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Involvement of B_2 receptors in the bradykinin-induced relaxation of guinea-pig isolated trachea

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1 The aim of this study was to determine the receptor type and involvement of arachidonic acid metabolites in bradykinin-induced relaxation of the guinea-pig isolated trachea.

2 In the resting tracheal preparation, bradykinin (0.1 nM–30 μM induced a concentration-related contractile response ($pD_2 = 8.8 \pm 0.3$). The maximal tension (1056 ± 321 mg) was observed at 0.3 μM bradykinin. In contrast, when tracheal preparations were pre-contracted with histamine (30 μM leading to a half-maximum response), a concentration-related relaxation was observed with bradykinin. At the highest concentration of bradykinin used (3 μM), a reversal of 63 ± 13% of the contractile response to histamine was observed. Both effects of bradykinin were inhibited by the cyclo-oxygenase inhibitor, indomethacin (1 μM). In concentration-response curves, melittin (10 nM–1 μM), a direct activator of phospholipase A_2, mimicked both effects of bradykinin. The highest concentration of melittin used (1 μM), induced a tension of 813 ± 120 mg and led to the reversal of 41 ± 8% of the contractile response to histamine. The contractile effect of melittin was inhibited in the presence of both indomethacin (1 μM) and AA861 (1 μM), a 5-lipoxygenase inhibitor.

3 [Des Arg^9]-bradykinin (1 nM–3 μM), a B_1-receptor agonist, was unable to relax precontracted guinea-pig tracheal preparations. The relaxation induced by bradykinin was antagonized by the B_2 receptor antagonists, Hoe 140 (D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) and NPC 17761 (D-Arg^0[Hyp^3,D-HypE(*trans*-thiophenyl)^7,Oic^8]bradykinin). Hoe 140 (0.1 μM to 0.6 μM) behaved as a non-competitive antagonist with an apparent $pA_2 = 7.2 \pm 0.4$, whereas NPC 17761 (0.3 to 1 μM) competitively antagonized bradykinin-induced relaxation with a $pK_B = 7.3 \pm 0.2$. The Schild regression slope did not differ from unity, 0.96 ± 0.20, $P < 0.05$.

4 These data demonstrate that bradykinin-induced relaxation of guinea-pig trachea occurs via the activation of bradykinin B_2-receptors. The stimulation of B_2-bradykinin receptors induces the activation of the cyclo-oxygenase pathway, leading either to contraction or relaxation depending on the tone of the trachea.

Keywords: Guinea-pig trachea; bradykinin; melittin; contraction; relaxation; bradykinin B_2-receptors; phospholipase A_2; Hoe 140; NPC 17761

Introduction

Bradykinin is a potent mediator of inflammation and is implicated in asthma (for a review see Barnes, 1992 and Trifilieff *et al.*, 1993b). On the basis of agonist potencies, the major airway receptors for bradykinin causing bronchoconstriction in asthmatic subjects belong to the B_2 type (Polosa & Holgate, 1990). In the human airway, the contraction of smooth muscle is mediated via cholinergic (Fuller *et al.*, 1987) and peptidergic mechanisms (Dixon & Barnes, 1989; Ichinose *et al.*, 1992). Inhibition of the cyclo-oxygenase pathway did not modify the bronchoconstrictor effect of inhaled bradykinin in human subjects (Fuller *et al.*, 1987; Polosa & Holgate, 1990).

