British Journal of Pharmacology (1996) 117, 1619–1624

The longitudinal muscle of rat ileum as a sensitive monoreceptor assay for bradykinin B_1 receptors

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1 Various bradykinin derivatives, acting preferentially at B_1 or B_2 receptors, were tested in the isolated longitudinal smooth muscle of rat ileum. Experiments were carried out in the presence of chlorpheniramine and atropine (both 1 μM), guanethidine and indomethacin (both 3 μM) and of the peptidase inhibitors (captopril, bestatin and thiorphan, all 1 μM).

2 The rank order of potency was (pD_2 values ± s.e.mean, $n=5$ in parentheses, at 5 h from set-up): [des-Arg^9]-BK (8.27 ± 0.11) ≥ [des-Arg^{10}]-kallidin (7.67 ± 0.24) > bradykinin (6.69 ± 0.25). The B_2 receptor selective agonist, [Hyp^3,Tyr(Me)^8]-BK, was approximately 10 fold less active than bradykinin. Contractile responses to all agonists increased with time. The maximal response to the B_1 receptor agonist, [desArg^9]-BK at 5 h (94 ± 2%) was significantly ($P<0.05$) greater than that measured at 2 h (74 ± 2%).

3 The B_2 receptor antagonist, D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK (Hoe 140, 0.1 μM) did not affect responses to the B_1 receptor agonist [des-Arg^9]-BK (0.1 nM – 1 μM) nor those to the B_2 receptor agonist, [Hyp^3,Tyr(Me)^8]-BK (1 nM – 10 μM). In control experiments performed in the longitudinal smooth muscle of guinea-pig ileum and rat isolated urinary bladder as bioassays for B_2 receptors, the B_2 receptor antagonist Hoe 140 (0.1 μM) antagonized bradykinin-induced contractions.

4 In the rat isolated ileum the B_1 receptor antagonist, D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8, des-Arg^9]-BK ([des-Arg^{10}]-Hoe 140, 0.3 – 10 μM) competitively antagonized contractile responses to [des-Arg^9]-BK with an estimated pK_B of 6.74 ± 0.08 (Schild plot slope with confidence limits 1.22, (0.70 – 1.73) $n=13$). In control experiments in the guinea-pig isolated ileum and rat isolated urinary bladder, [des-Arg^{10}]-Hoe 140 (1 – 10 μM) did not inhibit B_2 receptor-mediated contractile responses.

5 The putative B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK, behaved as a partial agonist when responses were determined 2 h from set-up (pD_2 6.43 ± 0.21, $n=5$; E_{max} 30% of that evoked by [des-Arg^9]-BK); at 5 h from set-up it behaved as a full agonist (pD_2 7.48 ± 0.12, $n=5$; E_{max} 90% of that evoked by [des-Arg^9]-BK). At this time the response to [Leu^8,des-Arg^9]-BK was antagonized in a concentration-dependent manner by [des-Arg^{10}]-Hoe 140, which at 1 μM and 10 μM, produced dose-ratios of 6.33 ± 3.66 ($n=4$) and 103 ± 40 ($n=4$).

6 In view of the rank order of potency of agonists, the antagonist activity by [des-Arg^{10}]-Hoe 140 and the lack of antagonist activity of Hoe 140, we conclude that the longitudinal smooth muscle of rat ileum, after histamine, acetylcholine, noradrenaline, and prostanoid production blockade, is a sensitive monoreceptor assay for studying the pharmacology of bradykinin B_1 receptors. Further the preparation can also be used as a sensitive bioassay to identify partial agonist activity of B_1 receptor antagonists such as [Leu^8,desArg^9]-BK.

Keywords: Kinins; bradykinin B_1 receptor; [des-Arg^9]-BK; (rat) ileum; [des-Arg^{10}]-Hoe 140; [Leu^8,des-Arg^9]-BK

Introduction

Two types of bradykinin receptors, termed B_1 and B_2, have been proposed by Regoli & Barabé (1980) on the basis of the different rank order of potency of agonists and of the affinity of various bradykinin analogues with antagonist activity. This proposal has been recently substantiated by the isolation and cloning of B_1 and B_2 receptors (Hess *et al.*, 1992; Menke *et al.*, 1994) as distinct molecular entities showing the appropriate pharmacological profiles after expression in suitable cell systems.

From a pharmacological point of view, bradykinin B_2 receptors have been extensively studied and a number of receptor-selective agonists and antagonists are available (see Hall, 1992 for review). B_2 receptors appear to be constitutively expressed in normal tissues by a number of cell types and this characteristic has facilitated the development of B_2 receptor pharmacology since a number of sensitive bioassays are available for studying B_2 receptor ligands.

