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Pharmacological evidence for a single bradykinin B_2 receptor in the guinea-pig

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1 The present study addresses the possibility of the existence of different kinin B_2 receptor subtypes in the guinea-pig by evaluating the affinity of peptide and nonpeptide receptor antagonists. For this purpose, jugular vein rings, ileum segments, lung parenchymal and trachea strips were set up in organ baths for isometric tension measurements. The experiments were conducted in the presence of indomethacin (3 μM), atropine (10 μM) and captopril (10 μM).

2 BK contracted jugular vein (JV), ileum (GPI), parenchyma (LP) and trachea (GPT) with an EC_{50} of 13.2 ± 1.4 nM ($n=27$), 11.2 ± 2.1 ($n=26$), 23.6 ± 6.3 nM ($n=26$) and 33.0 ± 6.5 ($n=27$), respectively. Thiorphan, a neutral endopeptidase (EC 3.4.24.11) inhibitor and MERGETPA (DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid), a carboxypeptidase inhibitor, had no effect on the BK-induced contractions of JV, GPI and LP. In the GPT, thiorpan potentiated the contractile response to BK and was thus added in the corresponding experiments.

3 The peptide B_2 receptor antagonist, Hoe 140 and the nonpeptide compound, WIN 64338, behaved as noncompetitive antagonists against contractile responses to cumulative BK in the four tissues although Hoe 140 appeared as a competitive inhibitor in the GPT only. In order to compare the inhibitory potency of these compounds between tissues, pK_B values were determined. Mean values of pK_B for Hoe 140 were 8.05 ± 0.07, 8.43 ± 0.11, 8.13 ± 0.18 and 8.52 ± 0.25 in the JV, GPI, GPT and LP, respectively. WIN 64338 gave mean pK_B values of 6.89 ± 0.10, 7.57 ± 0.12, 7.36 ± 0.12 and 7.51 ± 0.28 in the JV, GPI, LP and GPT, respectively.

4 D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and D-Arg [Hyp^3, D-Phe^7]BK (NPC 567) inhibited in a competitive fashion the concentration-response curves to BK. Values of pA_2 for each compound were not significantly different in the four tissues and were between 5.81 and 6.31 for D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and between 5.55 and 5.65 for NPC 567.

5 We conclude that the contractile response to BK in guinea-pig vascular, intestine and lung tissue is mediated by a unique B_2 receptor. Thus, our results do not support the existence of a B_3 receptor in the trachea and we suggest that the previously reported B_{2B} receptor subtype simply represents the guinea-pig isoform.

Keywords: Bradykinin B_2 receptors; guinea-pig bradykinin receptors; Hoe 140; jugular vein; ileum; trachea; lung parenchyma

Introduction

Bradykinin receptors have been primarily divided into B_1 and B_2 subtypes based on relative potencies of agonists and antagonists (Regoli & Barabé, 1980). For example, B_2 receptors are preferentially activated by bradykinin (BK), [Tyr(Me)^8]-BK and kallidin whilst B_1 receptors exhibit a high affinity for des-Arg^9-BK and related peptides lacking C-terminal arginine (Regoli & Barabé, 1980). Recently, the cloning of both human B_2 and B_1 receptor cDNA has confirmed the existence of these two subtypes (Hess *et al.*, 1992; Menke *et al.*, 1994).

Within the last decade, a number of reports have suggested the existence of bradykinin receptors other than B_1 and B_2. Bradykinin B_3 receptors have been described in smooth muscle cells from guinea-pig trachea (Farmer *et al.*, 1989) whilst B_4 and B_5 subtypes were proposed as mediating responses to BK of the opossum oesophageal sphincter (Saha *et al.*, 1990; 1991). These proposals were essentially based on pharmacological differences between kinin peptide derivatives as observed in isolated organ functional experiments and binding competition assays using [^{3}H]-bradykinin. In addition, two subtypes of bradykinin B_2 receptor, have been proposed (Regoli *et al.*, 1992; 1993; Gobeil & Regoli, 1994). These putative B_2 receptor subtypes were described as having a different pharmacological profile in the rabbit jugular vein and in the guinea-pig ileum (Regoli *et al.*, 1992; 1993; Gobeil & Regoli, 1994).

The intronless coding sequence for the mouse B_2 receptor (Hess *et al.*, 1993) is 92% identical to the rat B_2 receptor and 84% identical to the human B_2 receptor (McEachern *et al.*, 1991; Hess *et al.*, 1992). Although there is a high degree of identity between the mouse and human bradykinin B_2 receptor, significant differences in the binding of certain synthetic peptide antagonists to both bradykinin receptors have been reported (Hess *et al.*, 1993). These results suggest that species divergence can explain, at least in part, the differential pharmacology observed with peptide derivatives.

Thus, in order to clarify the issue of possible intraspecies B_2 receptor subtypes, we have compared the effects of Hoe 140, D-Arg [Hyp^3, D-Phe^7, Leu^8]BK, D-Arg [Hyp^3, D-Phe^7]BK (NPC 567) and of the recently described nonpeptide B_2 antagonist WIN 64338 (Sawutz *et al.*, 1994) on BK-induced contractions of jugular vein, ileum, lung parenchyma and trachea isolated from guinea-pig.

