ELSEVIER

European Journal of Pharmacology 282 (1995) 177–184

ejp

Mechanisms involved in the relaxant response of bradykinin in epithelium intact strips of the guinea-pig trachea

Valfredo Schlemper, João B. Calixto *

Department of Pharmacology, Centre of Biological Sciences, Universidade Federal de Santa Catarina, Rua Ferreira Lima, 72, 88015-420-Florianópolis, SC, Brazil

Received 2 January 1995; revised 23 May 1995; accepted 29 May 1995

Abstract

Kinins caused graded relaxations in guinea-pig trachea with epithelium under spontaneous or carbachol-induced tone. The order of potency was: [Tyr^8]bradykinin > lysyl-bradykinin > bradykinin > methionyl-lysyl-bradykinin. The bradykinin B_1 receptor agonist des-Arg^9-bradykinin (1 μM) was inactive. Relaxation in response to bradykinin (100 nM) was unaffected by tetrodotoxin (0.3 μM), nicardipine (1 μM), Ca^{2+}-free solution without or plus ryanodine (10 μM), propranolol (1 μM), glibenclamide (1 μM), staurosporine (0.3 μM), nickel chloride (100 μM) or [D-*p*-Cl-Phe^6,Leu^{17}]VIP (a vasoactive intestinal peptide receptor antagonist, 0.03 μM), but was partially inhibited by apamin (0.3–1 μM). Both HOE 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) and NPC 17761 (D-Arg^0[Hyp^3,D-Hype^E(*trans*-thiophenyl)^7,Oic^8]bradykinin) (0.1–1000 nM) caused graded, reversible and selective inhibition of the bradykinin (100 nM) relaxation, with IC_{50} values of 1.4 and 19.1 nM, respectively. HOE 140 and NPC 17761 (0.1–10 nM) produced a graded shift to the right of the bradykinin concentration-response curves associated with a reduction of the maximum relaxation. The kinin B_1 receptor antagonist, des-Arg^9-[Leu^8]bradykinin (1 μM), was inactive. Thus, bradykinin-induced relaxation in guinea-pig trachea results from activation of bradykinin B_2 receptors and can be antagonized with high affinity in a selective and reversible manner, through noncompetitive mechanism, by both HOE 140 and NPC 17761. In addition, the bradykinin response does not involve neural pathways, extracellular Ca^{2+} influx or mobilization of intracellular Ca^{2+} stores sensitive to ryanodine, but is modulated by small conductance Ca^{2+}-activated K^+ channels.

Keywords: Bradykinin; Trachea, guinea-pig; Epithelium; Bradykinin B_2 receptor; Relaxation; Ca^{2+}; Ryanodine; Apamin; HOE 140; NPC 17761

1. Introduction

Bradykinin and related kinins are proinflammatory and algesic peptides generated in the plasma and tissues from α_2-globulins, high and low molecular weight kininogens, by the action of kallikreins (Lewis, 1970; Garcia Leme, 1978; Regoli and Barabé, 1980; Marceau et al., 1983). Evidence indicates that kinins can be generated in upper airway secretions in allergic rhinitis, rhinovirus colds and asthma. Thus, pharmacological and biochemical studies suggest that bradykinin, acting on specific receptors, has an important role in the control of pulmonary function and may be implicated in several responses in the airways, such as neurogenic inflammation, venoconstriction, oedema and bronchoconstriction. In addition, kinins can release several inflammatory mediators including neuropetides and metabolites derived from arachidonic acid pathways (see for review: Farmer, 1991a,b, 1994; Hall, 1992; Barnes, 1992; Geppetti, 1993).

Bradykinin also causes contraction or relaxation of guinea-pig trachea, depending on the presence of the epithelium, these effects being mediated, at least in part, by epithelium-derived products of arachidonic acid metabolism (Mizrahi et al., 1982; Bramley et al., 1990). Recently, we reported that the relaxation induced by bradykinin in epithelium intact strips of guinea-pig trachea is mediated jointly by the release of L-arginine-derived nitric oxide or a nitric oxide-related substance and by a cyclo-oxygenase-derived product from the arachidonic acid pathway (Schlemper and

* Corresponding author. Department of Pharmacology, Universidade Federal de Santa Catarina, Rua Ferreira Lima 82, 88015-420, Florianópolis SC, Brazil. Fax (55482) 224164.

SSDI 0014-2999(95)00329-0

Calixto, 1994). However, the precise mechanism by which bradykinin causes relaxation in guinea-pig trachea remains unknown.

The present study was undertaken to investigate further the mechanisms and receptor subtypes underlying the relaxant responses induced by bradykinin in epithelium intact strips of guinea-pig trachea.

2. Materials and methods

2.1. Tissue preparation

Guinea-pig of both sexes (300–400 g) were killed by a blow on the head and exsanguinated. The trachea was rapidly removed and carefully dissected from adhering fat and connective tissues. Four preparations were obtained per animal, each containing 3–4 cartilaginous rings (3–4 mm wide) and the terminal trachea was discarded. The rings were opened and strips of about 8–10 mm in length with intact epithelium were suspended in individual 5 ml-jacketed organ baths containing Krebs-Henseleit solution maintained at 37°C, pH 7.2, gassed with 95% of O_2 and 5% of CO_2 and with the following composition (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $NaHCO_3$ 25; $MgSO_4$ 1.1; KH_2PO_4 1.1 and glucose 11. Preparations were allowed to equilibrate for at least 120 min before drug addition, under a resting tension of 1 g, during which time the bath solution was renewed each 15 min. A small increase in the resting tone was observed during the equilibration period. Isometric tension changes were recorded by means of a F-60 force transducer on a Narco 40 (Narco Biosystems) physiograph. Tissues were considered to contain a viable epithelium when bradykinin (100 nM) relaxed preparations under spontaneous tonus or precontracted with carbachol by at least 80% (approximately 200–350 mg) (Schlemper and Calixto, 1994). All the experiments with bradykinin and related kinins were carried out in the presence of captopril (3 μM), to avoid degradation of these peptides by kininase II. The kinin responses are expressed either in mg of tension or as percentage of relaxation.