On the other hand, the expression of B_1 receptors is rarely observed except when inflammation or tissue injury is produced. Consequently, there are few assays available for studying B_1 receptor-mediated responses. A time-dependent increase in the response to B_1 receptor agonists is observed in some isolated organs, which has been explained by *de novo* synthesis of the corresponding receptor protein (Regoli *et al.*, 1978). Some isolated smooth muscle assays used for the study of B_1 receptor pharmacology are complicated by the concomitant presence of constitutive B_2 receptors (rabbit urinary bladder, Butt *et al.*, 1995; mouse vas deferens, Maas *et al.*, 1995; rat oesophagus, Boxall *et al.*, 1995), whereby the effects of applied kinins are a mixture of responses produced by B_1 and B_2 receptors. Responses to B_1 receptor agonists can also be observed in preparations excised from animals in which inflammatory processes have been induced (Marceau *et al.*, 1980; Farmer *et al.*, 1991; Pruneau *et al.*, 1994; Roslan *et al.*, 1995) or after exposure of the isolated preparation to mediators of inflammation (Bouthillier *et al.*, 1987; DeBlois *et al.*, 1988; 1991). Indeed, it has recently been proposed that B_1 receptors mediate some of the actions of kinins during inflammation and hyperalgesia (Proud *et al.*, 1987; Chercuitte *et al.*, 1987; Dray &

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Perkins, 1993; Perkins & Kelly, 1993; Davis & Perkins, 1994). The availability of sensitive assays is therefore important for the development of potent and selective B_1 receptor antagonists.

In the present study we describe the kinin pharmacology of the contractile response of the longitudinal smooth muscle of rat isolated ileum using agonists selective for B_1 ((Lys-[des-Arg^9]-BK, [des-Arg^{10}]-kallidin)), for B_2 receptors ([Hyp^3, Tyr(Me)^8]-BK) and non selective (bradykinin) and selective B_1 ([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}]-Hoe 140) and a B_2 receptor antagonist, (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK, Hoe 140). We present evidence that the contractile response to kinins in this preparation is mediated by B_1 receptors, and that this preparation is a sensitive monoreceptor system for studying B_1 receptor pharmacology.

Methods

General

Male albino rats of Wistar strain (320–350 g) and male albino guinea-pigs (400–450 g), were stunned and bled. The small intestine was removed, placed in oxygenated and gassed (95% O_2, 5% CO_2) Krebs solution containing indomethacin, guanethidine (both 3 μM), clorpheniramine and atropine (both 1 μM). The peptidase inhibitors (thiorphan, bestatin and captopril, 1 μM) were added 15 min before determination of the concentration-response curves. Longitudinal muscle-myenteric plexus preparations were prepared as described for the guinea-pig ileum by Paton & Vizi (1969), for rat ileum by Barthó & Lefebvre (1994), and rat urinary bladder was prepared according to Patacchini *et al.* (1992). The composition of Krebs solution was as follows (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 0.5, NaH_2PO_4 1.0, $NaHCO_3$ 25 and glucose 11. The strips were transferred to organ baths (5 ml capacity) and prepared for isotonic recording (load 5 mN) of mechanical activity (Basile transducers) which was displayed on Basile 7050 pen recorder. Antagonists contact time was 15 min.

Longitudinal smooth muscle of rat ileum

After an equilibration period of 45 min, the preparations were exposed to KCl (40 mM), which was administered three times at intervals of 15 min as an internal standard. Two hours after tissue set-up, a cumulative concentration-response curve was constructed, with each concentration applied to the tissue when the effect of the preceding concentration had reached a steady state. At the end of each curve, KCl was again administered. The range of concentrations tested were 1 nM–10 μM for bradykinin and [Hyp^3,Tyr(Me)^8]-BK, 0.1 nM–1 μM for [des-Arg^{10}]-kallidin (Lys-[des-Arg^9]-BK) and [des-Arg9]-BK. After washout and recovery to baseline, a second concentration-response curve to the agonist (either in the absence or in the presence of the antagonist) was produced at 5 h from set-up. When antagonists studies were performed (concentrations: 0.3–10 μM for [des-Arg^{10}]-Hoe 140, 100 nM for Hoe 140) multiple strips from the same animal were studied in parallel, one of which served as control and the other receiving the stated concentration of antagonist. Concentration-response curves (range 0.1 nM–30 μM for [des-Arg^9]-BK, 1–30 μM for [Leu^8, des-Arg^9]-BK) in the presence of antagonists were performed at 5 h from set-up. Responses to different agonists were compared in multiple strips from the same animal.

Rat urinary bladder

After an equilibration period of 45 min, the preparations were exposed to KCl (40 mM); 45 min later a cumulative concentration-response curve to bradykinin (0.1 nM–10 μM) was constructed in the absence, or in the presence, of Hoe 140 (100 nM and 1 μM) or [des-Arg^{10}]-Hoe 140 (1 and 10 μM). At the end of the curve, a contractile response to KCl (40 mM) was obtained.

Longitudinal smooth muscle of guinea-pig ileum

After an equilibration period of 2 h, cumulative concentration-response curves to bradykinin or [Hyp^3, Tyr(Me)^8]-BK (0.1 nM–300 nM) were determined. After washout and recovery to baseline, concentration-response curves to bradykinin were determined in the presence of Hoe 140 (0.1 μM) or [des-Arg^9]-Hoe 140 (1 and 10 μM). Control experiments revealed no significant changes in the responses over two consecutive log concentration-response curves to bradykinin determined 45 min apart (data not shown).

Expression of results and statistical analysis

In rat ileum and urinary bladder contractile responses to kinin agonists were expressed as % of the response to KCl (40 mM) determined at the end of the experiment. In guinea-pig ileum responses to bradykinin either in the absence or presence of antagonist were normalized towards the maximal effect of bradykinin reached with the first curve.