Methods

Tissue preparation

Male Dunkin-Hartley guinea-pigs weighing 400 to 500 g (Charles River, Elboeuf, France) were killed by stunning. To avoid intravascular coagulation the animals were treated with heparin given i.p. at 2000 iu kg^{-1} 15 min before they were killed. Jugular veins were carefully isolated and a 0.96 mm external diameter polyethylene catheter (Biotrol No. 3, Paris, France) was introduced through the lumen. A jugular vein

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segment was then dissected out and the endothelium was rubbed off by gently moving the catheter back and forth twice. After opening of the abdomen and chest, the ileum, the lungs and the trachea were removed and cleared of surrounding fat and connective tissues whilst maintained in a Krebs solution of the following composition (in mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylenediaminetetracetic acid (EDTA) 0.026, glucose 5.5, bubbled with 95% O_2 plus 5% CO_2. The tracheal epithelium was rubbed off by gentle scraping with a cotton swab. Segments of ileum, lung parenchymal strips, 25 mm in length, and transverse strips of trachea as well as two to three rings of jugular vein, 4 mm in length, were prepared and suspended in 8 ml jacketed organ baths containing normal Krebs solution and maintained at 37°C. In accordance with previous work, the resting tension was set up at 0.5 g for the jugular vein and the lung parenchyma (Fleisch *et al.*, 1982; Gupta, 1992), 1 g for the ileum (Calixto *et al.*, 1988) and 2 g for the trachea (Rhaleb *et al.*, 1992). An initial load of 200 mg was applied to tracheal preparations and tissues were washed three times at 15 to 20 min intervals. Tracheas were then stretched in a stepwise fashion by 100 mg tension increments up to 2 g. This procedure previously described by Tschirhart *et al.* (1987) prevents the occurrence of large spontaneous changes in tension and allows a cumulative concentration-response curve to BK to be obtained.

Experiments were conducted in the presence of indomethacin (3 μM), atropine (10 μM) and captopril (10 μM). Atropine was omitted in JV experiments. Indomethacin has been described as inhibiting BK-induced contractions of the guinea-pig trachea (Farmer *et al.*, 1989) and was thus omitted in tracheal preparations. The effects of thiorphan (10 μM), an inhibitor of neutral endopeptidase (EC 3.4.24.11) and of DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (MERGETPA, 10 μM), a carboxypeptidase inhibitor were also evaluated against BK-induced contractions. These compounds which were found to potentiate responses to BK in the GPT (see Results) were subsequently used in this tissue only. To avoid degradation of D-Arg [Hyp^3, D-Phe^7]BK (NPC 567) MERGETPA (10 μM) was added in appropriate experiments.

Experimental protocol

Tissues were left under resting tension for 2 h. In order to obtain a reference contraction, a maximal contractile response was produced by adding histamine at 10 μM in the GPI and LP, histamine at 100 μM in the GPT and BK at 3 μM in the JV. After washings and return to the baseline, Hoe 140, D-Arg [Hyp^3, D-Phe^7, Leu^8]BK, D-Arg [Hyp^3, D-Phe^7]BK (NPC 567), WIN 64338 or the vehicle was added to organ baths. Fifteen min later, log concentration-response curves to BK were obtained in a cumulative fashion. In a separate series of experiments performed in the GPI, the antagonists were incubated for 15 or 45 min before the addition of BK. Only one concentration of antagonist and one bradykinin curve was obtained in each preparation. In some JV rings, acetylcholine was added at the end of the experiment to assess the accuracy of the endothelium removal.

Analysis of data

The concentration-response curves to BK (in the absence or presence of an antagonist) were expressed as percentages of the maximum contraction (100%) to BK (JV) or to histamine (GPI, LP and GPT). The concentration required to produce a half maximum contractile effect (EC_{50}) was calculated after fitting each curve according to a sigmoidal equation of the form:

$$Y = P_1 + P_2/[1 + e^{P_3(\log X - P_4)}]$$

in which, X = agonist concentration, P_1 = lower plateau response, P_2 = range between the lower and the maximal plateau of the concentration-effect curve, P_3 = a negative curvature index indicating the slope independently of the range and P_4 = log EC_{50} (Elghozi & Head, 1990). Amongst the antagonists tested, Hoe 140 and WIN 64338 appeared non competitive giving either Schild plot slopes different from unity and/or depressing significantly the maximum response. Thus, in order to evaluate the potency of these antagonists in each tissue, we