2.2. Relaxant effect of kinins in the epithelium intact strips of guinea-pig trachea

Following the equilibration period, complete non-cumulative concentration-response curves were obtained for bradykinin, lysyl-bradykinin, methionyl-lysyl-bradykinin, [Tyr^8]bradykinin or des-Arg^9-bradykinin (0.1–1000 nM), in preparations either under spontaneous tone or precontracted with carbachol (0.01–0.03 μM). Responses to bradykinin and related peptides were obtained by addition of single increasing concentrations of the peptide with 20-min intervals between doses. The contact time of the agonists with the preparations was restricted to 5–10 min.

2.3. Effect of selective kinin B_1 and B_2 receptor antagonists on bradykinin-induced relaxation in guinea-pig trachea

Following the equilibration period of the preparations, complete concentration-response curves were obtained for bradykinin (0.1–1000 nM) in the absence or in the presence of increasing concentrations of two selective bradykinin B_2 receptor antagonists, HOE 140 or NPC 17761 (0.1–10 nM) or in the presence of the bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]-bradykinin (1 μM). Antagonists were added to the preparations 10 min before challenge with bradykinin. Longer periods of incubation of the preparations with the antagonists were tested in previous experiments ($n = 4$), but they did not cause additional effects (data not shown). Only one complete concentration-response curve for each agonist was tested in each preparation. Therefore, separate control and test tissues were studied simultaneously. In order to correct for any spontaneous and/or agonist-induced changes in the response, control experiments were carried out in the presence of phosphate-buffered solution plus bovine serum albumin 0.25% (the vehicle used to dilute the bradykinin antagonists) throughout the experiments.

In other experiments following the equilibration period, preparations were stimulated with bradykinin (100 nM), a concentration producing about 80% of the maximal relaxant response. After at least two stable control relaxant responses to bradykinin (100 nM) were obtained, preparations were preincubated with different concentrations of the selective bradykinin B_2 receptor antagonists HOE 140 and NPC 17761 (0.1–1000 nM) for 10 min and new relaxant responses for bradykinin (100 nM) were obtained in their presence. The potencies (IC_{50} values) for these drugs in antagonizing the bradykinin relaxation response were determined. Only one antagonist was tested in each preparation.

2.4. Influence of different classes of drugs on bradykinin-induced relaxant responses.

In order to assess whether bradykinin-mediated relaxation in guinea-pig trachea involves direct ionic channel activation, release of neurotransmitters, and the participation of second messengers, preparations were stimulated with bradykinin (100 nM), and after at least two reproducible relaxant responses (control) were obtained, tissues were incubated with one of the following drugs: tetrodotoxin (a voltage-dependent Na^+ channel blocker, 0.3 μM), apamin (a Ca^{2+}-dependent K^+ channel blocker, 0.3–1 μM), glibenclamide (an

ATP-sensitive K^+ channel blocker, 1 μM), nickel chloride (a voltage-dependent T-type calcium channel blocker, 100 μM), nicardipine (a dihydropyridine L-type Ca^{2+} channel blocker, 1 μM), staurosporine (a protein kinase C inhibitor, 0.3 μM), propranolol (a β-adrenoceptor antagonist, 1 μM) or [D-*p*-Cl-Phe^6,Leu^{17}]VIP (a vasoactive intestinal peptide receptor antagonist, 0.03 μM). New relaxant responses for bradykinin (100 nM) were obtained in the presence of these compounds. All drugs remained in contact with the tissues for 10–20 min. In order to investigate the contribution of either extracellular or intracellular Ca^{2+} on bradykinin-mediated relaxation in guinea-pig trachea, in a separate group of experiments, preparations were mounted in normal Krebs solution. After the equilibration period and after at least two control responses to bradykinin (100 nM) were obtained, preparations were transferred to Ca^{2+}-free Krebs solution containing either EGTA (1 mM) or ryanodine (10 μM). Preparations remained 20 min in Ca^{2+}-free medium plus EGTA, during which time the bath solution was renewed every 5 min. After this, the guinea-pig trachea preparations remained 10 min in a Ca^{2+}-free solution without EGTA and new responses to bradykinin (100 nM), both in the absence or in the presence of ryanodine, were obtained. To correct for the possible time-dependent decrease of spontaneous tone of the preparation, resting tone was adjusted by addition of carbachol (0.1–0.3 μM).

2.5. Statistical analysis

Data are presented as means ± S.E.M., except the EC_{50} or IC_{50} values (i.e. the concentration of agonists causing half-maximal relaxant responses or the concentrations of antagonists required to inhibit the bradykinin-induced relaxation to 50% of the control response, respectively), which are given as geometric means accompanied by their respective 95% confidence limits. The IC_{50} or EC_{50} values were determined from individual experiments for the complete antagonist or agonist concentration-response curves by using a least-square regression analysis. Statistical analysis was performed either by means of unpaired Student's *t*-test or by analysis of variance followed by Dunnett's multicomparison test when appropriate. $P < 0.05$ or less was considered as indicative of significance.