In guinea-pig airways, the effect and the mechanism of action of bradykinin are complex and controversial. Bradykinin can produce both contraction and relaxation of the guinea-pig isolated trachea (Mizrahi *et al.*, 1982; Rhaleb *et al.*, 1988; Bramley *et al.*, 1990; Frossard *et al.*, 1990). The contractile response was first described as insensitive to early available B_2-receptor antagonists (Farmer *et al.*, 1989). Recently, the availability of new B_2-receptor antagonists such as Hoe 140 (Hock *et al.*, 1991; Wirth *et al.*, 1991) and NPC 17731 led to the demonstration of the involvement of B_2-receptors in the contractile response of bradykinin both *in vivo* (Wirth *et al.*, 1991) and *in vitro* (Farmer *et al.*, 1991a; Field *et al.*, 1992; Rhaleb *et al.*, 1992; Trifilieff *et al.*, 1992; 1993a). Bradykinin can also induce relaxation of guinea-pig trachea. A role for the epithelium has been suggested in this effect (Bramley *et al.*, 1990; Frossard *et al.*, 1990; Schlemper & Calixto, 1994). However, Bramley *et al.* (1990) noted that intact preparations (with epithelium) gained appreciable tone during the equilibration period, whereas rubbed trachea (without epithelium) developed no visible tone. We have therefore investigated whether the effects induced by bradykinin were related to the presence of epithelium or were dependent on the tone of the organ as has been described for prostaglandin E_2 (PGE_2) and arachidonic acid-induced responses (Braunstein *et al.*, 1988; Nijkamp & Folkerts, 1987; Tschirhart *et al.*, 1987). The relaxant effect of bradykinin has been observed in the guinea-pig isolated trachea precontracted with histamine (Mizrahi *et al.*, 1982). This relaxant effect was considered to be dependent on prostaglandins, without the involvement of B_1 or B_2 receptors, due to the inefficiency of bradykinin antagonists (Mizrahi *et al.*, 1982; Rhaleb *et al.*, 1988; Bramley *et al.*, 1989). A direct activation of one of the enzymes of the arachidonic acid cascade by bradykinin has been proposed (Rhaleb *et al.*, 1988). In the present study, the effects of new B_2-receptor antagonists (Hoe 140 and NPC 17761) on bradykinin-induced relaxation were investigated. The response to melittin, a direct activator of phospholipase A_2 (Shier *et al.*, 1979), was compared with that of bradykinin. The present study shows that the bradykinin-induced relaxation is mediated via a bradykinin B_2-receptor coupled with the activation of the arachidonic acid cascade. Thus, the products of the cyclo-oxygenase reaction can induce contraction or relaxation depending on the tone of the guinea-pig isolated trachea.

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Methods

Male albino Dunkin Hartley guinea-pigs (Elevage Saint-Antoine, Pleudaniel, France) weighing 250–350 g were anaesthetized with sodium pentobarbitone, 45 mg kg^{-1} i.p. The trachea was rapidly dissected out and immersed in a modified Krebs physiological solution (composition mM: NaCl 120, KCl 4.75, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.15, $NaHCO_3$ 25, glucose 10). The trachea was cleaned of connective tissue and opened longitudinally through the cartilage, opposite the smooth muscle. Fragments corresponding to four cartilaginous rings were cut from each trachea, discarding the upper and the lower part of the tissue. To prepare the epithelium-free tracheal preparations, the luminal surface was rubbed gently with a cotton swab as described previously (Da Silva *et al.*, 1992). Tracheal fragments were suspended in 10 ml organ baths containing the modified Krebs physiological solution at 37°C gassed with a mixture of O_2 (95%) and CO_2 (5%), pH = 7.4. Variations in smooth muscle tone were measured isometrically with Narco F60 Myographs connected to Narco MKIII Physiograph (Narco Bio-Systems, Inc, Houston, TX, U.S.A.) after washing (three times at 15 min intervals) and equilibration for 30 min at a tension of 2 g, which was found to be optimal for measuring changes in tension. The effects of melittin, bradykinin and two bradykinin-receptor antagonists (Hoe 140 and NPC 17761) in isolated tissues may be influenced by endogenous peptidases enzymes. Although many peptidases are able to hydrolyse kinins, endopeptidase 24.11 was found to be the most important modulator of bradykinin-induced effects in airways (Dusser *et al.*, 1988; Da Silva *et al.*, 1992). Therefore, all experiments were conducted in the presence of an inhibitor of endopeptidase 24.11, DL-thiorphan (10 μM). Inhibitors were added 30 min prior to bradykinin or melittin. Bradykinin receptor-antagonists were added 10 min after the addition of thiorphan to the organ bath. Concentration-response curves were constructed by the cumulative addition of bradykinin or melittin. We studied the effects of bradykinin and melittin on guinea-pig tracheal preparations set at 2 g tension (resting preparations) or set at 2 g tension and then precontracted by the addition of 30 μM histamine, which corresponds to the EC_{50} for this agonist (precontracted preparations). In all experiments, only one cumulative concentration-response curve was constructed in the presence or absence of a single concentration of bradykinin receptor-antagonist or enzyme inhibitor.