Since experiments dealing with activity of antagonist toward the response to kinins in rat ileum were performed in different strips from the same animal, we first checked whether the maximal effect (E_{max}) produced by kinin agonist (expressed as % of the KCl-induced contraction) in the presence of antagonist was significantly different from control, by use of two-way ANOVA, followed by Tukey's test. For estimation of antagonist affinities, data were then expressed as percentage of E_{max} obtained with the agonist. E_{max} was the effect at which the log concentration-response curve for a given agonist reached a plateau.

The nature of the interaction of antagonists with the bradykinin receptors was checked by Schild regression as follows: antagonist-induced parallel shifts of log concentration-response curves to the agonist were calculated graphically at the level of the half-maximal response as the ratio (dose-ratio) of equieffective concentrations of agonist. Estimates of log [dose-ratio -1] were plotted against log [antagonist concentration] (Arunlakshana & Schild, 1959). Antagonists providing plots with linear regression lines and slopes not significantly different from unity were considered to act in a competitive manner. The affinity of competitive antagonists was expressed in terms of pK_B calculated from the equation: $pK_B = \log$ [dose-ratio -1] $-\log$ [antagonist concentration] (Kenakin, 1984; Jenkinson, 1991).

When a complete Schild regression was not performed, the effect of the antagonist was expressed by dose-ratio values. pD_2 values were calculated through regression analysis made by the least squares method. All values in text, Figures and Tables are mean $\pm$ s.e.mean. Values reported in parentheses represent confidence limits (95%).

Drugs and solutions

Drugs used were: bestatin, bradykinin, [des-Arg^9]-BK, [des-Arg^{10}]-kallidin, [Leu^8,des-Arg^9]-BK, D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Hoe 140), D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8,des-Arg^9]-BK ([des-Arg^{10}]-Hoe 140) (Peninsula Laboratories Europe, Cheshire), atropine, clorpheniramine, guanethidine, indomethacin and captopril (Sigma, Dorset), thiorphan (Bachem, Essex). [Hyp^3,Tyr(Me)^8]-BK was synthesized by Dr L. Quartara in the Chemistry Department of Menarini Pharmaceuticals, Florence, Italy. All salts used were purchased from Merck (Darmstadt, Germany). Indomethacin was dissolved in dimethylsulphoxide (10^{-1} M), whereas all the other agents were dissolved in distilled water and peptides were stored at -20°C.

Results

Agonists potencies: bradykinin, [des-Arg^9]-BK, [des-Arg^{10}]-kallidin and [Hyp^3,Tyr(Me)^8]-BK in rat ileum

In the presence of atropine (1 μM), clorpheniramine (1 μM), guanethidine (3 μM), indomethacin (3 μM) and peptidase inhibitors, bradykinin, [des-Arg^9]-BK, [des-Arg^{10}]-kallidin evoked concentration-dependent, slowly developing, contractile responses (Figures 1 and 2). For each of the three agonists both E_{max} and pD_2 values tended to be higher when determined at 5 than at 2 h from set-up, although the difference was statistically significant only for E_{max} of the response to [des-Arg^9]-BK (Table 1). At both 2 and 5 h from set-up the rank order of potency was (pD_2 values at 5 h from set-up in parentheses) [des - Arg^9] - BK (8.27) $\geqslant$ [des - Arg^{10}]-kallidin (7.67) > BK (6.69). Thus [des-Arg^9]-BK is about 30 fold more potent than BK suggesting the involvement of B_1 receptors.

To check for a possible contribution of B_2 receptor, we studied the effect of the B_2-selective receptor agonist, [Hyp^3,-Tyr(Me)^8]-BK: this ligand produced contractile responses at micromolar concentrations and, as observed for the other agonists, the curve obtained at 5 h from set-up showed a greater sensitivity and E_{max} than that obtained at 2 h from set-up (Figure 2a and b). Although a maximal response was not obtained, the response to the highest concentration tested (10 μM) was significantly greater when determined at 5 h than at 2 h (28 $\pm$ 11 and 79 $\pm$ 6% of E_{max} to KCl at 2 and 5 h from set-up, respectively, $n = 5$, $P < 0.05$).

Effect of the B_2 receptor antagonist, Hoe 140

To assess the possible participation of B_2 receptors in evoking contraction of the longitudinal smooth muscle of rat ileum, the activity of the B_2 receptor antagonist Hoe 140 was tested against the responses produced by [des-Arg^9]-BK and [Hyp^3,-Tyr(Me)^8]-BK. As a positive control for B_2 receptors the two agonists were also tested in the guinea-pig ileum.

In the rat ileum, Hoe 140 (0.1 μM for 15 min) did not

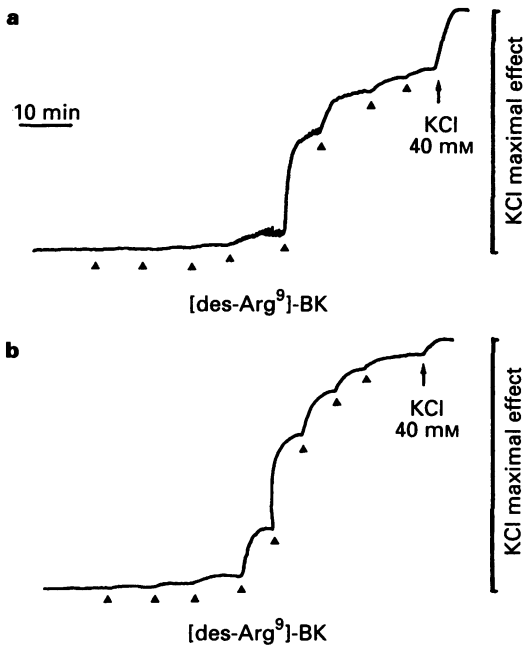

Figure 1 Representative original tracing of the contractile response to [des-Arg^9]-BK in the longitudinal smooth muscle of rat isolated ileum at 2 h (a) and 5 h (b) after preparation set-up. Addition of [des-Arg^9]-BK, in the concentration range 0.1 nM to 0.3 μM, is indicated by the arrows. KCl (40 mM) was administered at the end of each experiment as indicated. The trace is representative of 5 experiments.