2.6. Drugs

Bradykinin, lysyl-bradykinin, methionyl-lysyl-bradykinin, [Tyr^8]bradykinin, prostaglandin E_2, captopril, L-isoproterenol bitartrate, apamin, [D-*p*-Cl-Phe^6,Leu^{17}]VIP, DL-propranolol, staurosporine, tetrodotoxin, sodium nitroprusside (sodium nitroferricyanide), nicardipine hydrochloride, carbamylcholine chloride (carbachol), phosphate-buffered solution tablets (concentration: NaCl 137 mmol; KCl 2.7 mmol and phosphate-buffered 10 mmol) and EGTA (ethyleneglycol-bis-(β-amino-ethylether) *N,N'*-tetra-acetic acid (all obtained from Sigma Chemical Company, St. Louis, MO, USA), nickel chloride-6-hydrate (Riedel-de Haën, Germany), HOE 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) and glibenclamide (Hoechst, Frankfurt, Germany) and NPC 17761 (D-Arg^0[Hyp^3,D-Hype^E(*trans*-thiophenyl)^7,Oic^8]bradykinin) (Scios Nova, Baltimore, MD, USA), des-Arg^9-bradykinin and des-Arg^9[Leu^8]bradykinin (Peninsula, Belmont, CA, USA), ryanodine (Research Biochemicals, MA, USA).

Stock solutions of these drugs (1–100 mM) were prepared as follows: glibenclamide was dissolved in 0.01 N NaOH containing glucose 5%, nicardipine was

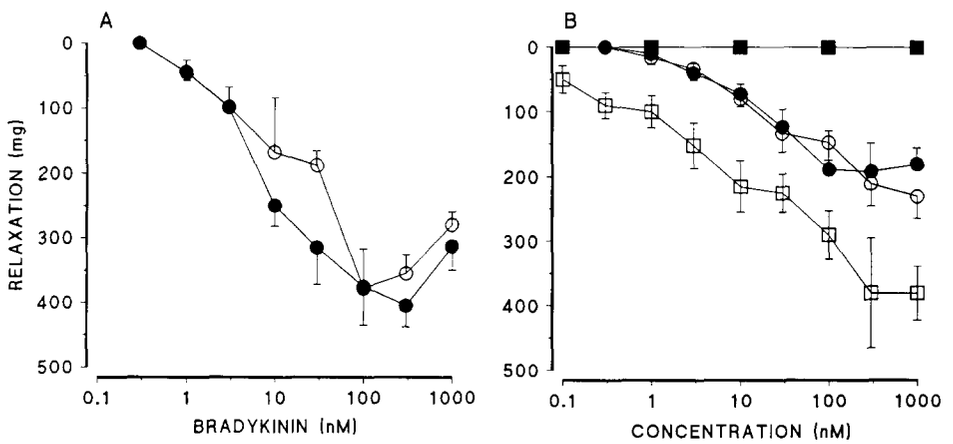

Fig. 1. (A) Relaxant effect of bradykinin in intact strips of guinea-pig trachea submitted to basal tone (1 g) (○) or in preparations pre-contracted by carbachol (Cb, 0.01–0.03 μM) (●). (B) Effect of lysyl-bradykinin (○), methionyl-lysyl-bradykinin (●), [Tyr^8]bradykinin (□) and des-Arg^9-bradykinin (■) in epithelium intact strips of guinea-pig trachea under basal tone (1 g). Each point represents the mean of 5–6 experiments and the vertical bars indicate the S.E.M.

dissolved in absolute ethanol, staurosporine in dimethyl sulfoxide (50%) and bradykinin B_2 receptor antagonists in albumin bovine 0.2% in phosphate-buffered solution. All other drugs were dissolved in phosphate-buffered solution. The final bath concentration of ethanol and dimethyl sulfoxide did not exceed 0.02%, which alone had no effect on the tonus of the preparation or on bradykinin-mediated relaxation. The experiments with nicardipine were protected from light to avoid its photo degradation.

3. Results

3.1. Analysis of kinin-induced relaxant response in intact epithelium of guinea-pig trachea

Addition of bradykinin and related peptides (0.1–1000 nM) to epithelium intact strips of guinea-pig trachea under basal tone caused small, transient contractions followed by concentration-dependent delayed relaxations (Fig. 1A and B). The relaxant bradykinin responses were reproducible, with no evidence of tachyphylaxis. The EC_{50} values and maximal relaxant responses caused by bradykinin and analogues are represented in Table 1. The potencies obtained for the relaxant responses caused by bradykinin and analogues in guinea-pig trachea under spontaneous tonus did not differ significantly (Table 1). Concerning the maximal relaxation, bradykinin and [Tyr^8]bradykinin produced a significantly greater relaxation when compared with lysyl-bradykinin and methionyl-lysyl-bradykinin. The selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin had no effect (Fig. 1B). When preparations with intact epithelium were precontracted with carbachol (0.01–0.03 μM), bradykinin (0.1–1000 nM) caused similar concentration-dependent relaxations (Fig. 1A). The EC_{50} and maximal response for bradykinin did not differ significantly from values reported for this peptide in preparations under spontaneous tonus (Table 1). About 30% of the tissues, either under spontaneous or in carbachol-induced tone, failed to produce relaxation when challenged with bradykinin (data not shown).