Reagents

Bradykinin and melittin (Sigma Chemical Co., St Louis, MO, U.S.A.) were prepared as 4 mM stock solutions and aliquots were stored at −20°C. DL-Thiorphan (Bachem, Bubbendorf, Switzerland), indomethacin (Merck, Sharp & Dohme, Riom, France) and AA861 (2-(12-hydroxydodeca-5,10-cliynyl)-3,5,6-trimethyl-1,4-benzoquinone) (Takeda Chemicals Industries Ltd, Japan) were dissolved in absolute ethanol at 20, 2 and 2 mM, respectively. D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140) was a gift from Hoechst AG (Frankfurt, Germany). D-Arg^0[Hyp^3,D-HypE(trans-thiophenyl)^7,Oic^8]-bradykinin (NPC 17761) was a gift from Scios Nova Inc. (Baltimore, MD, U.S.A.). Hoe 140 and NPC 17761 were prepared as 0.1 mM stock solutions in distilled water. Additional dilutions were performed in modified Krebs physiological solution.

Data analysis

pD_2 values (negative $\log_{10}$ of the molar concentration of agonist required to elicit 50% of the maximal response) were calculated (Van Rossum, 1963). Dose-ratios were calculated from the equi-effective molar concentration of bradykinin in the presence and absence of antagonist that led to 30% to the reversal of the contractile response to histamine. pA_2 and pK_B values were obtained by linear regression of 'Schild plots' derived from concentration-response curves (Arunlakshana & Schild, 1959). Differences between groups were assessed by Student's paired or unpaired t test and considered significant when $P<0.05$.

Results

Bradykinin (0.1 nM–30 μM) induced a concentration-related contraction in the resting tracheal preparations (Figure 1a). This response was prevented by 1 μM indomethacin, a cyclooxygenase inhibitor. A slight contractile effect remained in the presence of indomethacin with bradykinin concentrations greater than 0.1 μM (Figure 1a). A maximal tension of 1056 ± 321 mg was induced by 0.3 μM bradykinin. The estimated pD_2 for bradykinin was 8.8 ± 0.3 (n = 5). The same effect was observed in tracheal preparations without epithelium, the pD_2 and maximal tension being 7.4 ± 0.1 and