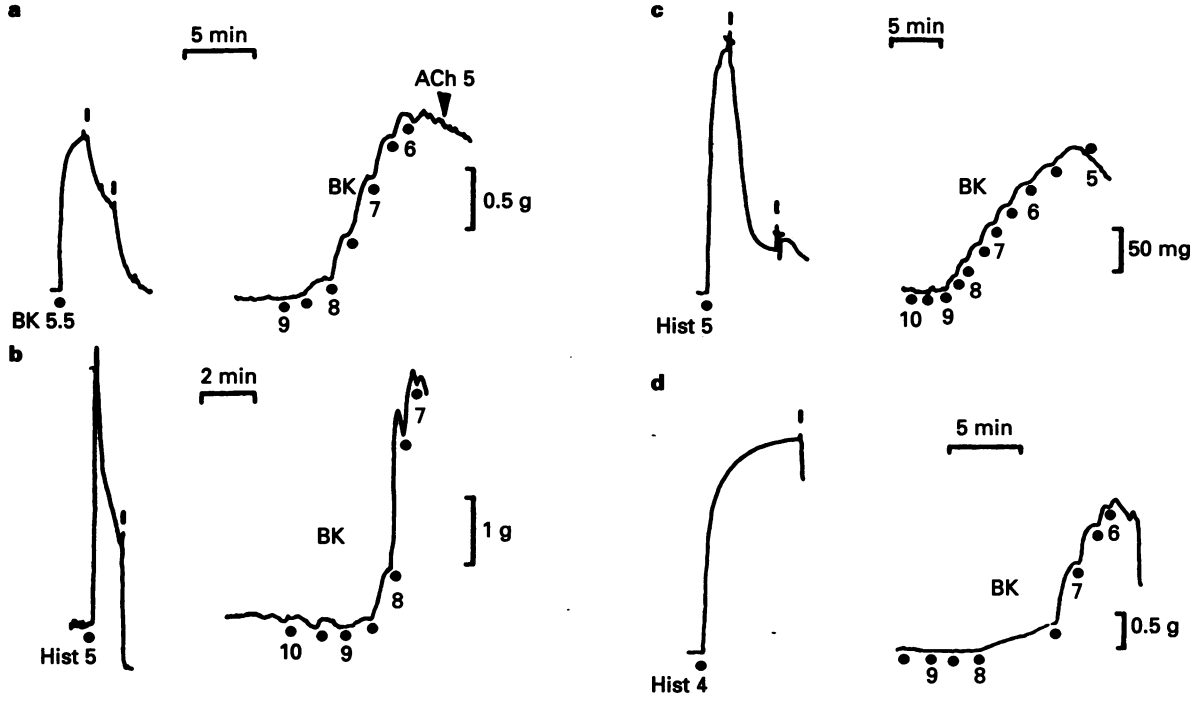

Figure 1 Original tracings showing the contractile effect of bradykinin in guinea-pig isolated jugular vein (a), ileum (b), lung parenchyma (LP) (c) and trachea (d). BK was added in a cumulative manner at least 1 h after tissue preparations were maximally contracted with either bradykinin (BK, 3 μM) or histamine (Hist, 10 μM). Vertical bars indicate washes (2×) with a fresh Krebs solution. In jugular vein (a), acetylcholine (ACh, 10 μM) had no effect confirming the absence of endothelium.

have calculated pK_B values and their s.e.mean by applying the following equation:

$$K_B = [B]/\text{slope} - 1$$

in which slope is that of the double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) and in the presence (1/A') of the antagonist (B) and [B] represents the antagonist concentration (Kenakin, 1993).

Schild analysis was used to calculate pA_2 values when Schild plot slopes did not differ from unity and when maximum responses to BK were not significantly affected whatever the concentration of antagonist.

A one-way analysis of variance followed by a Student's *t* test was used to establish significant differences between maximum responses and between pK_B or pA_2 values. A *P* value less than 0.05 was considered as statistically significant.

Drugs

Acetylcholine hydrochloride, atropine sulphate, bradykinin acetate, captopril, histamine hydrochloride, indomethacin and DL-thiorphan were purchased from Sigma Chemical Co (St Louis, MO, U.S.A.). MERGETPA (DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid) was obtained from Calbiochem (La Jolla, CA, U.S.A.). D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and D-Arg [Hyp^3, D-Phe^7]BK (NPC 567) were from Bachem (Bubendorf, Switzerland). Hoe 140 (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin) was obtained from Prof J. Martinez (EP CNRS 51, Montpellier, France) and WIN 64338 (phosphonium, [[4-[[2-[[*bis*(cyclohexylamino)methylene] amino]-3-(2-naphtalenyl) 1-oxopropyl]amino]-phenyl]-methyl]tributyl, chloride, monohydrochloride) was synthesised by Dr P. Dodey (Laboratoires Fournier, Daix, France).

Results

Response to BK and effect of degradation inhibitors

As illustrated in Figure 1, BK contracted in a concentration-dependent manner the JV, GPI, LP and GPT with a mean EC_{50} of 13.2±1.4 nM (n=27), 11.2±2.1 (n=26), 23.6±6.3 nM (n=26) and 33.0±6.5 (n=27), respectively. In Figure 1a, it can be seen that acetylcholine (ACh) did not relax the precontracted jugular vein indicating an accurate endothelium removal. In separate preparations with intact endothelium we found that ACh (10 μM) relaxed JV rings precontracted with BK by 70.3% (n=3). In the JV, GPI and LP, the sensitivity as well as the maximum of BK-induced response was not affected