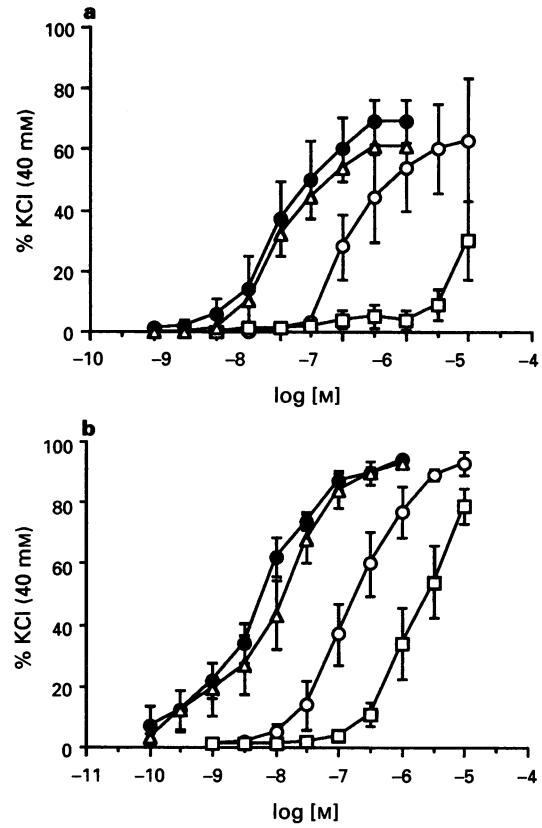

Figure 2 Concentration-related contractile responses of longitudinal smooth muscle of rat isolated ileum to bradykinin (○), [des-Arg^9]-BK (●), [des-Arg^{10}] kallidin (△), and [Hyp^3,Tyr(Me)^8]-BK (□). In (a) log concentration-response curves were obtained 2 h after the mounting of the preparations. In (b) log concentration-response curves were obtained 5 h after the mounting of the preparations. Data are expressed as % of responses to KCl (40 mM), with each point representing the mean $\pm$ s.e.mean of 5 experiments.

Table 1 Contractile potency estimates for agonists in the longitudinal smooth muscle of rat ileum

Agonist	*2 h* pD_2	E_{max} (%KCl 40 mM)	*5 h* pD_2	E_{max} (%KCl 40 mM)
Bradykinin	6.53 $\pm$ 0.29	63 $\pm$ 14	6.69 $\pm$ 0.25	92 $\pm$ 9
[des-Arg^9]-BK	7.58 $\pm$ 0.36	74 $\pm$ 6	8.27 $\pm$ 0.11	93 $\pm$ 2 (a)
[des-Arg^{10}]-kallidin	7.27 $\pm$ 0.37	70 $\pm$ 9	7.67 $\pm$ 0.24	93 $\pm$ 2
[Leu^8, desArg^9]-BK	6.43 $\pm$ 0.21	23 $\pm$ 7 (b)	7.48 $\pm$ 0.12	84 $\pm$ 2 (a)

Potencies of kinin derivatives are shown in terms of pD_2 estimates. First and second curves were performed 2 and 5 h after the mounting of the preparations (see Methods). Values are the mean $\pm$ s.e. mean of 5 experiments.
(a) $P < 0.05$ vs first curve (2 h), t test for paired data.
(b) $P < 0.05$ vs bradykinin, [des-Arg^9]-BK, and [des-Arg^{10}]-KD, Tukey's test.

produce any significant shift of the log concentration-response curve to [des-Arg^9]-BK and [Hyp^3,Tyr(Me)^8]-BK. Bradykinin and the B_2 receptor agonist, [Hyp^3,Tyr(Me)^8]-BK, produced concentration-related contraction of the guinea-pig ileum yielding pD_2 values of 7.71 ± 0.13 ($n=8$) and 8.02 ± 0.25 ($n=8$), respectively. The B_1 receptor agonist, [des-Arg^9]-BK had little effect in this assay: at a concentration of 3 μM it produced a weak contractile effect ($14 \pm 5\%$ of the maximal effect to bradykinin, $n=5$). Hoe 140 (0.1 μM) produced a marked rightward shift of the log concentration-response curve to bradykinin in the longitudinal smooth muscle of guinea-pig ileum, yielding a dose-ratio of 101 ± 23 ($n=6$) without depressing the maximal response to the agonist (maximal response was $91.5 \pm 7\%$ of control).