Table 1
Potency and maximal responses of kinins in epithelium intact strips of guinea-pig trachea

Agonist	EC_{50} (nM)	Relaxation (mg)
Bradykinin [a]	14.7 (3.1–69.4)	405 ± 33
Lysyl-Bradykinin [b]	18.0 (11.0–29.0)	230 ± 34
Bradykinin [b]	21.1 (6.3–69.6)	380 ± 49
Methionyl-lysyl-Bradykinin [b]	30.0 (13.0–70.0)	191 ± 43
[Tyr^8]Bradykinin [b]	31.0 (17.0–53.0)	380 ± 42

Values are means ± S.E.M. of at least 6–7 experiments. [a] Preparations precontracted with carbachol (0.01–0.1 μM). [b] Preparations under basal tonus (1 g).

3.2. Inhibition by selective bradykinin B_2 receptor antagonists

Incubation of epithelium intact strips of guinea-pig trachea with the selective bradykinin B_2 receptor antagonists HOE 140 and NPC 17761 (0.1–10 nM) for 10 min did not affect basal tone, but caused a concentration-dependent displacement to the right of the bradykinin-induced relaxant concentration-response curve, and a significant reduction of the maximal relaxant response to bradykinin (Fig. 2A and B). In contrast, results from Fig. 3 show that the selective bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]-bradykinin (1 μM) did not cause any significant effect

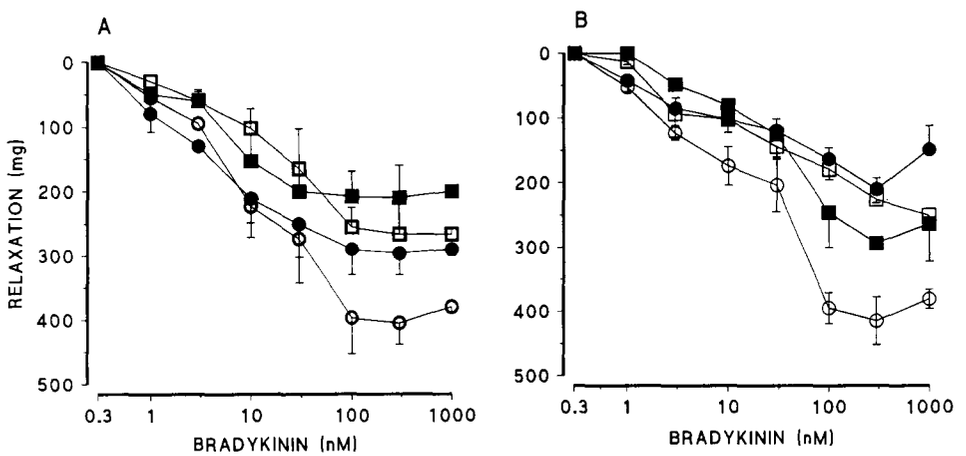

Fig. 2. Relaxant response obtained for bradykinin in the absence (○) or in the presence of (A) HOE 140 (nM): 0.1 (●), 0.3 (□) and 1 (■) or (B) NPC 17761 (nM): 1 (●), 3 (□) and 10 (■) in epithelium intact strips of guinea-pig trachea. Each point represents the mean of 4–5 experiments and the vertical lines indicate the S.E.M.

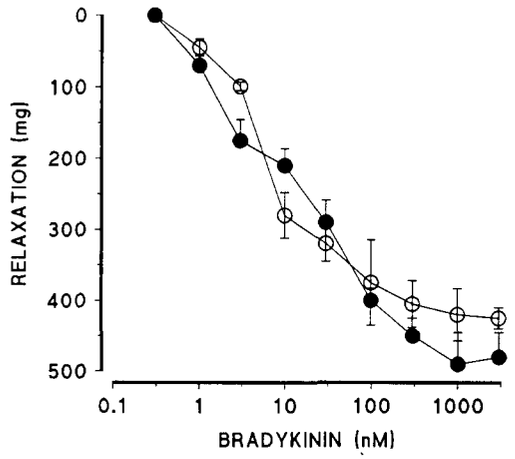

Fig. 3. Effect of kinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin on bradykinin-induced relaxation in epithelium intact strips of guinea-pig trachea. Responses obtained in the absence (○) or in the presence of des-Arg^9-[Leu^8]bradykinin 1 μM (●). Each point represents the mean of 8 experiments and the vertical bars indicate the S.E.M.

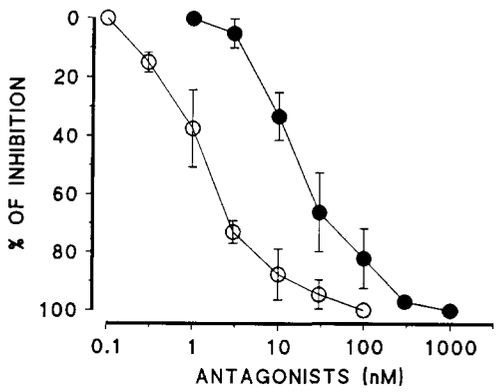

Fig. 4. Effect of bradykinin B_2 receptor antagonists HOE 140 (○) and 17761 (●) (0.1–3000 nM) on bradykinin (100 nM)-induced relaxation in epithelium intact strips of guinea-pig trachea. Each point represents the mean of 7 experiments and the vertical lines indicate the S.E.M.

on bradykinin-mediated relaxation. The calculated EC_{50} values (and 95% confidence limits) were: control group 12.4 (4.3/18.9) nM and in the presence of des-Arg^9-[Leu^8]bradykinin 12.9 (4.2/22.3) nM.