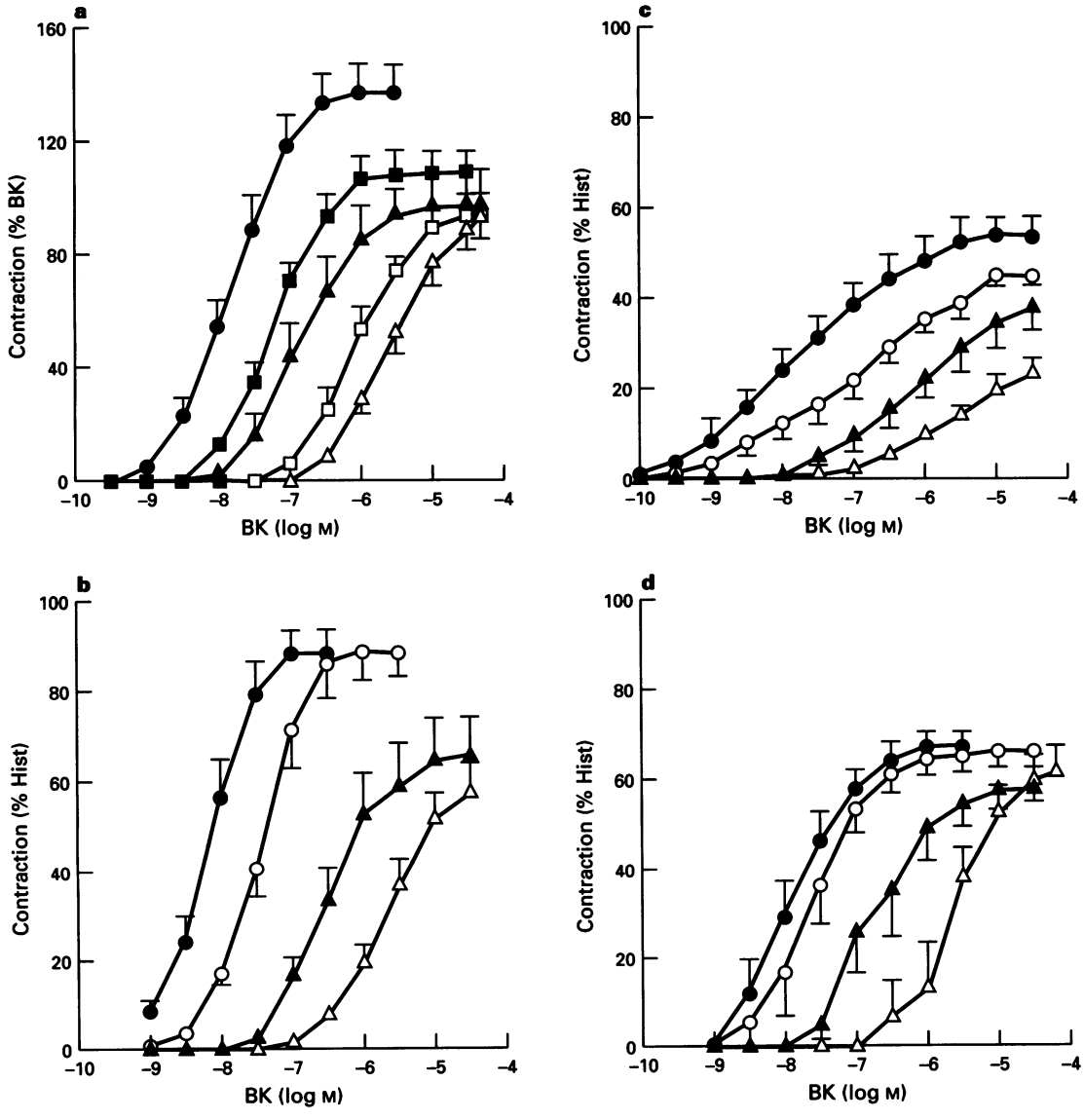

Figure 2 Effect of Hoe 140 on the concentration-response curve to bradykinin in guinea-pig jugular vein (a), ileum (b), lung parenchyma (c) and trachea (d). Vehicle (●); Hoe 140, 0.01 μM (○), 0.03 μM (■), 0.1 μM (▲), 0.3 μM (□) and 1 μM (△). Values represent means±1 s.e.mean of 6 experiments.

by thiorphan ($n=3$) or MERGETPA ($n=3$). In the GPT, thiorphan (10 μM) increased significantly both the sensitivity and the maximum of the concentration-response curve to BK, suggesting that neutral endopeptidase participated in the degradation of BK in this tissue ($n=6$). The carboxypeptidase inhibitor, MERGETPA ($n=6$) potentiated the BK-induced contractions to a lower extent than thiorphan, whilst thiorphan and MERGETPA added together ($n=6$) had no significant additive effect.

Effects of bradykinin antagonists

Hoe 140 and WIN 64338 had no agonist effect in the various tissues. In GPI, GPT and LP neither Hoe 140 (1 μM) nor WIN 64338 (10 μM) had an effect on histamine (100 μM)-induced contractions ($n=2$).

Hoe 140 produced an insurmountable antagonism of BK-induced responses in JV, GPI and LP whilst the antagonism was apparently competitive in the GPT (Figure 2). Calculated

Table 1 Values of mean pK_B for Hoe 140 and WIN 64338 and of pA_2 for D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and D-Arg [Hyp^3, D-Phe^7]BK (NPC 567) obtained against BK-induced response in guinea-pig tissues

	JV	*GPI*	*GPT*	*LP*
Hoe 140	8.05±0.07	8.43±0.11	8.13±0.18	8.52±0.25
WIN 64338	6.89±0.10*	7.57±0.12	7.36±0.12	7.51±0.28
D-Arg [Hyp^3, D-Phe^7, Leu^8]BK	5.81±0.15 (1.09±0.18)	6.31±0.14 (0.79±0.19)	6.02±0.10 (1.00±0.10)	6.06±0.18 (0.80±0.13)
NPC 567	5.65±0.31 (0.73±0.19)	5.57±0.09 (0.88±0.11)	5.58±0.25 (0.86±0.23)	5.55±0.18 (0.85±0.20)

*Indicates significant differences with GPI, GPT and LP.
In parenthesis Schild plot slope (not significantly different from 1); $n=4$ to 10 animals/group.