Activity of the B_1 receptor antagonist, [des-Arg^{10}]-Hoe 140

The B_1 receptor antagonist, [des-Arg^{10}]-Hoe 140 (0.3 ± 10 μM) produced a concentration-related rightward shift of the log concentration-response curve to the B_1 receptor-selective agonist, [des-Arg^9]-BK without depressing the maximal response (Figure 3a). Schild regression indicated a slope that did not differ significantly from unity (1.22 (0.70–1.73), $n=13$, $P>0.05$) compatible with competitive antagonism (Figure 3b), so unity slope was imposed; the pK_B value was 6.74 ± 0.08 ($n=13$). [des-Arg^{10}]-Hoe 140 produced a slight and erratic agonist effect of its own: at 10 μM it produced a contractile response averaging $11 \pm 5\%$ of that evoked by KCl ($n=4$).

At 10 μM, [des-Arg^{10}]-Hoe 140 was without effect on the bradykinin-induced responses in the guinea-pig ileum (data not shown).

In order to exclude possible species-related differences in the pharmacology of [des-Arg^{10}]-Hoe 140, the activity of this antagonist was compared with that of Hoe 140 against bradykinin-induced contraction of the rat isolated urinary bladder. A submaximal contractile response to bradykinin (10 nM) was not inhibited by [des-Arg^{10}]-Hoe 140 (1 and 10 μM). In contrast, Hoe 140 (0.1 and 1 μM) significantly inhibited the contractile response to 10 nM bradykinin (in the presence of 1 and 10 μM [des-Arg^{10}]-Hoe 140, the contractile response to bradykinin averaged 105 ± 16 and $108 \pm 20\%$, of the control response, respectively; in the presence of 0.1 and 1 μM Hoe 140, the effect of bradykinin averaged 16 ± 8 and $3 \pm 3\%$ of the control response, respectively ($n=4$, $P<0.05$)).

Effect of [Leu^8,des-Arg^9]-BK

The effect of the putative B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK, was also tested in the rat ileum. Since [Leu^8,des-Arg^9]-BK possessed a significant agonist contractile activity, we used the same experimental protocol as with the other kinin agonists. As shown in Figure 4a, the agonist activity of [Leu^8,des-Arg^9]-BK was markedly time-dependent: at 2 h from set-up its maximal effect did not exceed 20% of the response to KCl and the estimated pD_2 value corresponded to 6.43 ± 0.21 ($n=5$) (Table 1); at 5 h from set-up, [Leu^8,des-Arg^9]-BK (3 nM – 3 μM) produced a full concentration-response curve and its E_{max} ($84 \pm 3\%$ of response to KCl) was not statistically

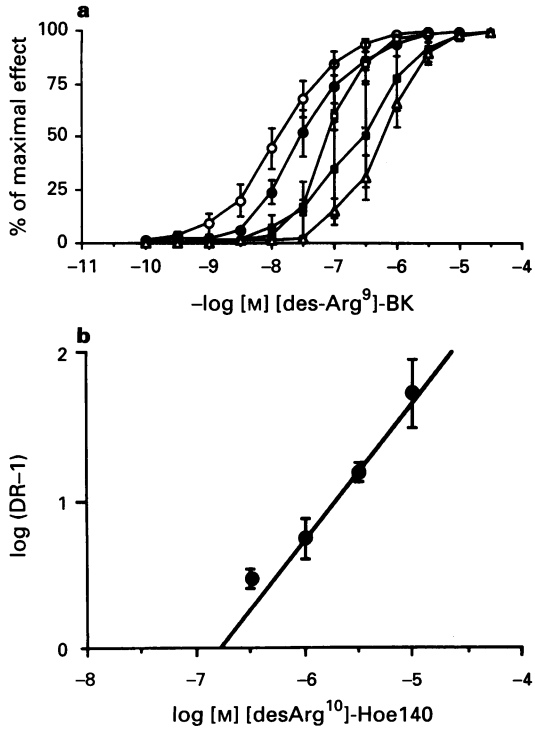

Figure 3 B_1 receptor antagonism in rat isolated ileum smooth muscle. (a) Lateral shift of the log concentration-response curves to [des-Arg^9]-BK (control, ○) by [des-Arg^{10}]-Hoe 140 at 0.3 μM (●), 1 μM (□), 3 μM (■), or 10 μM (△). Each point is the mean ± s.e.mean of 3–4 experiments. (b) Schild analysis: the line fitted has a slope of unity since the regression did not differ significantly from unity (slope 1.22 (0.70–1.73), $n=13$, $P>0.05$). The pK_B estimate is 6.74 ± 0.08, $n=13$.