We next analysed the effect of several concentrations of the selective B_2 bradykinin receptor antagonists HOE 140 and NPC 17761 (1–1000 nM) against bradykinin (100 nM)-induced relaxation in preparations with intact epithelium and under spontaneous tone. The results of Fig. 4 show that the two bradykinin B_2 receptor antagonists caused a concentration-dependent inhibition of bradykinin-induced relaxation. IC_{50} values (and their 95% confidence limits) were 1.4 (0.7/2.7) and 19.1 (7.6/47.0) nM, respectively. The inhibitory actions of both bradykinin B_2 receptor antagonists were completely reversible after washing (data not shown). Bradykinin B_2 receptor antagonists were selective in antagonizing bradykinin-induced relaxation, as even at higher concentrations up to 100 nM they did not affect the relaxant response produced by isoproterenol (1 μM) or sodium nitroprusside (1 μM) (Fig. 5).

3.3. Effect of several drugs against bradykinin-induced relaxation in the guinea-pig trachea

The results summarized in Table 2 and the data of Fig. 6 show that preincubation of epithelium intact strips of guinea-pig trachea with apamin (0.3 and 1 μM) caused a partial, although significant concentration-dependent antagonism of the relaxation caused by bradykinin (100 nM). However, even at a higher con-

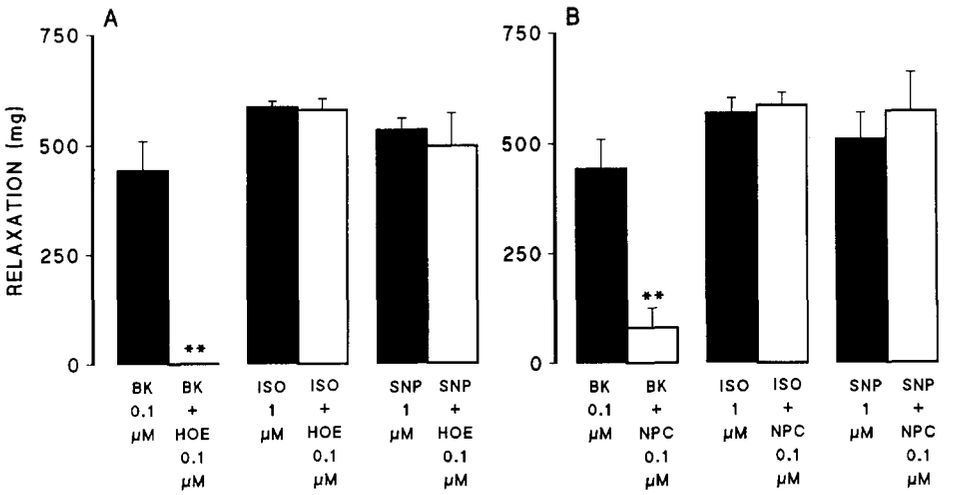

Fig. 5. Effect of bradykinin B_2 receptor antagonists HOE 140 (100 nM) (A) and NPC 17761 (100 nM) (B) on bradykinin (BK, 100 nM)-, isoproterenol (ISO, 1 μM)- and sodium nitroprusside (SNP, 1 μM)-mediated relaxations in epithelium intact strips of guinea-pig trachea. Each column represents the mean of 5–7 experiments and the vertical bars indicate the S.E.M. Significant difference from control groups: $^{**}P < 0.01$.

Table 2
Effect of different classes of drugs on bradykinin (100 nM)-induced relaxation in epithelium intact strips of guinea-pig trachea

Drug	Concentration (μM)	Relaxation (mg)	
		Absence	Presence
Glibenclamide	1	236±20	254±23
Tetrodotoxin	0.3	329±36	313±35
Nickel chloride	100	400±30	355±40
[D-*p*-Cl-Phe^6,Leu^{17}]VIP	0.03	405±56	350±29
Propranolol	1	384±75	441±60
Staurosporine	0.3	258±35	313±66
Nicardipine	1	278±25	280±21
Ca^{2+}-free plus EGTA	–	390±58	395±64
Ryanodine	10	326±33	376±54

Values are means±S.E.M. of at least 5–10 experiments.

centration (1 μM) apamin failed to inhibit prostaglandin E_2 (300 nM)-and isoproterenol (100 nM)-induced relaxation. Bradykinin-induced relaxation was not significantly affected by tetrodotoxin (0.3 μM), nicardipine (1 μM) or Ca^{2+}-free solution either in the absence or in the presence of ryanodine (10 μM), glibenclamide (1 μM), nickel chloride (100 μM), propranolol (1 μM), staurosporine (0.1 μM) or [D-*p*-Cl-Phe^6,Leu^{17}]VIP (0.03 μM) (Table 2). In addition, none of these drugs affected per se the resting tone of the preparations (results not shown).