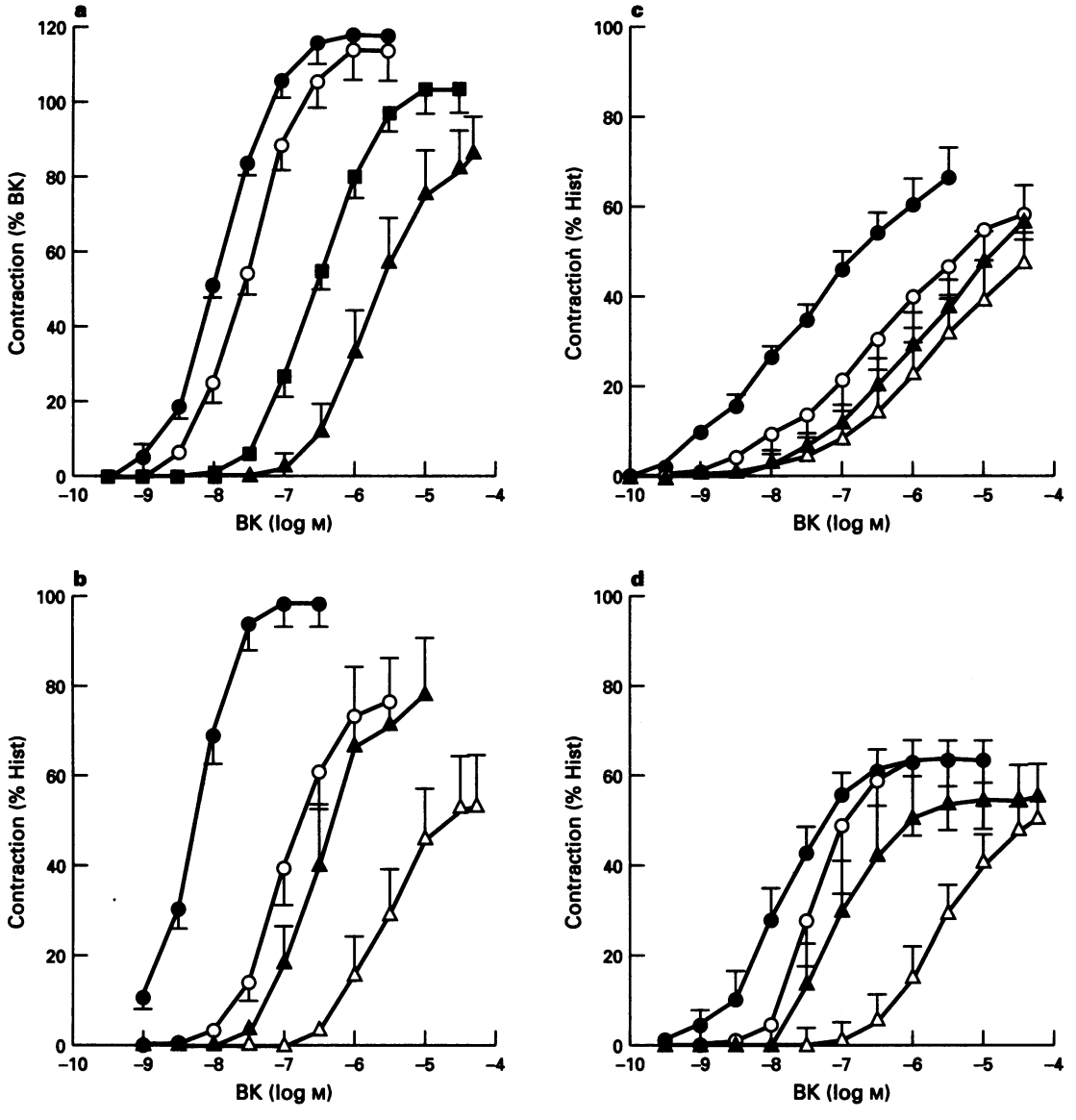

Figure 3 Effect of WIN 64338 on the concentration-response curve to bradykinin in guinea-pig jugular vein (a), ileum (b), lung parenchyma (c) and trachea (d). Vehicle (●); WIN 64338, 0.3 μM (○), 1 μM (▲), 3 μM (■) and 10 μM (△). Values represent means ± 1 s.e.mean of 6 experiments.

pK_B mean values of Hoe 140 in JV, GPI, GPT and LP are given in Table 1. The affinity of Hoe 140 was not significantly different between tissues.

WIN 64338 inhibited in an insurmountable manner contractions to cumulative BK (Figure 3). Mean values of pK_B of WIN 64338 in JV, GPI, LP and GPT are given in Table 1. WIN 64338 appeared significantly less potent ($P<0.05$) against BK in JV than in GPI and LP.

D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and NPC 567 were both competitive antagonists of BK-induced contractions in the GPI, LP, JV and GPT (Figures 4 to 7). Corresponding values of pA_2 are given in Table 1. Schild plot analysis gave values of slope that were not different from unity. There was no difference in potency of D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and NPC 567 between tissues.