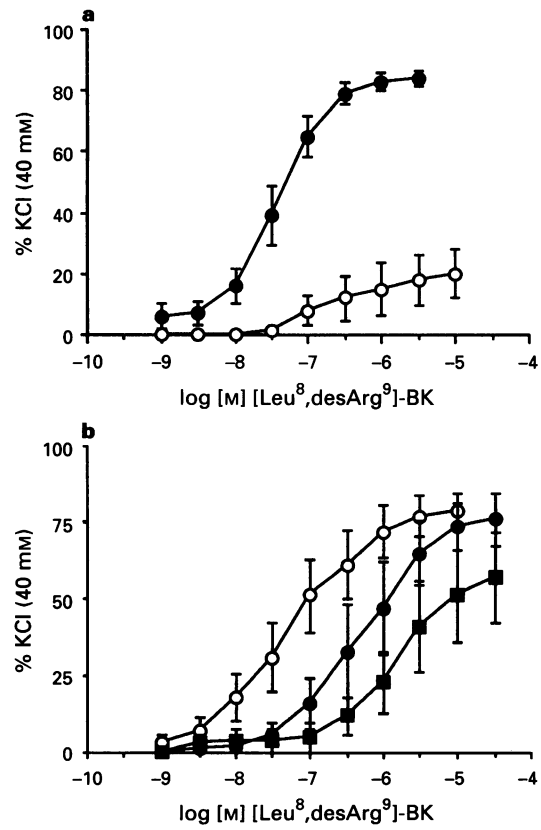

Figure 4 Agonist activity of the [Leu^8, des-Arg^9]-BK in the longitudinal smooth muscle of rat isolated ileum. (a) Concentration-related contractile responses of longitudinal smooth muscle of rat isolated ileum to [Leu^8, des-Arg^9]-BK after 2 h (○) and 5 h the mounting of the preparations (●). (b) Antagonism of contractile responses to [Leu^8, des-Arg^9]-BK (control: ○) by the BK B_1 receptor antagonist, [des-Arg^{10}]-Hoe 140 (●: 1 μM; ■: 10 μM). The dose-ratio (ratio between equieffective agonist concentrations, in the presence and in the absence of the antagonist) was 6.4 ± 3.7 and 103 ± 40 at 1 μM and 10 μM [des-Arg^{10}]-Hoe 140, respectively. Data are expressed as % of responses to KCl (40 mM), with each point representing the mean ± s.e.mean of 4 experiments.

different from the maximal effects reached by the other agonists (Table 1); the pD_2 averaged 7.48 ± 0.12 ($n = 5$).

The B_1 receptor antagonist, [des-Arg^{10}]-Hoe 140 (1 and 10 μM) produced a concentration-dependent rightward shift of the log concentration-response curve to [Leu^8,des-Arg^9]-BK at 5 h from set-up: dose-ratios calculated from data presented in Figure 4b (6.4 ± 3.7 and 103 ± 40 at 1 and 10 μM, respectively) yielded apparent pK_B values of 6.7 and 7.0 for [des-Arg^{10}]-Hoe 140 at 1 and 10 μM, respectively (Figure 4b).

Discussion

The present results indicate that kinins contract the longitudinal smooth muscle of the rat isolated ileum through B_1 receptors. Walker & Wilson (1979) showed that bradykinin potently and directly contracts the longitudinal smooth muscle of the rat isolated intact ileum when perfused onto its serosal surface. When bradykinin was perfused on the mucosal surface, the contractile activity was exerted through production of prostaglandins. In the present study we analysed bradykinin pharmacology on the longitudinal smooth muscle of rat isolated ileum, and to minimize problems related to possible release of other mediators, experiments were performed in the presence of indomethacin, atropine, guanethidine, and chlorpheniramine. The rank order of potency we obtained with the bradykinin analogues ([des-Arg^9]-BK $\geq$ [des-Arg^{10}]-kallidin > bradykinin > > [Hyp^3,Tyr(Me)^8]-BK) agrees with the pharmacological profile of the B_1 receptor type (Regoli & Barabé, 1980). Under the present experimental conditions the sensitivity of the rat ileum longitudinal smooth muscle is quite high as indicated by the potency of [des-Arg^9]-BK (pD_2 8.27 at 5 h from set-up): this value is higher then pD_2 values obtained in rabbit aorta (7.3, at 7 h from set-up, Regoli *et al.*, 1977), rabbit detrusor (6.8, at 5 h from set-up, Butt *et al.*, 1995), rat duodenum (5.9, at 4 h from set-up, Boschcov *et al.*, 1984, 7.16, Paiva *et al.*, 1989), inflamed rat urinary bladder (7.77, Roslan *et al.*, 1995), rat oesophagus (7.30, Boxall *et al.*, 1995), and is comparable to that found in the dog renal artery (8.41, Rhaleb *et al.*, 1989). As observed in other B_1 receptor assays, the activity of the B_1 receptor agonists increases with the time of incubation after set-up which may be attributable to the *de novo* synthesis of B_1 receptors (Regoli *et al.*, 1978). The observation that the longitudinal smooth muscle of the rat ileum responds to B_1 receptor agonists at 2 h from set-up is not necessarily an indication of a constitutive origin of B_1 receptors, because of the surgical manipulation required for preparing the strips which could have induced the *de novo* synthesis of B_1 receptors.

Hoe 140 is a potent and selective B_2 receptor antagonist with reported pK_B values ranging between 8.5 to 10 in various assays for rat and guinea-pig B_2 receptors (Hock *et al.*, 1991; Perkins *et al.*, 1991; Hall *et al.*, 1992; Boxall *et al.*, 1995) and confirmed here in our guinea-pig ileum and rat bladder experiments. The lack of antagonist activity of Hoe 140 against responses to the B_1 receptor agonist [des-Arg^9]-BK or the B_2 receptor agonist [Hyp^3,Tyr(Me)^8]-BK on the rat ileum longitudinal muscle rules out any involvement of the B_2 receptor in this bioassay.

The fact that the selective B_2 receptor agonist, [Hyp^3,-Tyr(Me)^8]-BK, possesses some residual, non B_2-receptor-induced, contractile activity in rat ileum, while being inactive in the rabbit aorta (Rhaleb *et al.*, 1990) may be due to the interaction of this agonist with B_1 receptors in this sensitive assay.