4. Discussion

The results reported in the present study extend our previous finding (Schlemper and Calixto, 1994) and show that bradykinin and related peptides caused, with slow onset, reproducible, concentration-dependent relaxation in epithelium intact strips of guinea-pig trachea under basal tone. Furthermore, the IC_{50} of bradykinin-induced relaxation in preparations precontracted with carbachol was not statistically different from the IC_{50} obtained in experiments carried out under normal tonus. Contrasting with the results reported by Rhaleb et al. (1988), our data indicate that kinin-induced relaxation in epithelium intact strips of the guinea-pig trachea is mediated through stimulation of bradykinin B_2 receptors. These observations are substantiated by the following evidence: (1) the reported rank order of potency for kinins is compatible with preparations containing bradykinin B_2 receptors; (2) the selective kinin B_1 agonist des-Arg^9-bradykinin did not cause any detectable response in the preparations, nor did des-Arg^9-[Leu^8]bradykinin, a kinin B_1 selective receptor antagonist, affect the concentration-dependent relaxant response caused by bradykinin; and (3) the new generation of potent and highly selective bradykinin B_2 receptor antagonists, HOE 140 and NPC 17761, caused a complete and reversible concentration-dependent inhibition of the bradykinin-mediated relaxation of epithelium intact strips of guinea-pig trachea.

Of interest are the results showing that both HOE 140 and NPC 17761 caused a concentration-dependent rightward shift of the relaxant concentration-reponse curve for bradykinin. The two antagonists also caused inhibition of the maximal response to bradykinin, thus indicating a noncompetitive antagonism. A noncompetitive mechanism of the bradykinin B_2 receptor antagonists towards bradykinin-mediated relaxation in guinea-pig trachea could be explained by the fact that,

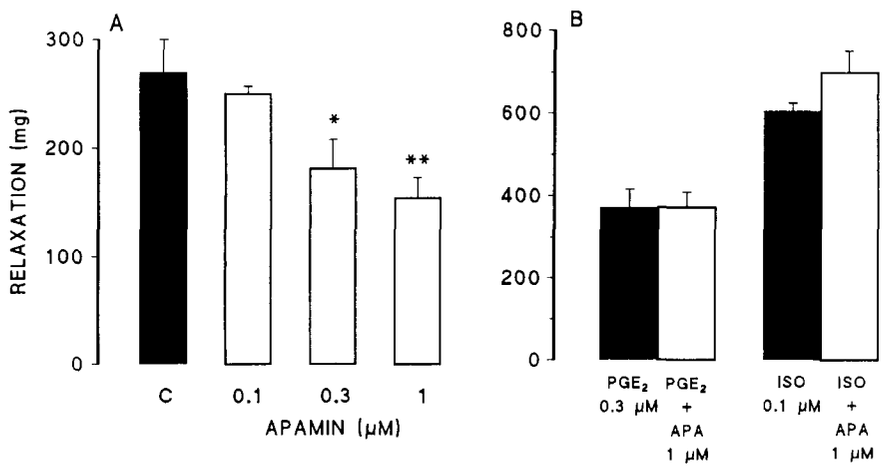

Fig. 6. Effect of Ca^{2+}-activated K^+ channel blocker apamin (APA) on (A) bradykinin (BK, 100 nM)-, (B) prostaglandin E_2 (PGE_2, 0.3 μM)- or isoproterenol (ISO, 100 nM)-mediated relaxation in epithelium intact strips of guinea-pig trachea. Each column represents the mean of 6–8 experiments and the vertical bars indicate the S.E.M. Significant difference from control group: $^*P < 0.05$, $^{**}P < 0.01$.

in this preparation, the bradykinin response is indirectely mediated by the release of prostanoids, possibly prostaglandin E_2 (Folkerts et al., 1989; Bramley et al., 1990; Proud et al., 1993), in addition to the release of nitric oxide from the epithelium (Schlemper and Calixto, 1994). Very similar noncompetitive antagonism was reported for propranolol in antagonizing the indirect responses to tyramine of electrically stimulated contractions of rat left atria (Black et al., 1981, see for review Kenakin, 1984). Recent radioligand binding studies carried out in guinea-pig tracheal epithelial cells have found two binding sites for bradykinin at B_2 receptors, a high-affinity site (estimated K_d of 0.44 nM) and a low-affinity site (estimated K_d of 10 nM) (Proud et al., 1993). Trifilieff et al. (1992) also reported two binding sites for bradykinin in epithelium intact strips of guinea-pig trachea (estimated K_d of 0.04 and 180 nM), while in guinea-pig trachea without epithelium only one binding site for bradykinin was observed (estimated K_d 55 pM). Interestingly, both bradykinin sites were competitively and completely antagonized with a higher affinity by the selective bradykinin B_2 receptor antagonists HOE 140 and NPC 17761, thus indicating the presence of B_2 receptors in the preparations (Trifilieff et al., 1992,1993; Proud et al., 1993). Furthermore, our results also demonstrated that the B_2 receptor antagonists studied were quite selective toward bradykinin, as they failed to affect isoproterenol- and sodium nitroprusside-mediated responses.