The antagonists had similar pK_B values when incubated with GPI preparations at a single concentration for 15 or 45 min. The respective pK_B values after 15 and 45 min were 8.4 ± 0.3 and 8.4 ± 0.3 for Hoe 140 (0.1 μM), 7.0 ± 0.2 and 7.0 ± 0.3 for WIN 64338 (1 μM), 5.7 ± 0.2 and 5.7 ± 0.3 for D-Arg [Hyp^3, D-Phe^7, Leu^8]BK (10 μM) and 5.0 ± 0.2 and 5.0 ± 0.2 for NPC 567 (10 μM).

Discussion

Our results show that BK-induced contractions of guinea-pig jugular vein, ileum, lung parenchyma and trachea were inhibited in a concentration-dependent manner by peptide and nonpeptide B_2 receptor antagonists. Although WIN 64338 appeared less potent in the JV than in other tissues we propose that BK-induced responses are mediated by a unique B_2 receptor.

The suggestion of B_2 receptor subtypes was first based on differences in the inhibitory potency of peptide antagonists towards BK-induced contractions of rabbit jugular vein and guinea-pig ileal preparations (Regoli *et al.*, 1992; 1993; Gobeil & Regoli, 1994). In this respect, D-Arg [Hyp^3, D-Phe^7, Leu8]BK and NPC 567 were proposed as B_{2A} antagonists since they inhibited BK-induced contractions of the rabbit jugular vein (respective pA_2, 8.86 and 8.0) more potently than contractions of the guinea-pig ileum, supposedly mediated by a B_{2B} subtype (respective pA_2, 6.77 and 5.41) (Regoli *et al.*, 1993; Gobeil & Regoli, 1994). In contrast, the potent antagonist, Hoe 140 did not discriminate between the two B_2 subtypes (pA_2, 9.20 and 8.94) (Regoli *et al.*, 1993; Gobeil & Regoli, 1994). D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and NPC 567 have been well characterized as selective and competitive B_2 receptor antagonists (Regoli *et al.*, 1993; Gobeil & Regoli, 1994). In the present study, we have confirmed that D-Arg [Hyp^3, D-Phe^7, Leu^8]BK and NPC 567 are relatively weak antagonists at guinea-pig ileal B_2 receptors (see Results) but we also showed that they inhibit with a similar potency BK-induced contractions of guinea-pig isolated jugular vein, lung parenchyma and trachea. Hoe 140 inhibited with a similar potency BK-induced responses in the four tissues. In accordance with previous studies (Rhaleb *et al.*, 1992; Regoli *et al.*, 1993; Marceau *et al.*, 1994), we observed that the antagonism produced by Hoe 140 was insurmountable except in the GPT. Different possible mechanisms can account for an insurmountable antagonism:

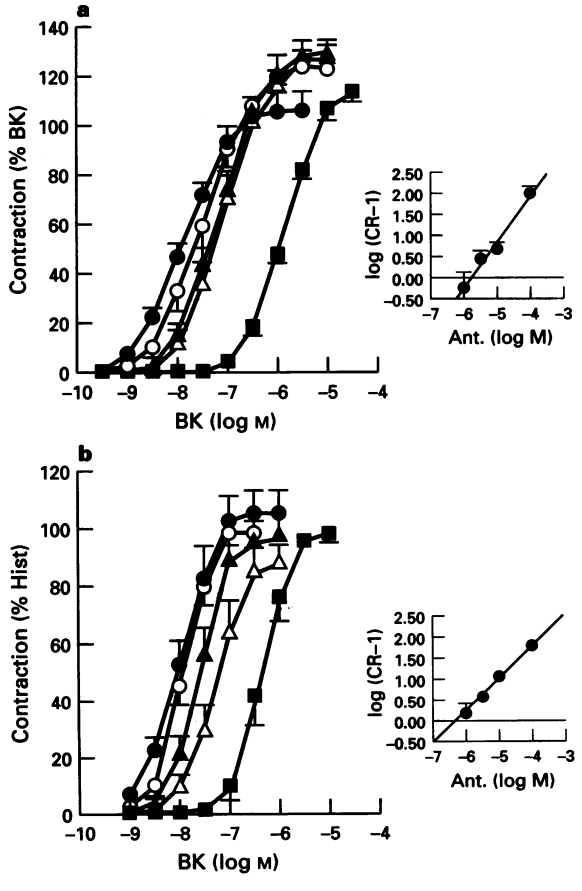

Figure 4 Effect of D-Arg [Hyp^3, D-Phe^7, Leu^8]bradykinin on the concentration-response curve to bradykinin in guinea-pig jugular vein (a) and ileum (b). Vehicle (●); D-Arg [Hyp^3, D-Phe^7, Leu^8]bradykinin, 1 μM (○), 3 μM (▲), 10 μM (△) and 100 μM (■). Corresponding Schild plots are inserted. Values represent means ± 1 s.e.mean of 4 to 10 experiments.