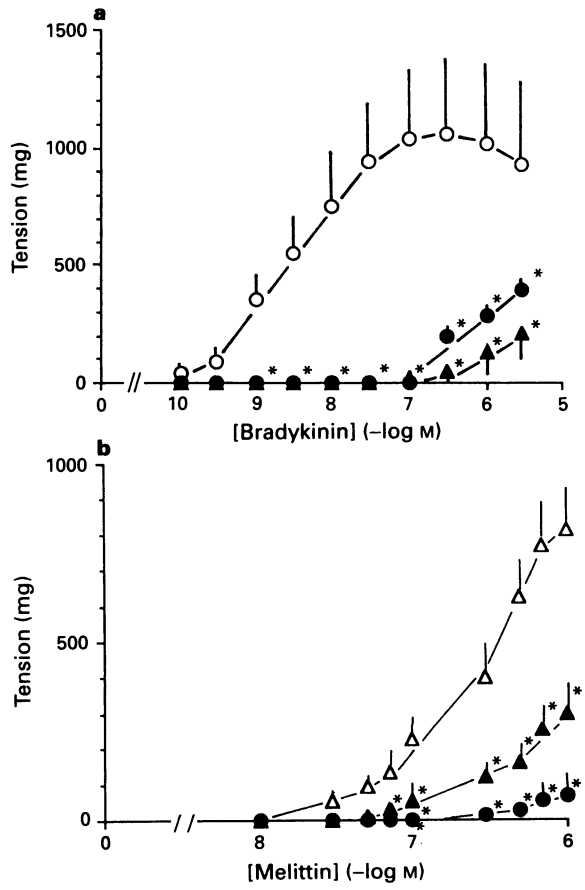

Figure 1 (a) Log concentration-response curves for bradykinin in guinea-pig tracheal preparations in the presence of thiorphan (10 μM). Tracheal preparations were suspended under 2 g tension before starting the concentration-response curve for bradykinin (○). Values are means ± s.e.mean for 5 experiments. Experiments were performed in the absence (○), in the presence of 1 μM indomethacin (▲) or in the presence of 1 μM indomethacin plus 1 μM AA861 (●) added 30 min before the first concentration of bradykinin. *Values significantly different between control preparations and preparations in the presence of indomethacin: $P<0.05$. (b) Log concentration-response curves for melittin in guinea-pig tracheal preparations in the presence of thiorphan 10 μM. Tracheal preparations were suspended under 2 g tension before starting the concentration-response curve for melittin (△). Values are means ± s.e.mean for 6 experiments. Experiments were performed in the absence (△), or in the presence of 1 μM indomethacin (▲) or in the presence of 1 μM indomethacin plus 1 μM AA861 (●) added 30 min before the first concentration of melittin. *Values significantly different between control preparations and preparations in the presence of indomethacin or with indomethacin plus AA861: $P<0.05$.

830 ± 77 mg, respectively ($n = 5$). AA861 (1 μM), a 5-lipoxygenase inhibitor (Ashida *et al.*, 1983), did not modify the responses to bradykinin in the presence or absence of indomethacin (Figure 1a). A contractile response of resting tracheal preparations was observed at concentrations from 30 nM to 1 μM melittin which was partly prevented by 1 μM indomethacin (Figure 1b) or 1 μM AA861 (not shown). Complete inhibition of the contractile response was observed in the combined presence of indomethacin and AA861 (Figure 1b).

A relaxant effect of bradykinin was observed with 3 nM of the peptide, following a pre-contraction of tracheal preparations with histamine (Figure 2a). The highest bradykinin concentration used (3 μM) led to the reversal of 63 ± 13% of the contractile response to histamine. In tracheal preparations without epithelium, the highest bradykinin concentration used (3 μM) led to the reversal of 42 ± 4% ($n = 5$) of the histamine-induced contraction. Indomethacin (1 μM) fully prevented this relaxant effect of bradykinin but did not significantly modify the precontraction by histamine (Figure 2a). The selective B_1-receptor agonist [des-Arg$_9$]-bradykinin did not reverse the histamine-induced tone (Figure 2a). Melittin relaxed the precontracted guinea-pig isolated trachea at concentrations greater than from 30 nM (Figure 2b). The highest melittin concentration used (1 μM) reversed 41 ± 8% ($n = 6$) of the histamine-induced contraction. This effect of melittin was entirely prevented by 1 μM indomethacin (Figure 2b).

The effect of selective B_2-receptor antagonists on the relaxant response of precontracted tracheal preparations to bradykinin is shown on Figure 3. Hoe 140 decreased the bradykinin-dependent relaxation, but is effect was insurmountable even on increasing the concentration of bradykinin. The apparent pA_2 was determined as 7.2 ± 0.4. In contrast, NPC 17761 led to a shift to the right of the bradykinin concentration-response curve (Figure 3). The corresponding pK_B value was 7.3 ± 0.2 with a Schild's plot slope of 0.96 ± 0.20 which did not differ from unity, $P < 0.05$ (Figure 3). The responses to melittin were unaffected by bradykinin antagonists (Figure 4).

