, as well as those evoking contractions in a high- K^+ depolarizing-medium and in a Ca^{2+} -free medium. The receptors involved in these five responses are not B_1 receptors, since the B_1 -selective agonist, [des-Arg ^{9}]-BK, was inactive in all experiments and the B_1 -selective antagonist [Leu ^{8} , des-Arg ^{9}]-BK did not inhibit any facets of the responses to bradykinin. In contrast, the B_2 antagonist D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK, inhibited all types of bradykinin response with a similar affinity (pK_B 8.4–8.5; Table 1). This antagonist, and several related [D-Phe ^{7}]-BK antagonists, were also able to displace [^3H]-bradykinin in competition binding experiments in the taenia caeci (Field *et al.*, 1992c). The affinity of D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK (and related B_2 antagonists; Field *et al.*, 1988; 1992b,c) for the guinea-pig taenia caeci receptor, is similar to that in other guinea-pig preparations, though lower than its affinity at B_2 receptors in several other species (Field *et al.*, 1992b,c). These observations support the existence of species-homologues of the B_2 receptor (see Hall, 1992; Hall *et al.*, 1993). These receptors in the guinea-pig taenia caeci are likely to be present on the smooth muscle cells, since tetrodotoxin and ω -conotoxin, and cyclooxygenase inhibitors, did not inhibit bradykinin responses; further these experiments were carried out in Krebs solution containing autonomic blockers and histamine receptor antagonists.

Regarding the mechanism of contraction, it was of interest that bradykinin B_2 receptor-induced contractile responses were considerably attenuated in the high- K^+ medium, suggesting that an important component of the bradykinin response depends on membrane potential-dependent events (electromechanical coupling), such as depolarization and calcium-entry via voltage-sensitive channels. Electromechanical coupling-mechanisms for bradykinin were not studied in detail here, but there is some evidence from this preparation suggesting a contribution from Na^+ -entry to depolarization: from electrophysiological observations (Aarsen & Van Caspel-De Bruyn, 1970), $^{24}\text{Na}^+$ -permeability determinations (Morton *et al.*, 1987), and functional experiments (present study).

The bradykinin B_2 receptor-mediated response remaining in high- K^+ depolarizing conditions must result from membrane potential-independent mechanisms (pharmacomechanical coupling); for example, release of intracellular Ca^{2+} . Further, the observation that bradykinin B_2 receptor stimulation could still produce transitory contractions in the absence of extracellular Ca^{2+} , indicates that bradykinin is indeed able to release Ca^{2+} from intracellular stores. It may well be relevant that a recent study in bovine tracheal smooth muscle cells, which quantified intracellular Ca^{2+} by direct imaging with fura-2, demonstrated a bradykinin-induced B_2 receptor antagonist-sensitive Ca^{2+} -elevation. This corresponded to a graded-influx of Ca^{2+} in the presence of extracellular Ca^{2+} . In contrast, in the absence of extracellular Ca^{2+} , there was Ca^{2+} -release; but this was essentially quantal

and resulted in lower levels of intracellular Ca^{2+} (Marsh & Hill, 1993). As a possible mechanism for Ca^{2+} -release in the taenia caeci, the role of the phosphatidylinositol hydrolysis transduction-pathway in the mobilization of Ca^{2+} from intracellular stores is particularly well established, and its involvement in bradykinin receptor-mediated signalling has been described in a number of cell types (see Burch, 1990; Farmer & Burch, 1992 and Hall, 1992). Recently, a role has been claimed for this pathway in contraction by bradykinin of smooth muscle of the gut (Ransom *et al.*, 1992), and airways (Marsh & Hill, 1992; 1992). In the present study, bradykinin caused a concentration-related increase in accumulation of total [^3H]-IPs: further, this was antagonized by the B_2 antagonists, D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK and D-Arg-[Hyp ^{3} , D-Phe ^{7}]-BK with the same affinity (pK_B 8.4 and 5.4, respectively) as for the functional responses (Table 1, Field *et al.*, 1992b). These data are compatible with the same receptor being involved in these functional and biochemical aspects of the action of bradykinin, and may indicate a causal relationship.