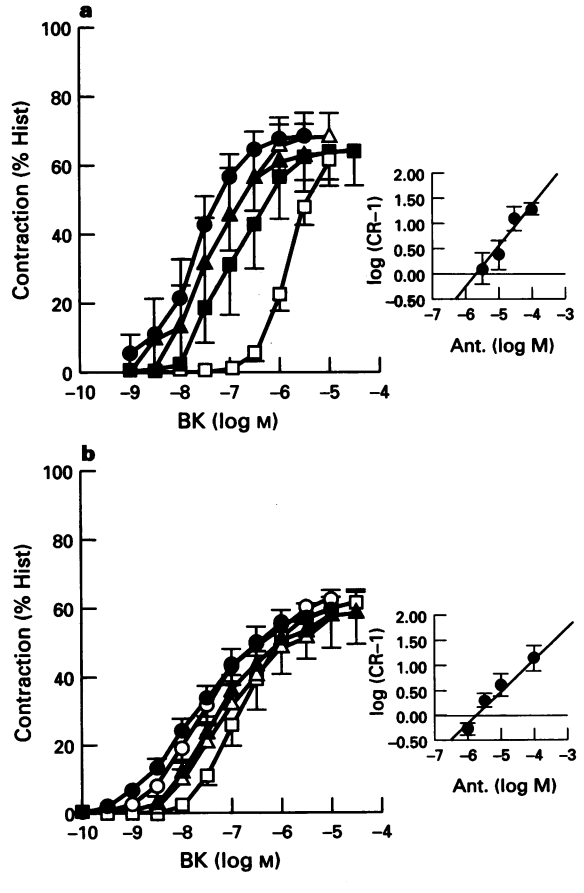

Figure 5 Effect of D-Arg [Hyp^3, D-Phe^7, Leu^8]bradykinin on the concentration-response curve to bradykinin in guinea-pig trachea (a) and lung parenchyma (b). Vehicle (●); D-Arg [Hyp^3, D-Phe^7, Leu^8]bradykinin, 1 μM (○), 3 μM (▲), 10 μM (△) and 100 μM (■). Corresponding Schild plots are inserted. Values represent means ± 1 s.e.mean of 4 to 10 experiments.

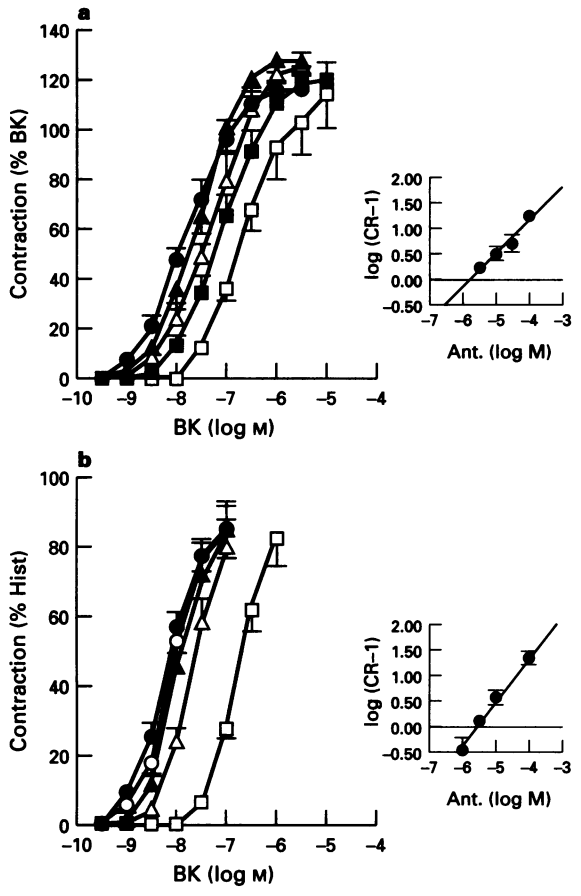

Figure 6 Effect of NPC 567 on the concentration-response curve to bradykinin in guinea-pig jugular vein (a) and ileum (b). Vehicle (●); NPC 567, 1 μM (○), 3 μM (▲), 10 μM (△), 30 μM (■) and 100 μM (□). Corresponding Schild plots are inserted. Values represent means ± 1 s.e.mean of 4 to 10 experiments.

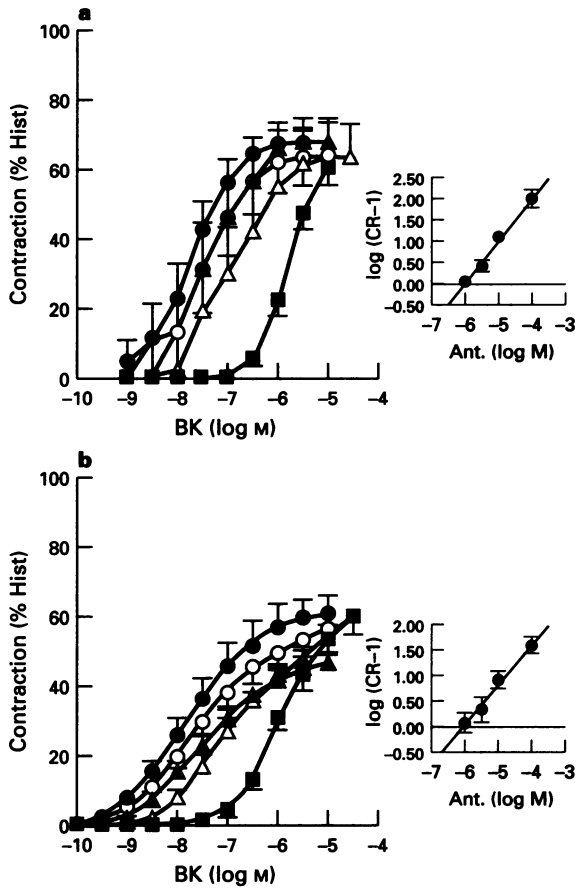

Figure 7 Effect of NPC 567 on the concentration-response curve to bradykinin in guinea-pig trachea (a) and lung parenchyma (b) Vehicle (●); NPC 567, 1 μM (○), 3 μM (▲), 10 μM (△), 30 μM (■) and 100 μM (□). Corresponding Schild plots are inserted. Values represent means ± 1 s.e.mean of 4 to 10 experiments.