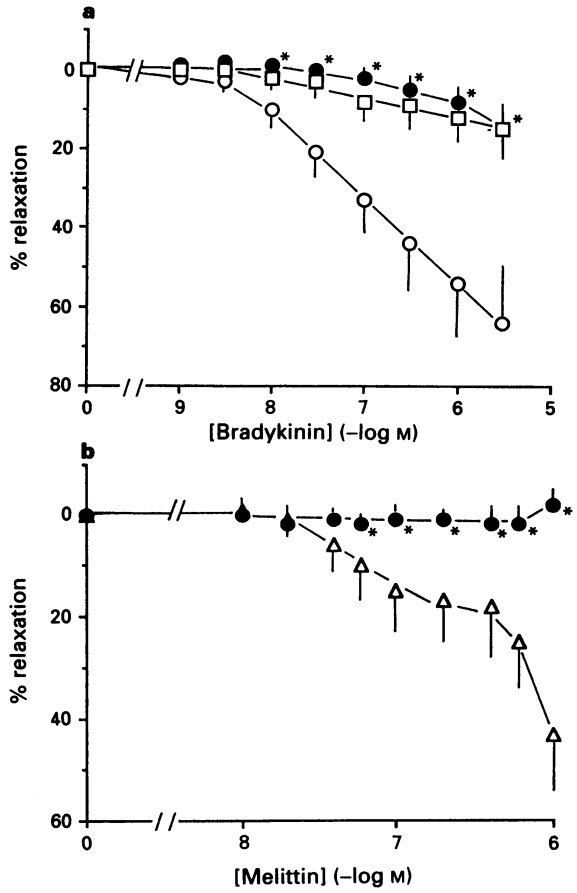

Figure 2 (a) Log concentration-response curves for bradykinin and [des-Arg^9]-bradykinin in precontracted guinea-pig tracheal preparations in the presence of thiorphan (10 μM). Tracheal preparations were suspended under 2 g tension then stimulated with histamine (30 μM) before starting the concentration-response curve for bradykinin (○) or [des-Arg^9]-bradykinin (□). Experiments with bradykinin were performed in the absence (○) or in the presence of 1 μM indomethacin (●) added 30 min before the first concentration of bradykinin. The tension induced by histamine was 1420 ± 174 mg. Values are means ± s.e.mean for 5 experiments. *Values significantly different between control preparations stimulated with bradykinin and preparations stimulated with bradykinin in the presence of indomethacin: $P < 0.05$. (b) Log concentration-response curves for melittin in precontracted guinea-pig tracheal preparations in the presence of thiorphan (10 μM). Tracheal preparations were suspended under 2 g tension then stimulated with histamine (30 μM before starting the concentration-response curve for melittin (△). Experiments were performed in the absence (△) or in the presence of 1 μM indomethacin (●) added 30 min before the first dose of melittin. The tension induced by histamine was 1583 ± 232 mg. Values are means ± s.e.mean for 6 experiments. *Values significantly different between control preparations stimulated with melittin and preparations stimulated with melittin in the presence of indomethacin: $P < 0.05$.