It is the presence of an initial relaxant component in the normal functional responses to bradykinin in the taenia caeci which distinguishes bradykinin from spasmogens such as carbachol, substance P and histamine, which give only a fast contraction (Hall & Morton 1986; 1991a; Hall *et al.*, 1986). In fact, biphasic responses to bradykinin have been reported in a number of other cell types (see Hall, 1992), and the present study was prompted, in part, by the description in NG108-15 cells (Higashida & Brown, 1986) of an initial apamin-sensitive hyperpolarization followed by a slower, phorbol ester mimicked, depolarization phase. The present study showed that the initial relaxant phase with bradykinin is mediated via B_2 receptors with the same properties as those for the contractile phase, since D-Arg-[Hyp ^{3} , Thi ^{5} , D-Tic ^{7} , Oic ^{8}]-BK showed a concentration-related antagonism, again yielding an apparent pK_B of 8.4 (Table 1). The relaxation phase is abolished by a low concentration of apamin (this study; Gater *et al.*, 1985; Carter *et al.*, 1986), indicating that it is caused by the opening of Ca^{2+} -activated K^+ -channels: a proposal supported by ion-flux and electrophysiological studies. Thus, in depolarized taenia caeci preparations, bradykinin caused an apamin-sensitive increase in K^+ -permeability (measured as efflux of ^{86}Rb in a medium containing 121 mM KMeSO_4 ; Gater *et al.*, 1985). Similarly, in the rat duodenum, we have demonstrated that B_2 receptors also mediate apamin-sensitive relaxation, and an apamin-sensitive increase in K^+ -permeability (Hall & Morton, 1991b). In the guinea-pig taenia caeci, we found that relaxant responses to bradykinin depended on electromechanical coupling-mechanisms, in as much as that in high- K^+ depolarizing-media, where hyperpolarization cannot mediate responses, relaxant responses to bradykinin are abolished. In normal Krebs solution, electrophysiological studies have demonstrated an initial apamin-sensitive hyperpolarization associated with a membrane conductance increase (Den Hertog *et al.*, 1988). This Ca^{2+} -dependent hyperpolarization,

through opening of K^+ -channels, is independent of extracellular Ca^{2+} , and is supposed to use Ca^{2+} mobilized from an intracellular store (Den Hertog *et al.*, 1988). The observations from the present study do not contribute direct evidence regarding the origin of this Ca^{2+} ; but do support an intracellular source for the Ca^{2+} contributing both to relaxation and contraction since both these actions of bradykinin could be demonstrated in zero- Ca^{2+} media. The hypothesis may be advanced that a common B_2 receptor, acting through the polyphosphoinositide pathway, is responsible for a release of intracellular Ca^{2+} which contributes to electrophysiological or mechanical events.

A question raised by this study is the mechanism of the divergence observed in the receptor-coupling processes in the taenia caeci. One group of receptors (e.g. muscarinic M_3 , tachykinin NK_1 and histamine H_1) elicit simple monophasic contractions (both in normal and high- K^+ media), and activate the polyphosphoinositide pathway (Hall & Morton, 1986; Hall *et al.*, 1986; Gardner *et al.*, 1988; Hall & Morton, 1991a). The second group, which includes α_1 -adrenoceptors and P_{2Y} -purinoceptors (Jenkinson & Morton, 1967a; Banks *et al.*, 1979; Den Hertog *et al.*, 1985; Nelemens & Den Hertog, 1987; O'Connor *et al.*, 1991) also activate the polyphosphoinositide pathway, but nevertheless cause an initial relaxation associated with opening of apamin-sensitive Ca^{2+} -activated K^+ -channels. The bradykinin B_2 receptor-effector mechanism bears more resemblance to the latter group, though the initial relaxation is quickly followed by contraction. Further work is needed to explain this divergence. A speculative possibility is that it arises at the G protein level, so that different stores of Ca^{2+} are mobilized, or ion channels modulated.

In conclusion, our data show that in the smooth muscle of the guinea-pig taenia caeci, activation of bradykinin B_2 receptors causes a biphasic mechanical response. This response is due to a direct action of the smooth muscle and does not depend on the release of neurally-derived mediators or prostanooids. Although the full sequence of events leading to the biphasic responses cannot be determined from these experiments, the hypothesis advanced is that receptor-induced stimulation of phosphatidylinositol hydrolysis leads to Ca^{2+} -mobilization from intracellular stores which contributes to responses. The initial relaxant phase involved membrane potential-dependent mechanisms, *viz.* those involving the opening of Ca^{2+} -activated K^+ -channels and hyperpolarization. The subsequent contraction may involve both membrane potential-dependent and -independent mechanisms, utilizing both extracellular and intracellular Ca^{2+} .

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