The derivative of the B_2 receptor antagonist, Hoe 140, lacking the C-terminal Arg, [des-Arg^{10}]-Hoe 140, has been proposed as a B_1 receptor antagonist with an IC_{50} of 12 nM in the rabbit aorta (Wirth *et al.*, 1991). The activity of this antagonist has also been assessed in other preparations expressing the B_1 receptor: in the rabbit urinary bladder (pK_B 7.1, Butt *et al.*, 1995), rat oesophagus (pK_B 6.4, Boxall *et al.*, 1994), and rat inflamed urinary bladder (IC_{50} 0.103 μM, Roslan *et al.*, 1995). In all cases the affinity of [des-Arg^{10}]-Hoe140 is comparable to what we found (pK_B 6.74) in the rat ileum longitudinal smooth muscle.

A quite surprising result of the present study was the agonist activity of the reported B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK. Partial agonist activity has previously been shown in the study of Paiva and coworkers (1989) in the rat isolated duodenum, where [Leu^8,des-Arg^9]-BK produced contractile responses (pD_2 7.65) with a maximum response approaching 40% of that evoked by bradykinin. In the present study in the rat ileum longitudinal smooth muscle, [Leu^8,des-Arg^9]-BK exerted partial agonist activity at 2 h from set-up, though it exerted a full agonist activity at 5 h, when a pD_2 value comparable to that of [des-Arg^9]-BK was obtained. Moreover, its contractile activity was blocked, in a concentration-dependent fashion, by [des-Arg^{10}]-Hoe 140. Although we did not carry out a complete Schild analysis, the dose-ratios determined with the two antagonist concentrations tested, indicate that the affinity of [des-Arg^{10}]-Hoe 140 when tested against [Leu^8,des-Arg^9]-BK is comparable to that determined against [des-Arg^9]-BK. We speculate that [Leu^8,des-Arg^9]-BK is a partial agonist at B_1 receptors, thus in preparations exhibiting a low sensitivity to B_1 receptor agonists, [Leu^8,des-Arg^9]-BK antagonizes responses to full agonists.

We conclude that the longitudinal smooth muscle of rat isolated ileum is a sensitive and useful monoreceptor assay for studying the pharmacology of bradykinin B_1 receptor and is a suitable preparation for studying novel B_1 receptor antagonists.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BARTHÓ, L. & LEFEBVRE, R.A. (1994). Nitric oxide causes contraction in the rat isolated small intestine. *Eur. J. Pharmacol.*, **259**, 101–104.

BOSCHCOV, P., PAIVA, A.C.M., PAIVA, T.B. & SHIMUTA, S.I. (1984). Further evidence for the existence of two receptor sites for bradykinin responsible for the diphasic effect in the rat isolated duodenum. *Br. J. Pharmacol.*, **83**, 591–600.

BOUTHILLIER, J., DEBLOIS, D. & MARCEAU, F. (1987). Studies on the induction of pharmacological response to des-Arg^9-bradykinin *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **92**, 257–264.

BOXALL, S.J., WHEELDON, A. & BIRCH, P.J. (1995). Characterisation of the bradykinin receptors that mediate contraction of the rat oesophagus *in vitro*. *Br. J. Pharmacol.*, **114**, 225P.

BUTT, S.K., DAWSON, L.G. & HALL, J.M. (1995). Bradykinin B_1 receptors in the rabbit urinary bladder: induction of responses, smooth muscle contraction, and phosphatidylinositol hydrolysis. *Br. J. Pharmacol.*, **114**, 612–617.

CHERCUITTE, F., BEAULIEU, A.D., POUBELLE, P. & MARCEAU, F. (1987). Carboxypeptidase N (kininase I) activity in blood and synovial fluid from patients with arthritis. *Life Sci.*, **41**, 1225–1232.

DAVIS, A.J. & PERKINS, M.N. (1994). Induction of B_1 receptors *in vivo* in a model of persistent inflammatory mechanism hyperalgesia in the rat. *Neuropharmacol.*, **33**, 127–133.

DEBLIOS, D., BOUTHILLIER, J. & MARCEAU, F. (1988). Effect of glucocorticoids, monokines and growth factors on the spontaneously developing responses of the rabbit isolated aorta to des-Arg^9-bradykinin. *Br. J. Pharmacol.*, **93**, 969–977.

DEBLOIS, D., BOUTHILLIER, J. & MARCEAU, F. (1991). Pulse exposure to protein synthesis inhibitors enhances vascular responses to des-Arg^9-bradykinin: possible role of interleukin-1. *Br. J. Pharmacol.*, **103,** 1057–1066.

DRAY, A. & PERKINS, M.N. (1993). Bradykinin and inflammatory pain. *Trends Neurosci.*, **16,** 99–104.

FARMER, S.G., MCMILLAN, B.A., MEEKER, S.N. & BURCH, R.M. (1991). Induction of vascular smooth muscle bradykinin B_1 receptors *in vivo* during antigen arthritis. *Agents Actions*, **34,** 191–193.

HALL, J.M. (1992). Bradykinin receptors: pharmacological properties and biological roles. *Pharmacol. Ther.*, **56,** 131–190.

HALL, J.M., MITCHELL, D., CHIN, S.Y., FIELD, J.L., EVERETT, C.M. & MORTON, I.K.M. (1992). Differences in affinities of bradykinin B_2 receptors antagonists may be attributable to species. *Neuropeptides*, **22,** 28–29.