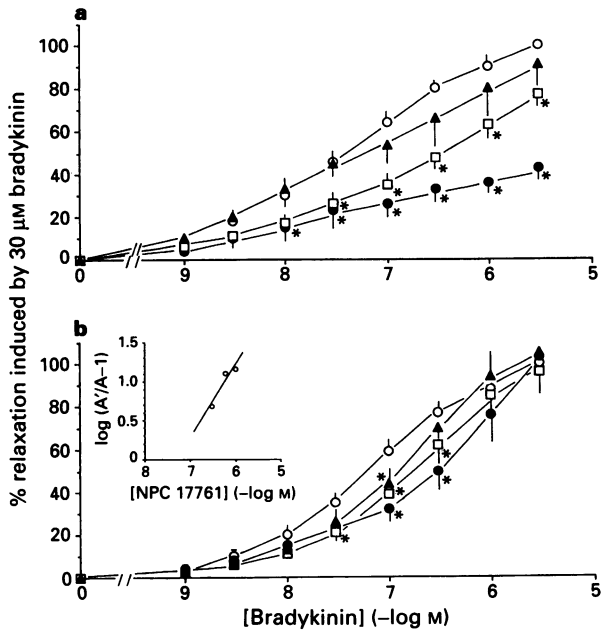

Figure 3 Effect of bradykinin receptor antagonists on bradykinin-induced relaxation on precontracted guinea-pig tracheal preparations. (a) Tracheal preparations were suspended under 2 g tension then stimulated with histamine (30 μM) before starting the concentration-response curve for bradykinin in the absence (○) or in the presence of Hoe 140 (▲, 0.1 μM; □, 0.3 μM and ●, 0.6 μM on bradykinin-induced relaxation. The tension induced by histamine was 1418 ± 315 mg. Values are means ± s.e.mean for 5 experiments. *Values significantly different between control preparations stimulated with bradykinin and preparations stimulated with bradykinin in the presence of Hoe 140: $P < 0.05$. (b) Tracheal preparations were suspended under 2 g tension then stimulated with histamine (30 μM) before starting the concentration-response curve for bradykinin in the absence (○) or in the presence of NPC 17761 (▲, 0.3 μM; □, 0.6 μM and ●, 1 μM) on bradykinin-induced relaxation. The tension induced by histamine was 1706 ± 173 mg. Insert: Schild plot for NPC 17761-induced relaxation. Values are means ± s.e.mean for 11 experiments. *Values significantly different between control preparations stimulated with bradykinin and preparations stimulated with bradykinin in the presence of NPC 17761: $P < 0.05$. Bradykinin effects are expressed as % of the relaxation induced by bradykinin (30 μM) in the absence of antagonist.

Discussion

We demonstrate that bradykinin can induce relaxation or contraction of guinea-pig isolated trachea both in the presence or absence of the epithelium. The effects of bradykinin are inhibited by the cyclo-oxygenase inhibitor, indomethacin and by the B_2 receptor antagonists, Hoe 140 and NPC 17761. In previous studies it had been suggested that bradykinin-induced relaxation was related to the presence of the epithelium (Bramley *et al.*, 1990; Schlemper & Calixto, 1994). Bramley *et al.* (1990) showed that in the presence of indomethacin the response of the guinea-pig tracheal preparation to bradykinin was changed from a relaxation to a contraction. Therefore, it was suggested that bradykinin-induced relaxation was related to an epithelium-dependent cyclo-oxygenase mechanism (Bramley *et al.*, 1990) or to a cyclo-oxygenase and nitric oxide mechanism (Schlemper & Calixto, 1994). However, in these studies, the experimental conditions were not the same for the tracheal preparations with or without epithelium. Intact tracheal preparations were allowed to develop a spontaneous tone. Treatment with indomethacin reduced the release of prostaglandins (Bramley *et al.*, 1990) and the spontaneous tone (Schlemper & Calixto, 1994). Tracheal preparations without epithelium did not develop a spontaneous tone (Bramley *et al.*, 1990). Moreover, after the induction of tone by acetylcholine, the contractile response elicited by bradykinin was changed to a relaxation (Bramley *et al.*, 1990). The results of the present study show that when tracheal preparations with or without epithelium were equilibrated at a resting tone, bradykinin induced a concentration-related contraction. When tracheal preparations were precontracted, bradykinin induced a concentration-related relaxation. However, in the absence of epithelium, the relaxation induced by bradykinin was smaller (63 ± 13% relaxation in the presence of epithelium vs. 42 ± 6% relaxation in the absence of epithelium). This may be related to a reduced production of prostaglandins in tracheal preparations without epithelium (Bramley *et al.*, 1990). Such a dual behaviour, related to the tone of the guinea-pig isolated trachea and independent of the epithelium, has also been reported for prostaglandin E_2 (Braunstein *et al.*, 1988). Melittin, a direct activator of phospholipase A_2, can also induce contraction or relaxation in guinea-pig tracheal preparations with or without epithelium. Melittin-induced effects were unaffected by bradykinin-receptor antagonists. Another difference between the effects of bradykinin and melittin is the sensitivity of the contractile response to melittin to 5-lipoxygenase inhibition (Yamamoto *et al.*, 1990; Shikada & Tanaka, 1993). This observation is in agreement with the activation of phospholipase A_2 by melittin, since arachidonic acid is the substrate of both cyclo-oxygenase and 5-lipoxygenase.