these include, multiple receptor subtypes, allostery and irreversible antagonism. Recently, a two-state receptor model has been proposed which appears to match the present results (Robertson *et al.*, 1994). The apparent competitive antagonism of Hoe 140 in the GPT might be related to a large number of spare receptors in this tissue so that the non competitive antagonist, Hoe 140, although reducing significantly agonist-receptor occupancy will not produce a depression of the maximal response (Kenakin, 1993). An alternative explanation might be that an incubation of 15 min was not sufficient to reach equilibrium, due to a slow onset of action of Hoe 140 (Field *et al.*, 1992). However, it is unlikely since Hoe 140 incubated either 15 or 45 min exhibited a similar inhibitory potency in the GPI suggesting that 15 min was sufficient to reach an equilibrium, at least in this tissue. WIN 64338 has been recently described as a nonpeptide B_2 receptor antagonist giving a pA_2 value of 7.97, 7.9 or 8.19 against BK-induced response in the guinea-pig ileum (Farmer & DeSiato, 1994; Gobeil & Regoli, 1994; Sawutz *et al.*, 1994). In agreement, we have found that WIN 64338 behaved as a non competitive antagonist giving a pK_B value of 7.57 in the GPI which was not significantly different from pK_B values in the GPT and LP. A possible explanation for the reduction in the maximum response to BK observed in the presence of WIN 64338 is that this compound is an irreversible or slowly reversible antagonist. In addition, we cannot rule out that WIN 64338 at high concentrations had some non-selective inhibitory effects against muscular contraction. In this respect, WIN 64338 has been reported to inhibit [^{3}H]-nitrendipine binding giving a K_i of 12 μM (Sawutz *et al.*, 1994). However, in the present study we found that WIN 64338 did not affect the maximal response to histamine indicating that this compound did not interfere with the signal transduction mechanism. WIN 64338 appeared less potent in the JV than in the other preparations. Although we have no adequate explanation for such a difference, it is certainly not sufficient to support the existence of a different receptor subtype in the JV. As a consequence, definition of B_2 receptor subtypes based on pharmacological data obtained from different tissues and from different species need to be re-evaluated. Cloning and sequencing of the cDNA encoding the guinea-pig B_2 receptor will certainly help to understand intra- and interspecies pharmacological differences. In this respect, it must be considered that so far, efforts to identify and isolate cDNA encoding various subtypes of rat or human B_2 receptors have failed (Park *et al.*, 1994).

According to Farmer *et al.* (1989), bradykinin receptors in the GPT smooth muscle have a peculiar pharmacology and may represent a new bradykinin B_3 receptor. These authors have shown that BK-induced contractions of guinea-pig trachea and lung parenchyma were resistant to inhibition by several [D-Phe^7]BK and analogues previously described as B_2 antagonists (Farmer *et al.*, 1989). In addition, NPC 567 did not displace BK from tracheal binding sites and, in lung membranes, it displaced only 60% of total specifically bound [^{3}H]-BK. In contrast, Trifilieff *et al.* (1991) showed two high affinity binding sites for BK in guinea-pig lung membranes and demonstrated that NPC 567 totally displaced [^{3}H]-BK from its binding sites. In addition, Field *et al.* (1992) showed that NPC 567 was a full competitor of [^{3}H]-BK binding to tracheal smooth muscle cells and inhibited with a similar potency BK-induced responses of guinea-pig taenia caeci and trachea. However, a recent report showing that WIN 64338 at 1 μM was inactive against BK-induced tracheal contractions whilst it inhibited in a concentration-dependent manner responses of

the ileum further supported the existence of a B_3 receptor (Farmer & DeSiato, 1994). In sharp contrast to the last results, we observed that WIN 64338 markedly inhibited in a concentration-dependent manner the contractile response to BK of the guinea-pig trachea. Although we have no adequate explanation for such a discrepancy, there are some technical differences that have to be pointed out. In the study by Farmer & DeSiato (1994), BK exhibited a weak affinity (EC_{50} of about 100 nM) in the trachea, a phenomenon possible due to the experimental protocol. These authors did not use inhibitors of degradation of BK and as shown in the present study, it appears particularly important to prevent degradation of BK by neutral endopeptidase in tracheal preparations. In accordance with Trifilieff *et al.* (1991), we found that NPC 567 behaved as a weak competitive antagonist of bradykinin receptors in the trachea and lung parenchyma giving similar pA_2 values to other tissues. In addition, D-Arg [Hyp^3, D-Phe^7, Leu^8]BK as well as Hoe 140 inhibited the BK-induced response of the trachea similarly to jugular vein. Thus, we suggest that the BK-induced response in GPT is mediated by activation of B_2 receptors.

In conclusion, we have demonstrated that a single type of B_2 receptor mediates BK-induced contractions in guinea-pig vascular and non-vascular tissues and we suggest that previously described B_{2B} receptor subtype simply represents the guinea-pig isoform of the B_2 receptor. In addition, we propose that the BK-induced contraction of the trachea is essentially dependent on B_2 receptors.

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