HESS, J.F., BOROKOWSKI, J.A., YOUNG, G.S., STRADER, C.D. & RANSOM, R.W. (1992). Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem. Biophys. Res. Commun.*, **184,** 260–268.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, S., BREIPHOL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin antagonist: *in vitro* studies. *Br. J. Pharmacol.*, **102,** 769–773.

JENKINSON, D.H. (1991). How to describe competitive antagonists: three questions of usage. *Trends Pharmacol. Sci.*, **12,** 53–54.

KENAKIN, T.P. (1984). The classification of drugs and drug receptors in isolated tissues. *Pharmacol. Rev.*, **36,** 165–222.

MAAS, J., RAE, G.A., HUIDOBRO-TORO, J.P. & CALIXTO, J.B. (1995). Characterization of kinin receptors modulating neurogenic contractions of the mouse vas deferens. *Br. J. Pharmacol.*, **114,** 1471–1477.

MARCEAU, F., BARABÉ, J., ST-PIERRE, S. & REGOLI, D. (1980). Kinin receptors in experimental inflammation. *Can. J. Physiol. Pharmacol.*, **58,** 536–542.

MENKE, J.G., BORKOWSKI, J.A., BIERILO, K.K., MacNEIL, T., DERRICK, A.W., SCHNECK, K.A., RANSOM, R.W., STRADER, C.D., LINEMEYER, D.L. & HESS, J.F. (1994). Expression cloning of a human B_1 bradykinin receptor. *J. Biol. Chem.*, **269,** 21583–21586.

PAIVA, A.C.M., PAIVA, T.B., PEREIRA, C.C. & SHIMUTA, S.I. (1989). Selectivity of bradykinin analogues for receptors mediating contraction and relaxation of the rat duodenum. *Br. J. Pharmacol.*, **98,** 206–210.

PATACCHINI, R., SANTICIOLI, P., ASTOLFI, M., ROVERO, P., VITI, G. & MAGGI, C.A. (1992). Activity of peptide and non-peptide antagonists at peripheral NK_1 tachykinin receptors. *Eur. J. Pharmacol.*, **215,** 93–98.

PATON, W.D.M. & VIZI, E.S. (1969). The inhibitory action of noradrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmacol.*, **35,** 10–28.

PERKINS, M.N., BURGESS, G.M., CAMPBELL, E.A., HALLETT, A., MURPHY, R.J., NAEEM, S., PATEL, I.A., PATEL, S., RUEFF, A. & DRAY, A. (1991). Hoe 140: a novel bradykinin analogue that is a potent antagonist at both B_2 and B_3 receptors *in vitro*. *Br. J. Pharmacol.*, **102,** 171P.

PERKINS, M.N. & KELLY, D. (1993). Induction of bradykinin B_1 receptors *in vivo* in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. *Br. J. Pharmacol.*, **110,** 1441–1444.

PROUD, D., BAUMGARTEN, C.R., NACLERIO, R.M. & WARD, P.E. (1987). Kinin metabolism in human nasal secretion during experimentally induced allergic rhinitis. *J. Immunol.*, **138,** 428–434.

PRUNEAU, D., LUCCARINI, J.M., ROBERT, C. & BÉLICHARD, P. (1994). Induction of kinin B_1 receptor-dependent vasoconstriction following balloon catheter injury to the rabbit carotid artery. *Br. J. Pharmacol.*, **111,** 1029–1034.

REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32,** 1–46.

REGOLI, D., BARABÉ, J. & PARK, W.K. (1977). Receptors for bradykinin in rabbit aortae. *Can. J. Physiol. Pharmacol.*, **55,** 855–867.

REGOLI, D., MARCEAU, F. & BARABÉ, J. (1978). *De novo* formation of vascular receptors for bradykinin. *Can. J. Physiol. Pharmacol.*, **56,** 674–677.

RHALEB, N-E., DION, S., BARABÉ, J., ROUISSI, N., JUKIC, D., DRAPEAU, G. & REGOLI, D. (1989). Receptors for kinins in isolated arterial vessels of dogs. *Eur. J. Pharmacol.*, **162,** 419–427.

RHALEB, N-E., DRAPEAU, G., DION, S., JUKIC, D., ROUISSI, N. & REGOLI, D. (1990). Structure-activity studies on bradykinin and related peptides: antagonists. *Br. J. Pharmacol.*, **99,** 445–448.

ROSLAN, R., CAMPBELL, E.A. & DRAY, A. (1995). The induction of bradykinin B_1 receptors in the non-inflamed and inflamed rat urinary bladder. *Br. J. Pharmacol.*, **114,** 228P.

WALKER, R. & WILSON, K.A. (1979). Prostaglandins and the contractile action of bradykinin on the longitudinal muscle of rat isolated ileum. *Br. J. Pharmacol.*, **67,** 527–533.

WIRTH, K., BREIPOHL, G., STECHL, J., KNOLLE, J., HENKE, S. & SCHÖLKENS, B. (1991). Des-Arg^9-D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8] bradykinin (desArg10-[Hoe140]) is a potent bradykinin B_1 receptor antagonist. *Eur. J. Pharmacol.*, **205,** 217–218.

(Received July 31, 1995
Revised December 10, 1995
Accepted December 19, 1995)