Another important finding of the present study is the fact that indomethacin can suppress the relaxation induced by bradykinin. These results suggest that, upon blockade of prostaglandin synthesis, bradykinin cannot induce the release of nitric oxide or that nitric oxide alone is unable to induce tracheal relaxation (Schlemper & Calixto, 1994). In the presence of indomethacin, the bradykinin-induced contraction was reduced, although a small contraction persisted for bradykinin concentrations up to 1 μM. These results agree with previous reports that bradykinin-induced effects *in vitro* are largely related to prostaglandin synthesis in guinea-pig tracheal preparations (Farmer *et al.*, 1989; Bramley *et al.*, 1990).

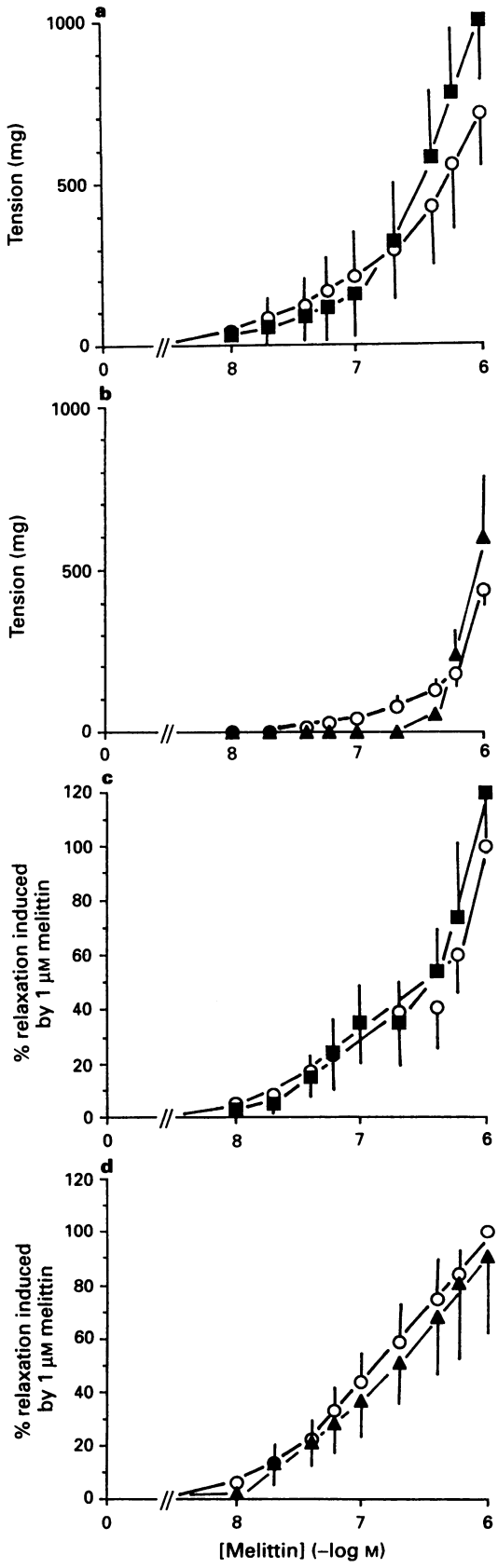

Figure 4 Effect of bradykinin receptor antagonists on melittin-induced contraction and relaxation on guinea-pig tracheal preparations. Tracheal preparations were suspended under 2 g tension before starting the concentration-response curve for melittin in the absence (○) or (a) in the presence of Hoe 140 (■, 0.6 μM, $n = 6$) or (b) in the presence of NPC 17761 (▲, 1μM, $n = 3$). Alternatively, tracheal preparations were suspended under 2 g tension then stimulated with histamine (30 μM) before starting the concentration-response curve for melittin in the absence (○) or (c) in the presence of Hoe 140 (■, 0.6 μM, $n = 6$) or (d) in the presence of NPC 17761 (▲, 1μM, $n = 5$). The tensions induced by histamine were 1367 ± 195 mg (c) and 1290 ± 447 mg (d).