The relaxation caused by bradykinin in epithelium intact strips of guinea-pig trachea was unaffected by tetrodotoxin, nicardipine or Ca^{2+}-free medium, either in the absence or in the presence of ryanodine, glibenclamide, the selective VIP receptor antagonist [D-*p*-Cl-Phe^6,Leu^{17}]VIP, propranolol, nickel chloride or staurosporine. These data suggest that the neural release of neurotransmitters in response to the action potential-dependent, activation of ATP-sensitive K^+ channels, mobilization of extracellular Ca^{2+} influx through L-or T-type channels, mobilization of intracellular Ca^{2+} stores sensititive to ryanodine, stimulation of β_2-adrenoceptors and VIP receptors or activation of a protein kinase C-dependent mechanism does not seem to be involved in the relaxant response produced by bradykinin. The question as to whether bradykinin-mediated relaxation in guinea-pig trachea involves Ca^{2+} mobilization from intracellular sources through mechanisms distinct from ryanodine-sensitive receptors (reviewed by Sorrentino and Volpe, 1993; McPherson and Campbell, 1993; Meissner, 1994), as it has been suggested to occur for the relaxation response to bradykinin in guinea-pig taenia caeci (Field et al., 1994), was not further investigated in the present study.

Bradykinin-mediated relaxation in guinea-pig trachea was partially but significantly antagonized by apamin, thus suggesting that, as reported for bradykinin-mediated relaxation in other smooth muscle tissues (Hall and Morton, 1991; Griesbacher, 1992; Field et al., 1994), bradykinin-mediated relaxation in guinea-pig trachea is modulated by small conductance Ca^{2+}-activated K^+ channels. However, apamin did not significantly affect the relaxation responses caused by exogenous administration of prostaglandin E_2 or isoproterenol, suggesting that inhibition of bradykinin-induced relaxation did not occur at the smooth muscle, but probably at the epithelium level controlling prostaglandin E_2 and/or nitric oxide release. However, evidence now suggests that nitric oxide or nitric oxide releasing substances directly activate Ca^{2+}-sensitive K^+ channels in vascular smooth muscles, a response which is antagonized by potent and selective Ca^{2+}-activated K^+ channel blockers, such as apamin, charybdotoxin and iberiotoxin (Khan et al., 1993; Suzuki et al., 1993; Bolotina et al., 1994; Kitamura et al., 1993; Maggi and Giuliani, 1994). Thus, we cannot completely rule out the possibility that apamin inhibits bradykinin relaxation through a direct action at the smooth muscle cells from guinea-pig trachea. Further studies will be required to clarify this point.

While this paper was under revision, Da Silva et al. (1995) reported that bradykinin induced graded relaxation, in epithelium intact strips of guinea-pig trachea pre-contracted with histamine (30 μM), through stimulation of bradykinin B_2 receptors, but not B_1 receptors. This effect involves the activation of phospholipase A_2 and the release of products from the cyclo-oxygenase pathway. The authors also reported that, in contrast to our results, NPC 17761 caused a pure competitive antagonism of bradykinin-mediated relaxation, yielding a pA_2 value of 7.3 ± 0.4 and slope of unity, whereas the antagonism afforded by HOE 140 was non-competitive. A comparison between our results and those reported by Da Silva et al. (1995) is difficult due to marked differences between the methodologies used, such as the periods of equilibration, presence or absence of previous tone with histamine, or of the endopeptidase inhibitor thiorphan. Also, Da Silva et al. (1995) obtained concentration-response curves by cumulative addition of bradykinin, whereas single addition was used in our experiments.

In summary, the findings of the present study confirm and extend those of previous studies (Bhoola et al., 1989; Bramley et al., 1990; Schlemper and Calixto, 1994; Da Silva et al., 1995), and provide functional evidence that bradykinin induces concentration-dependent relaxation of epithelium intact strips of the guinea-pig trachea through stimulation of bradykinin B_2 receptors, a response which is antagonized in a selective and reversible but noncompetitive manner by the highly potent and selective bradykinin B_2 receptor antagonists, HOE 140 and NPC 17761. Furthermore, bradykinin-induced relaxation of guinea-pig trachea

does not involve a neuronal pathway, but is modulated, at least in part, by activation of small conductance Ca^{2+}-activated K^+ channels.

Acknowledgements

The authors are grateful to Elizabet T. Ramos Ganzer for secretarial help in preparing the manuscript and the pharmaceutical companies for the donation of some of the drugs used in this study. This work was supported by grants from CNPq and FINEP (Brazil). V.S. is an M.Sc. student receiving a grant from CAPES (Brazil).

References

Barnes, P.J., 1992, Bradykinin and asthma, Thorax 47, 979.

Bhoola, K.D., J. Bewley, D.M. Crothers, M.I. Cingi and C.D. Figueroa, 1989, Kinin receptors on epithelial cells and smooth muscle of the trachea, Adv. Exp. Med. Biol. 247, 421.

Black, J.W., D.H. Jenkinson and T.P. Kenakin, 1981, Antagonism of an indirectly acting agonist: block by propranolol and sotalol of the action of tyramine on rat heart, Eur. J. Pharmacol. 65, 1.

Bolotina, V.M., S. Najibi, J.J. Palacino, P.J. Pagano and R.A. Cohen, 1994, Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle, Nature 368, 850.

Bramley, A.M., M.N. Samhoun and P.J. Piper, 1990, The role of the epithelium in modulating the responses of guinea-pig trachea induced by bradykinin in vitro, Br. J. Pharmacol. 99, 762.

Da Silva, A., Y. Amrani, A. Trifilieff and Y. Landry, 1995, Involvement of B_2 receptors in the bradykinin induced relaxation of guinea-pig isolated trachea, Br. J. Pharmacol. 114, 103.

Farmer, S.G., 1991a, Role of kinins in airway diseases, Immunopharmacology 22, 1.