Under the conditions used in the present study, the responses obtained were fully reproducible. We therefore investigated the possibility of the activation of a bradykinin receptor leading to an induction of relaxation. Kinins receptors have been subdivided into two types (Regoli & Barabé, 1980): B_1, activated by [des-Arg^9]-bradykinin, with a higher potency than bradykinin, and antagonized by [Leu^8, des-Arg^9]-bradykinin, and B_2 for which these agents have low affinity. The first B_2-receptor antagonist, [D-Phe^7]-bradykinin, was described by Vavrek & Stewart (1985) and has since been followed by many analogues with improved affinities and selectivities (see Hall, 1992). However, these B_2-receptor antagonists maintain low affinity and partial agonist activity especially in the guinea-pig trachea (Stewart & Vavrek, 1990; Regoli *et al.*, 1990). Such compounds led Farmer *et al.* (1989), to suggest the presence of a putative bradykinin B_3-receptor in airway smooth muscle. Recently, Farmer & DeSiato (1994) described a novel nonpeptide bradykinin B_2 receptor antagonist (WIN 64338) selective for ileal receptors, i.e. without effect on bradykinin-induced contraction of guinea-pig trachea. B_1-receptor agonists and B_2-receptor antagonists have been described as having no effect on bradykinin-induced relaxation (Rhaleb *et al.*, 1988). Therefore, it was proposed that bradykinin may activate directly one of the enzymes involved in the arachidonic acid cascade, including phospholipase A_2. Alternatively, these authors proposed that the guinea-pig trachea may contain a new type of receptor for kinins which is not blocked by the B_1- or B_2-receptor antagonists. Bramley *et al.* (1989) have reported that bradykinin-induced contraction was inhibited by the B_2-receptor antagonist, D-Arg,[Hyp^3,Thi^{5,8},D-Phe$_7$]-bradykinin whereas the bradykinin-induced relaxation was potentiated. These observations led them to suggest that this antagonist may act as a partial agonist for other bradykinin receptors. The results of the present study do not agree with a direct activation of phospholipase A_2 by bradykinin nor with the involvement of a non-B_1, non-B_2 bradykinin receptor. We show that bradykinin-induced relaxation is reduced in the presence of B_2-receptor antagonists, Hoe 140 or NPC 17761. As for the contraction induced by bradykinin (Trifilieff *et al.*, 1992; 1993a), Hoe 140 behaved as a non-competitive antagonist and NPC 17761 as a competitive antagonist on bradykinin-induced relaxation. The B_1-receptor agonist, [des Arg^9]-bradykinin was without effect on guinea-pig trachea. The non-competitive properties of Hoe 140 in the guinea-pig trachea remain unexplained. The existence of B_2-receptor subtypes cannot be excluded considering the recent results of Farmer & DeSiato (1994). Also, a low-affinity binding site has been detected in tracheal preparations with epithelium but not in preparations without epithelium (Trifilieff *et al.*, 1992). This binding site may be related to the activation of a receptor in epithelial cells which is not involved in bradykinin-induced tone or may belong to non-receptor proteins such as enzymes involved in the degradation of kinins. For example, endopeptidase 24.11 has been shown to be located mainly in the epithelium (Johnson *et al.*, 1985).

In summary, the present study provides consistent evidence supporting the view that both bradykinin-induced contraction or relaxation are mediated by a B_2-receptor in guinea-pig trachea. The response, relaxation or contraction, is not related to the presence or absence of the epithelium but to the tone of the isolated trachea.

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