Farmer, S.G., 1991b, Airway pharmacological bradykinin, in: Bradykinin Antagonists: Basic and Clinical Research, ed. R.M. Burch (Marcel Dekker, New York) p. 213.

Farmer, S.G., 1994, The pharmacology of bradykinin in human airways, in: Drugs and the Lung, eds. C.P. Page and W.J. Metzger (Raven Press, New York).

Field, J.L., S.K. Butt, I.K.M. Morton and J.M. Hall, 1994, Bradykinin B_2 receptors and coupling mechanisms in the smooth muscle of the guinea-pig taenia caeci, Br. J. Pharmacol. 113, 2.

Folkerts, G., F. Engels and F.P. Nijkamp, 1989, Endotoxin-induced hyperreactivity of the guinea-pig isolated trachea coincides with decreased prostaglandin E_2 production by the epithelial layer, Br. J. Pharmacol. 96, 388.

Garcia Leme, J., 1978, Bradykinin system, in: Handbook of Experimental Pharmacology, Vol. 50, Inflammation, eds. J.R. Vane and S.H. Ferreira (Springer-Verlag, Berlin) p. 464.

Geppetti, P., 1993, Sensory neuropeptide release by bradykinin; mechanisms and pathophysiological implications, Regul. Pept. 47, 1.

Griesbacher, T., 1992, Kinin-induced relaxations of the rat duodenum, Naunyn-Schmied. Arch. Pharmacol. 346, 102.

Hall, J.M., 1992, Bradykinin receptors: pharmacological properties and biological roles, Pharmacol. Ther. 56, 131.

Hall, J.M. and K.M. Morton, 1991, Bradykinin B_2 receptor evoked K^+ permeability increase mediates relaxation in the rat duodenum, Eur. J. Pharmacol. 193, 231.

Kenakin, T.P., 1984, The classification of drugs and drug receptors in isolated tissues, Pharmacol. Rev. 36, 165.

Khan, S.A., W.R. Mathews and K.D. Meisheri, 1993, Role of calcium-activated K^+ channels in vasodilation induced by nitroglycerine, acetylcholine and nitric oxide, J. Pharmacol. Exp. Ter. 267, 1327.

Kitamura, K., Q. Lian, A. Carl and H. Kuriyama, 1993, *S*-Nitrosocysteine, but not sodium nitroprusside, produces apamin-sensitive hyperpolarization in rat gastric fundus, Br. J. Pharmacol. 109, 415.

Lewis, G.P., 1970, Kinin in inflammation and tissue injury, in: Handbook of Experimental Pharmacology, Vol. 25, ed. E.G. Erdös (Springer- Verlag, Berlin) p. 516.

Maggi, C.A. and S. Giuliani, 1994, Multiple inhibitory mechanisms mediate non-adrenergic non-cholinergic relaxation in the circular muscle of the guinea-pig colon, Naunyn-Schmied. Arch. Pharmacol. 347, 630.

Marceau, F., A. Lussier, D. Regoli and G.P. Giroud, 1983, Pharmacology of kinins: their relevance to tissue injury and inflammation, Gen. Pharmacol. 14, 209.

McPherson, P.S. and K.P. Campbell, 1993, The ryanodine receptor/Ca^{2+} release channel, J. Biol. Chem. 268, 13765.

Meissner, G., 1994, Ryanodine receptor/Ca^{2+} release channel and their regulation by endogenous effectors, Annu. Rev. Physiol. 56, 485.

Mizrahi, J., R. Couture, S. Caranikas and D. Regoli, 1982, Pharmacological effects of peptides on tracheal muscle, Pharmacology 25, 39.

Proud, D., C.J. Reynolds, J. Broomfield, D.W. Goldman and J.M. Bathon, 1993, Bradykinin effects in guinea-pig tracheal epithelial cells are mediated through a B_2 kinin receptor and can be inhibited by the selective antagonist HOE 140, J. Pharmacol. Exp. Ther. 264, 1124.

Regoli, D. and J. Barabé, 1980, Pharmacology of bradykinin and related kinins, Pharmacol. Rev. 32, 1.

Rhaleb, N.-E., S. Dion, P. D'Orléans-Juste, G. Drapeau, D. Regoli and R. Browne, 1988, Bradykinin antagonism: differentiation between peptide antagonists and inflammatory agents, Eur. J. Pharmacol. 151, 275.

Schlemper, V. and J.B. Calixto, 1994, Nitric oxide pathway-mediated relaxant effect of bradykinin in the guinea-pig isolated trachea, Br. J. Pharmacol. 111, 83.

Sorrentino, V. and P. Volpe, 1993, Ryanodine receptors: how many, where and why, Trends Pharmacol. Sci. 14, 98.

Suzuki, K., K.M. Ito, Y. Minayoshi, H. Suzuki, M. Asano and K. Ito, 1993, Modification by charybdotoxin and apamin of spontaneous electrical and mechanical activity of the circular smooth muscle of the guinea-pig stomach, Br. J. Pharmacol. 109, 661.

Trifilieff, A., A. Da Silva, Y. Landry and J.-P. Gies, 1992, Effect of HOE 140, a new B_2 noncompetitive antagonist, on guinea-pig tracheal bradykinin receptors, J. Pharmacol. Exp. Ther. 263, 1377.

Trifilieff, A., Y. Amrani, Y. Landry and J.-P. Gies, 1993, Comparative action of new highly potent bradykinin receptor antagonists in the guinea-pig trachea, Eur. J. Pharmacol. 239, 227.